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Soil microbial activity and crop sustainability in a long-term experiment with three soil-tillage and two crop-rotation systems

Mariangela Hungria [a,*], Julio Cezar Franchini [a], Osvaldino Brandão-Junior [b], Glaciela Kaschuk [a], Rosinei Aparecida Souza [a]

[a] Embrapa Soja, Londrina, Paraná, Brazil
[b] Universidade Estadual de Campinas, Faculdade de Engenharia Agrícola, Cx. Postal 6011, 13083-875 Campinas, SP, Brazil

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ABSTRACT

Reduction in soil disturbance can stimulate soil microbial biomass and improve its metabolic efficiency, resulting in better soil quality, which in turn, can increase crop productivity. In this study we evaluated microbial biomass of C (MB-C) by the fumigation-extraction (FE) or fumigation-incubation (FI) method; microbial biomass of N (MB-N); basal respiration (BR) induced or not with sucrose; metabolic quotient (obtained by the ratio BR/MB-C) induced (qCO_2(S)), or not with sucrose (qCO_2); and crop productivity in a 14-year experiment in the state of Paraná, southern Brazil. The experiment consisted of three soil-tillage systems [no-tillage (NT), conventional tillage (CT) and no-tillage using a field cultivator every 3 years (FC)] and two cropping systems [a soybean–wheat-crop sequence (CS), and a soybean–wheat–white lupin–maize–black oat–radish crop rotation (CR)]. There were six samplings in the 14th year, starting at the end of the winter crop (wheat in the CS and lupin in the CR plots) and finishing at full flowering of the summer crop (soybean in the CS and maize in the CR). Differences in microbiological parameters were greater than those detected in the total C (TCS) and total N (TNS) contents of the soil organic matter (SOM). Major differences were attributed to tillage, and on average NT was higher than the CT in the following parameters: TCS (19%), TNS (21%), MB-C evaluated by FE (74%) and FI (107%), and MB-N (142%). The sensibility of the microbial community and processes to soil disturbance in the tropics was highlighted, as even a moderate soil disturbance every 3 years (FC) affected microbial parameters but not SOM. The BR was the parameter that most promptly responded to soil disturbance, and strong differences were perceived by the ratio of qCO_2 evaluated with samples induced and non-induced with sucrose. At plowing, the qCO_2(S):qCO_2 was five times higher under CT, indicating a C-starving low-effective microbial population in the C-usage. In general, crop rotation had no effect on microbial parameters or SOM. Grain yield was affected by tillage and N was identified as a limiting nutrient. Linear regressions between grain yields and microbial parameters showed that soybean was benefited from improvements in the microbial biomass and metabolic efficiency, but with no significant effects observed for the maize crop. The results also indicate that the turnover of C and N in microbial communities in tropical soils is rapid, reinforcing the need to minimize soil disturbance and to balance inputs of N and C.

1. Introduction

Sustainability is an important goal for modern agriculture, i.e. maintenance of productivity of food and feed crops and of animals coupled with efficient cycling of nutrients and with biological control of pathogens. Most arable soils worldwide have been cultivated continuously for decades with limited deposition of residues, and now show relatively poor biological diversity, resulting in decreased fertility and gradual loss of plant/animal productivity (Giller et al., 1997; Nogueira et al., 2006; Roscoe et al., 2006). Unfortunately, even with reduced soil disturbance, cropping may affect soil biological activity as well as physical and chemical attributes (Balota et al., 1998, 2003, 2004; Alvarenga et al., 1999). Therefore, despite improvements that have been achieved from soil-conservation methods – such as the no-till (NT) system – the search continues for agricultural practices with effects that mimic the equilibrium of natural ecosystems.

Soil biomass is partly composed by diverse microbial species – fungi, bacteria, actinomycetes, protozoa, nematodes, algae – that

* Corresponding author at: Embrapa-Soja, Cx. Postal 231, 86001-970 Londrina, PR, Brazil. Tel.: +55 43 33716206; fax: +55 43 33716100.
E-mail addresses: hungria@cnpso.embrapa.br (M. Hungria), franchin@cnpso.embrapa.br (J.C. Franchini), dinoagr@yahoo.com.br (O. Brandão-Junior), glaciela.kaschuk@gmail.com (G. Kaschuk), rosyquimica@yahoo.com.br (R.A. Souza).

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are responsible for nutrient cycling, nutrient foraging (e.g. mycorrhizal fungi), nutrient fixation (e.g. diazotrophic bacteria), biological suppressiveness, decomposition of xenobiotics, etc. (Höflich et al., 1999; Hungria and Vargas, 2000; Pankhurst et al., 2002; Lupwayi et al., 2004). Although activity of microorganisms is influenced by environmental and soil chemical–physical properties, soil microbial biomass responds positively to sustainable cropping and soil-management practices, suggesting that it is a parameter that diagnoses soil quality (Doran and Parkin, 1994; Balota et al., 1998; Roscoe et al., 2006; Franchini et al., 2007; Souza et al., 2008a,b).

In tropical soils, turnover of nutrients and microbial biomass occurs more rapidly (e.g. Balota et al., 1998, 2003; Franchini et al., 2007) than in temperate regions (e.g. Wardle and Ghani, 1995; Wardle et al., 1999), and for that reason, it is important to implement practices of management that minimize soil disturbance, which has drastic effect on soil microorganisms. As a strategy to control soil erosion in Brazil that started with agricultural expansion in the 1960s, no-tillage (NT) was adopted in 1971 in the southern state of Paraná and, by 2007, was used on over 26 million hectares, more than half of the country's cropped area (FEBRAPDP, 2008); concomitant improvements in soil physical, chemical and biological properties have been reported (Sidiras et al., 1982; Balota et al., 1998; Sá et al., 2001; Bayer et al., 2002; Castro Filho et al., 2002; Franchini et al., 2007).

We hypothesize that reduction in soil disturbance [e.g. NT in contrast to conventional tillage (CT)] favors the survival and activity of soil microorganisms with an improved metabolic efficiency (less CO_2 produced per unit of microbial biomass). With time, such improved metabolic efficiency results in improved soil quality, which in turn, favors crop productivity and sustainability. In this study, our objectives were to measure soil microbial biomass-C (MB-C) and activity [basal respiration (BR) and related parameters] through one cropping season at the 14th year of a field experiment with three soil-tillage and two crop-rotation systems and to relate them with crop productivity.

2. Materials and methods

2.1. Experiment design and soil sampling

The study was performed in a 14-year-old experiment established at the experimental station of Embrapa Soja, located at an altitude of 620 m, in Londrina (23°11′S, 51°11′W), state of Paraná, southern Brazil. The climate is subtropical (*Cfa*, according to Koppen's classification), with an annual average temperature of 21 °C, and mean maximum and minimum temperatures of 28.5 °C in February and 13.3 °C in July, respectively. Mean annual precipitation of 1651 mm, with January being the wettest month (217 mm) and August the driest (60 mm). The trial is established on an oxisol (Latossolo Vermelho Eutroférrico, Brazilian classification; Rhodic Eutrudox, USA classification), with the following physical composition: 710 g kg^{-1} of clay, 82 g kg^{-1} of silt and 208 g kg^{-1} of sand. The soil chemical properties at the first evaluation are shown in Table 1.

The experiment consists of plots, 7.5 m in width × 30.0 m in length (four replicates per treatment), distributed in a completely randomized block design comparing three soil-management practices [(1) no-till: NT, sowing directly through the residue of the previous crop, with the opening of only a narrow channel in the sowing row; (2) conventional till: CT, where the soil is prepared yearly with one pass with disc plough and disc harrow; (3) field cultivated: FC, with the subsoil scarified to a depth of 15–20 cm in the winter every 3 years, leaving residues on the surface] and two crop-rotation systems [with the following species, in the winter and in the summer, respectively: (1) soybean (*Glycine max* (L.)

Table 1
Chemical properties of the soils[a] (0–10 cm) evaluated at the first sampling.

Management		pH $CaCl_2$	H + Al ($cmol_c\ dm^{-3}$)	K ($cmol_c\ dm^{-3}$)	Ca ($cmol_c\ dm^{-3}$)	Mg ($cmol_c\ dm^{-3}$)	BS[b] ($cmol_c\ dm^{-3}$)	CEC[c] ($cmol_c\ dm^{-3}$)	P ($mg\ dm^{-3}$)	C ($g\ dm^{-3}$)	N ($g\ dm^{-3}$)	SBS[d] (%)
NT	CR	5.87 (0.22)	2.57 (0.24)	0.75 (0.05)	5.62 (0.56)	2.67 (0.23)	9.03 (0.65)	11.61 (0.52)	39.19 (3.92)	28.11 (2.76)	2.38 (0.26)	77.78 (2.54)
	CS	6.06 (0.19)	2.40 (0.11)	0.69 (0.08)	6.32 (0.54)	2.62 (0.34)	9.63 (0.90)	12.03 (1.00)	36.17 (9.24)	25.28 (1.77)	2.01 (0.15)	79.98 (0.80)
CT	CR	5.19 (0.30)	2.89 (0.23)	0.55 (0.12)	4.32 (0.83)	1.51 (0.10)	6.38 (0.96)	9.26 (0.74)	16.66 (2.45)	23.05 (1.07)	2.10 (0.04)	68.58 (4.60)
	CS	5.52 (0.11)	2.62 (0.21)	0.55 (0.17)	5.07 (0.42)	1.83 (0.12)	7.45 (0.41)	10.07 (0.50)	14.94 (2.10)	21.71 (1.15)	1.54 (0.23)	74.00 (1.71)
FC	CR	5.59 (0.26)	2.77 (0.34)	0.79 (0.01)	5.13 (0.91)	2.25 (0.21)	8.17 (1.10)	10.93 (0.77)	32.20 (4.99)	25.05 (2.52)	2.73 (0.20)	74.45 (4.88)
	CS	5.90 (0.26)	2.45 (0.28)	0.63 (0.21)	5.57 (0.33)	2.50 (0.40)	8.70 (0.61)	11.15 (0.64)	25.14 (3.71)	23.98 (3.10)	2.42 (0.19)	78.00 (2.38)

[a] Means of four replicates; and standard deviation is shown in parenthesis.
[b] Base sum (BS) = K + Ca + Mg.
[c] Cation exchange capacity (CEC).
[d] Soil base saturation = (BS/CEC) × 100, where CEC = K + Ca + Mg + total acidity at pH 7.0 (H + Al).

Merr.) and wheat (*Triticum aestivum* L.), designated as a crop sequence: CS; and (2) a crop rotation: CR—with soybean, wheat, white lupine (*Lupinus albus* L.), maize (*Zea mays* L.), black oat (*Avena strigosa* Schreb.) and radish (*Raphanus sativus* L.)]. Therefore, six treatments were tested: NT (CS or CR), CT (CS or CR) and FC (CS or CR). In the year that the samples were taken, the CS-plots were cropped with the soybean cultivar BRS 133, which was preceded by wheat, and the CR-plots were cropped with maize hybrid Pionner 3041, preceded by lupin. Every year, summer and winter crops received the same amount of fertilizer in NT and CT, based on the soil analysis and on recommendations for the cultivar used, as follows: for the wheat, no fertilizers in the first 8 years, and after that, 12.8–20 kg ha^{-1} of N, 44.8–70 kg ha^{-1} of P, and 24–32 kg ha^{-1} of K; for the maize, starting in the 5th year, 12.8–15 kg ha^{-1} of N, 28–60 kg ha^{-1} of P, and 56–60 kg ha^{-1} of K; no fertilizers were applied to the oat, lupin or radish. For the soybean, fertilizers were applied every year, as follows: zero N, and 40–60 kg ha^{-1} of P and K, and in the 12th and 14th years, the soybean received no fertilizers. Other macro- and micronutrients were added equally to all treatments, according to the soil analysis, as well as pH correction with lime. Herbicides were used equally for all treatments as recommended for the CT or NT cropping systems, while insects were controlled in all treatments with biological and chemical insecticides.

Soil samples were collected from the top layer (0–10 cm) between cropped lines during the growing season of the 14th year, in the mornings of the following dates: (1) 4th of September (immediately after harvesting the winter crop); (2) 3rd of October (after soil preparation in the CT and in the FC managements); (3) 17th of October (after sowing); (4) 7th of November (early vegetative stage); (5) 5th of December (full vegetative stage); and (6) 16th of January (full flowering).

For the soil sampling, in each plot a 0.4 m^2 was delimited with a metal square and with the help of a ruler a surface layer of 0–10 cm was established. A soil sample of approximately 150 g was then taken from the central part of the square using a shovel. The procedure was repeated six times in each pot, in points spatially distributed to represent the whole area. Subsamples were joined such that each replicate of this study consisted of a composite soil sample (approximately 1 kg) of six subsamples. At the laboratory the sample was homogenized and sieved (4 mm, 5 mesh).

2.2. Determination of soil moisture

Ten grams of soil were taken from each composite sample and dried for 12 h at 105 °C; on the following day, on the basis of the actual dry weight, the samples were corrected to reach a moisture of 40% of water holding capacity (WHC) (Vance et al., 1987a) by adding distilled water.

2.3. Microbial evaluations

2.3.1. Microbial biomass-C and -N (fumigation-extraction method)

Microbial biomass-C and -N (MB-C and MB-N) were evaluated by the fumigation-extraction (FE) method after Vance et al. (1987a), with some modifications. Four subsamples (20 g) of each composite soil sample were weighed and placed in glass receptacles (300 mL). Two of those subsamples were submitted to fumigation as described by Vance et al. (1987a), and the other two were not fumigated. Fumigated and non-fumigated samples were kept in the dark at 25 ± 2 °C for 16 h. After that, the C was extracted from the samples by adding 50 mL of extractor solution (0.5 M K_2SO_4), shaking (175 rpm, 1 h), centrifuging (3000 rpm, 10 min) and filtering as described by Franchini et al. (2007). Carbon content in the extracts was determined by the oxidation with Mn^{3+}, and evaluation on a spectrophotometer (Bartlett and Ross, 1988). The MB-C was estimated from the difference between fumigated and non-fumigated samples, and although the correction factor of 0.38 was proposed by Vance et al. (1987a,b), we used a K_{CE} of 0.41, as recommended for tropical soils to avoid overestimation (Feigl et al., 1995; Oliveira et al., 2001).

MB-N was evaluated by adding to the 20 mL of extract 1.5 mL of 1 M H_2SO_4 and 0.5 g of catalyst (K_2SO_4 + $CuSO_4$, 10:1). After digestion, the product was diluted with distilled water and the N determined by spectrophotometric determination of NH_4-N using the indophenol blue method (Feije and Anger, 1972). The correction factor K_{NE} = 0.54 (Brookes et al., 1985) was used.

2.3.2. Microbial biomass-C and -N (fumigation-incubation method), basal respiration and metabolic quotient (qCO_2)

An adaptation of the method fumigation-incubation (FI) of Jenkinson and Powlson (1976a) and Jenkinson and Powlson (1976b) was used to evaluate MB-C and MB-N. Five subsamples (100 g) of each composite sample were placed in glass jars (300 mL); two of those jars were subjected to fumigation and incubation, two were not fumigated and the fifth was homogenized with 1 g of sucrose. The receptacles with soil to be fumigated were placed, in groups of five, in sealable chambers, a Petri dish containing 50 mL of chloroform was placed in the bottom of each chamber, and wet absorbent paper was placed inside each chamber to maintain humidity. The chloroform was purified to be alcohol free by distillation. The chambers were evacuated and sealed with vaseline. All treatments – fumigated and non-fumigated soil samples (with or without sucrose) – were kept in the dark at room temperature (25 ± 2 °C) for 16 h. Chloroform was then removed from the fumigated samples via a vacuum pump, opening and closing the chambers five times.

After the first period of incubation, all samples were moved to individual chambers (2 L) containing either a receptacle filled with 50 mL of 0.5 N NaOH to capture the evolved CO_2, or containing 20 mL of distilled water. For each treatment, two controls, including only NaOH and water, were added. The chambers were sealed with vaseline and incubated in the dark at 25 ± 2 °C for 10 days. After this, basal respiration was indirectly determined by the addition of saturated $BaCl_2$ to the NaOH solution, followed by titration of the non-consumed NaOH with 0.2 N HCl. The MB-C was estimated from the difference between fumigated and non-fumigated samples using a correction factor (K_C = 0.411) (Anderson and Domsch, 1978). The basal respiration was estimated in non-fumigated soil samples (with or without sucrose).

The metabolic quotient (qCO_2) (Anderson and Domsch, 1993) was obtained from the relation between basal respiration (with or without sucrose) per unit of MB-C.

2.4. Total soil carbon and nitrogen

Organic-C was determined after Walkley-Black by the oxidation with $Cr_2H_2O_7$ in the presence of H_2SO_4 and titration of the excess dicromate with $Fe(NH_4)_2(SO_4)_2{\cdot}6H_2O$ (Allison, 1965). Total N was determined by digestion of samples with H_2SO_4 in the presence of K_2SO_4 and $CuSO_4$ followed by colorimetric determination of NH_4-N using the indophenol blue method (Feije and Anger, 1972).

2.5. Crop yield

Seed yield at physiological maturity was determined by harvesting 3.0 m of the four central rows of each plot. Seeds were cleaned and weighed and values were corrected to 13% moisture content, after determination of the humidity level in a grain moisture tester.

Table 2
Microbial biomass of C (MB-C)[a] (mg C kg^{-1} dry soil) obtained by fumigation-extraction and fumigation-incubation methods in soils (0–10 cm) after the 14th year of a field experiment under three soil-management and two crop-rotation systems.

Management		Sampling date											
		Fumigation-extraction						Fumigation-incubation					
		1	2	3	4	5	6	1	2	3	4	5	6
NT	CR	1144.5 a	376.0 ns	467.0 a	465.3 a	614.0 a	623.7 ns	839.3 a	658.0 a	869.6 a	438.1 a	476.6 ab	541.9 ns
	CS	1216.3 a	361.5	442.0 ab	412.3 a	661.0 a	497.0	759.2 ab	547.6 a	501.7 b	480.0 a	590.3 a	450.8
CT	CR	772.2 b	198.0	157.0 c	101.0 c	318.0 b	510.2	416.9 d	285.9 b	127.6 c	198.0 b	308.1 b	352.1
	CS	850.2 b	168.0	136.5 c	101.0 c	213.7 b	666.5	372.5 d	293.6 b	190.5 c	239.8 b	249.3 b	427.8
FC	CR	901.7 b	288.0	359.0 b	302.3 ab	496.0 a	740.5	685.8 bc	392.0 b	551.0 b	532.4 a	335.9 ab	456.5
	CS	851.5 b	299.0	368.5 b	226.0 bc	517.7 a	811.5	565.7 c	413.9 b	623.0 b	509.2 a	499.0 ab	465.3
NT		1180.4 a	368.7 a	454.5 a	438.8 a	637.5 a	560.4 ns	799.2 a	602.8 a	685.6 a	459.1 a	533.5 a	496.4 ns
CT		811.2 b	183.0 b	146.7 c	101.0 c	265.9 c	588.4	394.7 c	289.8 c	159.0 b	218.9 b	278.7 b	389.9
FC		876.6 b	293.5 ab	363.7 b	264.2 b	506.9 b	776.0	625.8 b	403.0 b	587.0 a	520.8 a	417.4 ab	460.9
CR		939.5 ns	287.3 ns	327.7 ns	289.6 ns	476.0 ns	624.8 ns	647.3 a	445.3 ns	516.0 ns	389.5 ns	373.5 ns	450.2 ns
CS		972.7	276.2	315.7	246.4	464.2	658.3	565.8 b	418.4	438.4	409.7	446.2	448.0

[a] Statistical analysis considering all treatments ($n = 4$), soil management ($n = 8$) and crop rotation ($n = 12$), and means from a same column followed by different letters are significantly different ($p \leq 0.05$, Duncan's test), ns means statistically non-significant.

2.6. Statistical analysis

The experiments analyzed as a complete randomized block design with 4 blocks (Cochran and Cox, 1957). The data were analyzed using SAS for PC statistical package (SAS Institute, 2001), using PROC GLM. All assumptions required by the analysis of variance (ANOVA) were verified. The error normality, according to the experimental model design, was evaluated by Shapiro–Wilk's test (Shapiro and Wilk, 1965), the variance of homogeneity by Burr–Foster's test (Burr and Foster, 1972), and the non-additivity of the model by Tukey's method (Tukey, 1949). Coefficient of skewness and kurtosis were also checked. Linear regressions were applied to individual plot observation of grain yields ($n = 12$) and the averaged values of microbial parameters in each plot.

3. Results

3.1. MB-C and MB-N

Differences in the values of MB-C (evaluated by the FE method) among the plots under the three soil-tillage systems were clear after 14 years, with the highest values detected under NT, followed by the FC and the lowest in the CT treatment (Table 2). In addition, MB-C values were statistically different between tillage systems throughout the seasons, except for the 2nd (soil preparation), and the 6th (full flowering) samplings. In contrast, no differences in MB-C were attributed to crop rotation in any of the tillage systems (Table 2).

In all treatments, MB-C was higher at the winter harvesting (1st sampling), drastically decreasing in the harvest realized after that. Later in the growing season, MB-C increased again during early vegetative growth (5th sampling) (Table 2). In the 30-day period between the winter harvest and soil preparation for sowing (2nd sampling), the MB-C values corresponded to only 35% (FC with CS) to 20% (CT with CS) of those at the previous sampling. In general, decreases were greater in the CT and FC treatments than in the NT.

Significant correlations were found between the MB-C values obtained with the FE and the FI methods ($r = 0.66$; $p \leq 0.01$). Considering all treatments, values of MB-C evaluated by FI were on average 6% lower than those obtained by FE. Similarly, considering the three soil-tillage treatments, higher values of MB-C evaluated by the FI method were always observed in the NT treatment, and were statistically different from the CT treatment in all samplings except for the last one (Table 2). In relation to the two cropping systems, no differences were observed in MB-C evaluated by FI, the only exception being at the 1st sampling, with increased levels with lupin (Table 2).

In general, the estimates of MB-N showed differences among the treatments (Table 3) more clearly than did MB-C (Table 2).

Table 3
Microbial biomass of N (MB-N, fumigation-extraction method)[a] and the MB-C:MB-N ratio of the microbial biomass (fumigation-extraction method)in soils (0–10 cm) after the 14th year of a field experiment under three soil-management and two crop-rotation systems.

Management		Sampling date											
		MB-N, mg N kg^{-1} dry soil						MB-C:MB-N ratio, mg C mg^{-1} N					
		1	2	3	4	5	6	1	2	3	4	5	6
NT	CR	84.5 bc	101.5 a	46.5 ab	113.3 a	72.0 bc	104.2 a	13.54 b	3.70 ns	10.04 ns	4.11 ns	8.53 ab	5.99 b
	CS	113.2 a	95.2 ab	51.7 a	92.7 b	96.7 a	93.0 ab	10.74 bc	3.80	8.55	4.45	6.84 ab	5.34 b
CT	CR	45.7 d	45.5 d	17.2 c	14.3 d	32.0 e	46.0 b	16.90 a	4.35	9.13	7.06	9.94 a	11.09 ab
	CS	65.7 c	50.0 d	16.2 c	26.3 cd	35.0 de	44.7 b	12.94 b	3.36	8.43	3.84	6.11 b	14.91 a
FC	CR	68.5 c	75.5 c	39.2 b	73.3 b	53.7 dc	88.7 ab	13.16 b	3.81	9.16	4.12	9.24 ab	8.35 ab
	CS	98.7 ab	79.2 bc	40.7 b	45.0 c	69.0 ab	91.0 ab	8.63 c	3.78	9.05	5.02	7.50 ab	8.92 ab
NT		98.9 a	98.4 a	49.1 a	103.0 a	84.4 a	98.6 a	11.94 b	3.75 ns	9.26 ns	4.26 ns	7.55 ns	5.68 b
CT		55.7 c	47.7 c	16.7 c	20.3 c	33.5 c	45.4 c	14.56 a	3.84	8.78	4.98	7.94	12.96 a
FC		83.6 b	77.4 b	40.0 b	59.2 b	60.3 b	89.8 b	10.49 b	3.79	9.09	4.46	8.41	8.64 ab
CR		66.2 b	74.2 ns	34.3 ns	67.0 a	52.6 b	79.7 ns	14.19 a	3.87 ns	9.55 ns	4.32 ns	9.05 ns	7.84 ns
CS		92.6 a	74.8	36.2	54.7 b	66.7 a	76.2	10.50 b	3.69	8.72	4.50	6.96	8.64

[a] Statistical analysis considering all treatments ($n = 4$), soil management ($n = 8$) and crop rotation ($n = 12$), and means from a same column followed by different letters are significantly different ($p \leq 0.05$, Duncan's test), ns means statistically non-significant.

Table 4
Basal respiration (mg C kg^{-1} dry soil h^{-1}) not induced (BR)[a] and induced with addition of sucrose during incubation [BR(S)] in soils (0–10 cm) after the 14th year in a field experiment under three soil-management and two crop-rotation systems.

Management		Sampling date											
		Not induced						Induced					
		1	2	3	4	5	6	1	2	3	4	5	6
NT	CR	0.77 ab	0.87 ns	1.49 bc	1.42 a	0.68 a	1.19 a	5.49 ns	5.56 ns	4.80 ns	4.31 ns	5.05 ns	7.06 a
	CS	0.83 a	0.89	2.08 a	1.46 a	0.77 a	1.33 a	5.07	5.36	4.93	4.80	5.54	7.05 a
CT	CR	0.39 c	0.63	1.20 c	0.65 c	0.34 b	0.73 b	5.60	5.42	4.80	5.39	4.73	6.21 ab
	CS	0.52 bc	0.68	1.09 c	0.75 c	0.64 ab	0.82 b	4.95	4.88	4.93	4.20	4.53	5.17 b
FC	CR	0.55 bc	0.80	1.34 bc	1.14 b	0.74 a	1.14 a	5.65	5.14	5.47	5.07	4.84	6.83 a
	CS	0.62 ab	0.84	1.74 ab	1.02 b	0.77 a	1.20 a	5.80	5.13	5.25	5.75	5.27	7.07 a
NT		0.80 a	0.88 a	1.79 a	1.44 a	0.72 a	1.26 a	5.28 ns	5.46 ns	4.87 ns	4.56 ns	5.30 ns	7.05 a
CT		0.46 b	0.66 b	1.15 b	0.70 c	0.49 b	0.77 b	5.27	5.15	4.87	4.79	4.63	5.69 b
FC		0.58 b	0.82 ab	1.54 a	1.08 b	0.76 a	1.17 a	5.73	5.13	5.36	5.41	5.05	6.95 a
CR		0.57 ns	0.77 ns	1.34 ns	1.07 ns	0.59 ns	1.02 ns	5.58 ns	5.37 ns	5.02 ns	4.93 ns	4.87 ns	6.70 ns
CS		0.66	0.80	1.64	1.08	0.73	1.12	5.27	5.12	5.04	4.92	5.12	6.43

[a] Statistical analysis considering all treatments ($n = 4$), soil management ($n = 8$) and crop rotation ($n = 12$), and means from a same column followed by different letters are significantly different ($p \leq 0.05$, Duncan's test), ns means statistically non-significant.

Higher values of MB-N were verified in the NT system at all harvests, with the lowest value of MB-N obtained in the CT treatment (Table 3). In relation to cropping system, differences between the CS and CR treatments were detected in three of the six samplings. The MB-N was higher in the CS at the winter harvesting and full vegetative stages (1st and 5th samplings), but lower at the early vegetative stage (4th sampling) (Table 3), attributable to the application of N-fertilizer (15 kg of N ha^{-1}) to the maize crop (CR) at sowing, and 15 days before the 4th sampling.

The MB-C:MB-N ratio ranged from 3.36 to 16.9, but in general this parameter was not sufficiently sensitive to detect differences among soil and cropping treatments (Table 3). The main exceptions were for the CT treatment at the winter harvest (1st) and at full flowering (6th sampling), both of which showed a very high C:N ratio. For the cropping systems, only at the winter harvest was the C:N ratio of the CR plots with lupin higher than that of the CS plots with wheat, indicating that the legume had released no N during growth prior to its incorporation (Table 3).

3.2. Basal respiration and metabolic quotient (qCO_2)

In general, the highest values of BR were observed with NT and CS, and the lowest with CT (Table 4). A peak in BR was observed in all treatments around sowing (3rd sampling), indicating high sensitivity to soil disturbance (Table 4). However, the increase in BR was short-lived, with no effects on MB-C (Table 2). Interestingly, the peak was not observed when the basal respiration was evaluated in the samples amended with sucrose, except at the last evaluation, at full flowering (Table 4). Amendment with sucrose strongly increased BR and maximum rates were observed at full flowering (Table 4). The results indicate that the potential microbial activity of soils in all samplings till flowering was similar in NT and CT systems, but under CT the microbes were limited in C. Therefore at flowering, when the microbial community usually is stimulated, the potential activity with NT proved to be higher than that with CT.

The qCO_2 estimates with FI in non-amended soils varied mainly with sampling time (Table 5). The lowest qCO_2 values were observed at the winter harvest (1st sampling), indicating low microbial activity after the crop harvest. Increases in the qCO_2 – indicating lower efficiency in the use of the soil C by the microbial population – were then observed at sowing and early vegetative growth, associated with the soil preparation and sowing practices, decreasing again at full vegetative growth and flowering. In general, higher qCO_2 varied as a function of soil management and not of cropping system (Table 5). In relation to cropping system, differences in the qCO_2 were detected in three out of the six samplings, being higher with CR, probably due to the previous

Table 5
Metabolic quotient (μg C mg^{-1} MB-C h^{-1}) not induced (qCO_2)[a] and induced with addition of sucrose during incubation [qCO_2(S)] in soils (0–10 cm) after the 14th year in a field experiment under three soil-management and two crop-rotation systems.

Management		Sampling date											
		Non-induced (qCO_2)						Induced [qCO_2(S)]					
		1	2	3	4	5	6	1	2	3	4	5	6
NT	CR	0.92 b	1.32 b	1.72 d	3.24 a	1.42 bc	2.19 b	6.54 d	8.45 e	5.52 c	9.84 c	10.60 c	13.03 bc
	CS	1.10 b	1.62 ab	4.15 bc	3.05 a	1.30 c	2.94 a	6.68 d	9.79 d	9.83 c	10.00 c	9.39 c	15.64 ab
CT	CR	0.94 b	2.21 a	9.37 a	3.31 a	1.12 c	2.07 b	13.43 a	18.95 a	37.64 a	27.24 a	15.35 ab	17.64 a
	CS	1.40 a	2.32 a	5.75 b	3.15 a	2.56 a	1.92 b	13.28 a	16.62 b	25.91 b	17.50 b	18.18 a	12.09 c
FC	CR	0.80 b	2.03 ab	2.43 cd	2.15 b	2.20 ab	2.49 ab	8.24 c	13.10 c	9.92 c	9.53 c	14.41 b	14.96 ab
	CS	1.09 b	2.04 ab	2.79 cd	2.00 b	1.55 bc	2.58 ab	10.26 b	12.38 c	8.42 c	11.29 c	10.56 c	15.20 ab
NT		1.01 ns	1.47 b	2.93 b	3.14 b	1.36 ns	2.57 ns	6.61 c	9.12 c	7.68 b	9.92 b	9.99 b	14.33 ns
CT		1.17	2.26 a	7.56 a	3.23 a	1.84	1.99	13.35 a	17.78 a	31.78 a	22.37 a	16.77 a	14.87
FC		0.94	2.03 a	2.61 b	2.07 b	1.88	2.54	9.24 b	12.74 b	9.17 b	10.41 b	12.49 b	15.08
CR		0.88 ns	1.85 ns	4.51 ns	2.90 ns	1.58 ns	2.25 ns	10.07 ns	13.50 ns	17.69 a	15.54 a	13.46 ns	15.21 ns
CS		1.19	1.99	4.23	2.73	1.80	2.48	9.40	12.93	14.72 b	12.93 b	12.71	14.31

[a] Statistical analysis considering all treatments ($n = 4$), soil management ($n = 8$) and crop rotation ($n = 12$), and means from a same column followed by different letters are significantly different ($p \leq 0.05$, Duncan's test), ns means statistically non-significant.

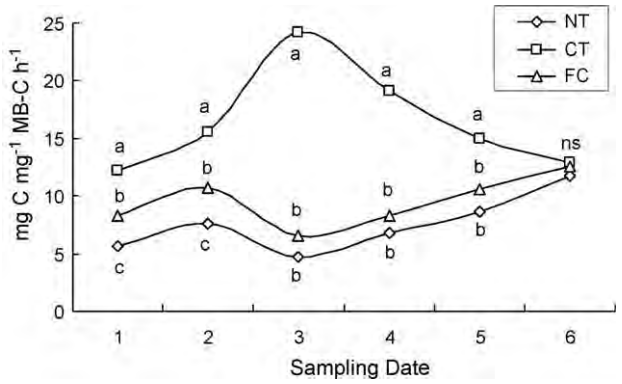

Fig. 1. Differences between induced qCO_2 and qCO_2 under normal soil conditions in a long-term experiment with three soil-tillage systems. Different letters indicate significant difference at $p \leq 0.05$ (Duncan) between treatments; ns = not significantly different.

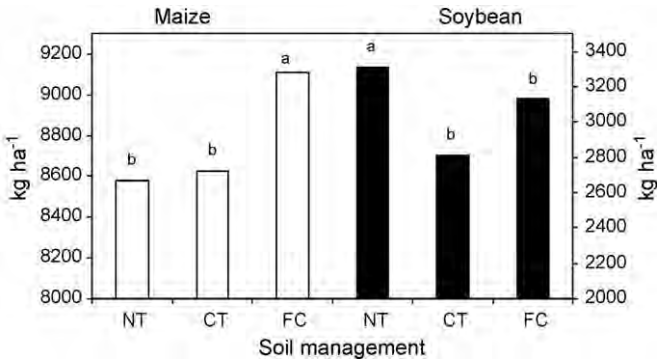

Fig. 2. Maize and soybean yields (kg ha^{-1}) as a function of three soil-management systems (no-till, NT; conventional till, CT; use of field cultivator every 3 years, FC), after 14 years of experiments. Soybean was cultivated in a cropping sequence cropping system and was preceded by wheat, while maize was cultivated in cropping rotation (as defined in Table 2) and preceded by lupin. Different letters indicate significant difference at $p \leq 0.05$ (Duncan) between treatments.

cropping with lupin. Later, at full vegetative stage of the summer crop, the qCO_2 was higher with maize growing in the CR system, in comparison with the CS growing soybean (Table 5).

As expected, the qCO_2 values obtained from the BR of the sucrose-amended soil were considerably higher than those of non-amended soils (Table 5); furthermore, the qCO_2 was higher under CT than in the NT in all samplings. The differences estimated between the qCO_2 obtained with sucrose and without amendment emphasize that, after 14 years of conventional practices of soil preparation, the microbes under CT were metabolically less active in using C reserves. Field cultivation every 3 years also decreased microbial metabolic activity at least for a period of time (Fig. 1).

Finally, the effects of tillage on the soil microbes were highlighted when the means of all samplings were estimated, indicating that, after 14 years, the MB-C and MB-N values with CT corresponded to about half of those with NT, whereas the MB-C:MB-N ratio increased considerably (Table 6). The changes were of greater magnitude than those observed in soil total C, as the values detected in the CT and FC treatments corresponded to 84 and 92% of the content of NT system, respectively. In relation to the soil total N, the content under CT corresponded to 83% of that of the NT; however, a value 17% higher was detected in the FC treatment, related to soil perturbation by the field cultivator. The ratio of qCO_2 estimated with and without supplemental sucrose was also very sensitive in detecting differences among the tillage systems, with a 40% increase in the CT in relation to the NT. Considering all samplings, no differences could be attributed to cropping system for any of the microbiological parameters, whereas a decrease in total N of the SOM was observed, due to CS (Table 6).

3.3. Crop yield

The summer crops were harvested on April of the 14th year. Grain yields of maize (CR system) cultivated under CT and NT were similar, and less than in the FC system (Fig. 2). An opposite situation was observed for the soybean crop in the CS system, in which, grain yield was significantly increased due to NT in comparison with FC and CT treatments.

Linear regressions were applied to test the relationships between grain yields and microbial parameters [MB-C (FE), MB-C (FI), qCO_2, $qCO_2(S)$ or $qCO_2(S)/qCO_2$; $n = 12$]. There was no significant relationship between maize yields and any of the microbial parameters evaluated. However, for the soybean crop the slope coefficients of linear regressions (b) were significantly affected by MB-C (FE) ($b = +1.97$; $p < 0.001$), MB-C (FI) ($b = +1.11$; $p = 0.05$), qCO_2 (S) ($b = -61.82$; $p = 0.003$) and $qCO_2(S)/qCO_2$ ($b = -154.02$; $p = 0.009$).

4. Discussion

It is well known that, in the long-term, reduction of tillage, as in the NT system, results in increased SOM in comparison to CT management (Sidiras et al., 1982; Alvarez et al., 1995; Bayer et al., 2002; Castro Filho et al., 2002; Franchini et al., 2007). In this study, after 14 years, the soil total C content was 19% higher in the NT system, and field cultivation every 3 years did not result in a detectable decrease in C content (Tables 1 and 6). Lupwayi et al. (2004) suggested that the higher soil-C content is not related to the production of residues, as – given the same nutritional

Table 6
Total N in the soil (g dm^{-3}), total C in the soil (g dm^{-3}), microbial biomass of C (MB-C, mg C kg^{-1} dry soil) evaluated by the fumigation-extraction (FE) or fumigation-incubation (FI) methods; MB-N (FE) (mg N kg^{-1} dry soil); ratio MB-C/MB-N (FE); basal respiration, amended [BR(+S)] or not with sucrose (BR) (mg C kg^{-1} dry soil h^{-1}); metabolic quotient, induced [$qCO_2(S)$] or not induced with sucrose (qCO_2) (μg C mg^{-1} MB-C h^{-1}), ratio $qCO_2(S)/qCO_2$, evaluated after the 14th year of a field experiment with three soil-management and two crop-rotation systems. Data represent the means of six samplings performed from the end of the winter crop to the full flowering of the summer crop.

Management	TNS	TCS	MB-C (FE)	MB-N (FE)	Ratio MB-C/MB-N	MB-C (FI)	BR	BR(S)	qCO_2	$qCO_2(S)$	Ratio $qCO_2(S)/qCO_2$
NT	2.20 b	26.70 a	606.8 a	88.8 a	6.84 b	596.1 a	1.15 ns	5.42 ns	2.08 b	9.61 c	4.62 c
CT	1.82 c	22.38 c	349.4 c	36.7 c	9.52 a	288.5 b	0.70	5.07	3.01 a	19.49 a	6.48 a
FC	2.58 a	24.52 b	513.6 b	69.7 b	7.37 ab	502.5 a	0.99	5.61	2.01 b	11.52 b	5.73 b
CR	2.40 a	25.40 ns	490.9 ns	62.39 ns	7.87 ns	470.3 ns	0.89 ns	5.41 ns	2.33 ns	14.13 ns	6.07 ns
CS	1.99 b	23.66	488.9	67.72	7.22	454.4	1.00	5.32	2.41	12.95	5.38

Statistical analysis considering soil management ($n = 8$) and crop rotation ($n = 12$), and means from a same column followed by different letters are significantly different ($p \leq 0.05$, Duncan's test), ns means statistically non-significant.

conditions – tillage should not affect the quality or quantity of crop residues. Benefits from NT may be related to decreased water runoff and erosion, to effects on soil temperature and humidity oscillations, to rate of decomposition of residues – due to less surface area in contact with microorganisms – among others, contributing to increased SOM (Kladviko, 2001; Franchini et al., 2007). With time, the effects of NT may result in increased production of plant biomass and yields, thus increasing residue deposition and, as a consequence, soil-C content (Hungria and Vargas, 2000; Sá et al., 2001; Santos et al., 2006; Franchini et al., 2007; Pereira et al., 2007). Increases in SOM with the adoption of NT in Brazil have been detected sooner than in this experiment, e.g. an increase of 25% was observed after 5 years of NT in the state of Paraná (Franchini et al., 2007), and of 25 and 29% in total soil C and N in the state of Rio Grande do Sul after 9 years (Bayer et al., 2002); apparently, increases continue with time, as in a 21-year-old experiment differences in soil total C reached 45% (Balota et al., 2004).

In this study, the cropping system did not alter soil total-C values, but a higher N content was obtained with CR (Tables 1 and 6). Absence of differences in SOM and TCS have also been reported for three different cropping systems after 5 years of experiments (Franchini et al., 2007), as well as in a 7-year experiment that included a soybean/wheat sequence and a lupin/oat/maize crop rotation (Franchini et al., 2002).

Soil microbial activity and composition may vary considerably with climate seasonality, plant growth stage, soil coverage, etc. (Balota et al., 1998; Wardle et al., 1999; Hungria and Vargas, 2000; Franchini et al., 2007). In this study, the microbial parameters also fluctuated during plant development: MB-C was higher with the deposition of plant residues at the harvest of the winter crop and, in the next season, was stimulated by the plant rhizosphere at the full-vegetative and full-flowering stages (Table 2). Those results corroborate reports of fast increases in microbial activity due to the deposition of fresh residues (Balota et al., 1998; Franchini et al., 2007), as well as of higher activity of microbial processes, e.g. N_2 fixation and mycorrhizal colonization, at flowering (Bethlenfalvay et al., 1982; Hungria and Neves, 1986). In addition, the high MB-C at the winter harvest was associated with a low BR (Table 4), possibly because of a more mature and stable microbial community, showing lower metabolic activity than by young cells (Balota et al., 1998). Basal respiration was a parameter very sensitive to soil disturbance, clearly demonstrated by the peak of activity at sowing of the summer crops, even under the NT system, although only a narrow channel was opened for seed placement with immediate closure.

In general, MB-N varied less throughout the season than did MB-C; however, it is noteworthy that low values were detected at sowing time (Table 3). The results obtained in this study clearly show differences between the microbial C- and N-related processes—the C-community promptly responded to the addition of fresh residues or to rhizospheric stimulus, and only after a period of activity of the C-related microorganisms was the N-community affected. The C:N ratio reflects those differences; the lowest C:N ratio of the microbial biomass occurred some time after the incorporation of the winter-crop residues.

In this long-term experiment, the major effects on microbial biomass were related to tillage. After 14 years, NT increased the MB-C and MB-N values, in comparison to CT, by averages of 74 and 142%, respectively (Table 6). In other studies in the state of Paraná, increases on those parameters were of 80 and 104%, respectively, after 5 years of NT (Franchini et al., 2007), of 118 and 101%, respectively, after 16 years of NT (Balota et al., 1998), and of 103 and 54%, respectively, in a 21-year experiment (Balota et al., 2003). These results emphasize the importance of soil-conservation practices in the tropics, where treatment effects on microbial biomass are far greater than those reported in temperate regions (Wardle and Ghani, 1995; Wardle et al., 1999).

Not only was MB-C higher in the NT system, but microbial metabolic efficiency tended to be increased (lower qCO_2) (Table 5), corroborating other reports from Brazil (Balota et al., 1998; Franchini et al., 2007; Pereira et al., 2007). The shift towards a more efficient consumption of the energy stored in SOM by microorganisms under NT might result from a higher efficiency of mineralization, caused by changes in enzymatic activity, as well as by changes in microbial-community composition. In relation to metabolic differences, in one example reported from the Brazilian Cerrados, a higher MB-C with NT was coupled with higher activities of β-glucosidase (EC 3.2.1.21), acid phosphatase (EC 3.1.3.2) and arysulphatase (EC 3.1.6.1) in comparison with CT (Mendes et al., 2003). In terms of changes in microbial community, NT favors fungi over bacteria (Beare et al., 1992; Frey et al., 1999; Bailey et al., 2002), probably because it precludes breakage of hyphal nets and contributes to stabilization of macroaggregates (Six et al., 2001). As fungi apparently have a lower maintenance energy requirement than bacteria, and a higher efficiency to transform substrate-C into microbial-C, accumulation of organic matter may be favored (Alvarez et al., 1995; Haynes, 1999).

Lastly, differences within bacterial and fungi communities leading to higher efficiency in the C usage in the latter may also play a role in increasing the soil-C reservoir with time. For example, rhizobial populations under NT may also change their capacity to exploit C and N sources, towards more-efficient use of those nutrients (Hungria et al., 2001). Previous studies performed in Brazil indicate that tillage changes the diversity of diazotrophic rhizobia, producing rhizobia of increased N_2-fixing efficiency (Ferreira et al., 2000; Hungria and Vargas, 2000; Hungria et al., 2001, 2006b; Kaschuk et al., 2006; Pereira et al., 2007). These mechanisms may have contributed to the lower qCO_2 observed under NT in this long-term experiment, when compared to the CT system.

Soil microorganisms are often limited by C and N resources and usually respond promptly to the addition of those nutrients (Nordgren, 1992; Gregorich et al., 2006). Also in this study, a prompt increase in microbial activity was observed with incubation with sucrose. Interestingly, with the amendment of a C source, similar values of BR were observed in NT and CT between the soil preparation and full-vegetative growth (Table 4). Those results indicate that the microbes were starving for C under CT and their potential activity was similar to that of the population under NT; however, that maximum activity occurred at full flowering emphasizes the higher potential of the community under NT. The vulnerability of microbial communities and processes in tropical soils was highlighted by some results. First, the effects of the field cultivator – an implement that leaves most of the residues on the soil surface and has direct impact only on the 15–20-cm-depth soil layer, where microbial numbers and activities are relatively low – immediately decreased MB-C and MB-N. Second, a peak in BR observed in all treatments around sowing, indicates that any disturbance of the soil immediately increases microbial respiration (Table 4).

The grain yields obtained in the 14th year of the experiment are consistent with the conclusion of Souza et al. (2008a,b), that N might be the nutrient most frequently limiting grain yields in Brazil. Since maize grain yield in the CR system was similar with CT and NT and enhanced in the FC system, we may conclude that N was immobilized with NT and depleted with CT, and, as the amount of N-fertilizer applied was low, plant growth was limited by N. Interestingly, in the CT system the plants were deficient in N even after the incorporation of N-rich lupin residues, the previous crop, indicating that the rate of N mineralization did not meet the N demand of the maize crop. On the other hand, the use of the FC

accelerated the mineralization of N accumulated in the soil organic matter, increased the TNS and resulted in higher maize yield, but as the enhancement was based mainly on exploitation of the N reservoir in the SOM, the soil might be depleted of N in the future.

An opposite situation was observed for the soybean crop in the CS system, in which, grain yield was significantly increased due to NT in comparison with FC and CT treatments. In fact, the N_2-fixing symbiosis is highly stimulated by NT soil environment, and therefore, can fulfill the plant's need for N (Hungria and Vargas, 2000; Hungria et al., 2006a). Although concerns over intensive cropping of soybean in the tropics are often raised, an intriguing observation in this experiment is that when the crop is grown largely based on the N_2-fixation process, the sustainability of the system is favored. Only 72 kg of N-fertilizer were added to the wheat crop in 14 years of experiment, or 36 kg of N assuming an efficiency of usage of 50%. Nevertheless high yields were obtained for winter and summer crops, as observed also for the soybean in the 14th year (Fig. 2). Therefore, the results suggest that N_2 fixation was not only capable of supplying all of the N needs of the legume, it also left N to the following crop, as found before (Hungria et al., 2006a).

As has been observed before (Balota et al., 1998; Franchini et al., 2007), the microbial parameters were not clearly affected by crop rotation (Table 6). Indeed, in another study performed in the state of Paraná, crop rotation did not affect MB-C even after 21 years (Balota et al., 2004), highlighting how complexities in plant-microbe interactions are. We observed that soybean crop (under CS system) was benefited from increased amounts in the MB-C and improvement in the metabolic activity of soil microorganisms whereas maize crop (under CR) was not affected. It is still unsure whether these differences were due to crop physiological differences or due to rotations. However, there should be no doubt about the benefits of crop rotation with several plant species, by increasing diversity of the bacterial community in general and of diazotrophs in particular, increased efficiency of microbial processes, e.g. N_2 fixation (Ferreira et al., 2000; Pereira et al., 2007), and suppression of pest-crop interactions (Pankhurst et al., 2002), although it is not easy to correlate crop rotation with increases in SOM or long-term yield.

The results presented in this paper corroborate with others which give evidence that increased soil microbial biomass is an indicator of improved soil quality, and in turn, favors agricultural sustainability and benefits yields (Balota et al., 1998; Roscoe et al., 2006; Franchini et al., 2007; Souza et al., 2008b). Our study shows that improvement in soil quality in the NT system is probably related to a change in the structure of soil microbial community. We suggest that further studies should characterize soil biodiversity in addition to measurements of soil biomass, because it may correlate with changes in metabolic activity and soil microbial biomass.

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