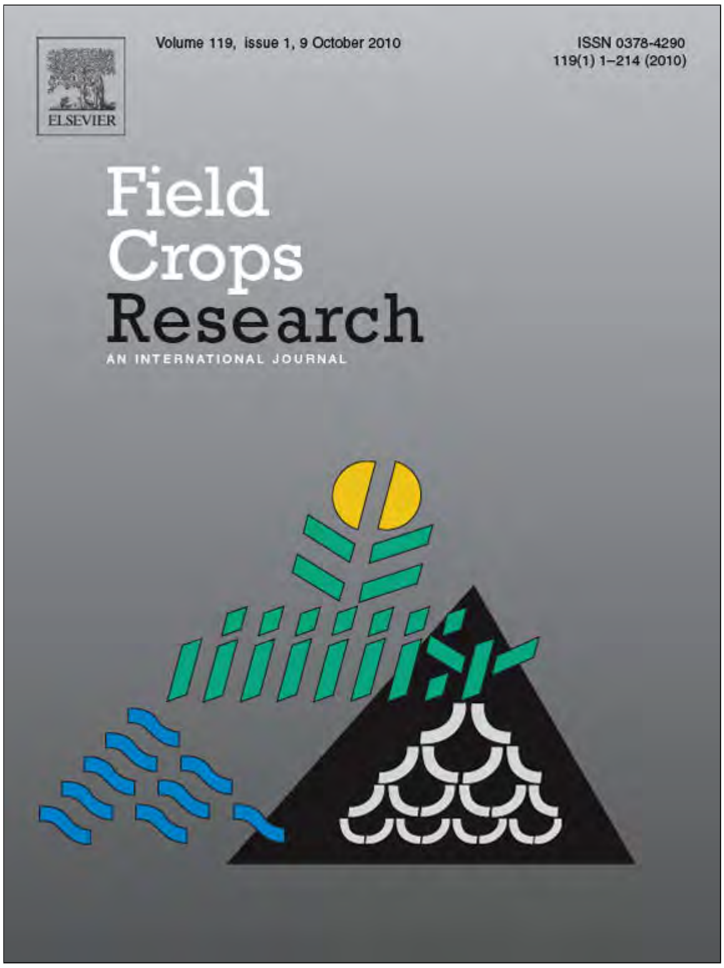
Volume 119, issue 1, 9 October 2010
ISSN 0378-4290
119(1) 1–214 (2010)
ELSEVIER
Field Crops Research
AN INTERNATIONAL JOURNAL

Field Crops Research 119 (2010) 20–26

Contents lists available at ScienceDirect

Field Crops Research

journal homepage: www.elsevier.com/locate/fcr

Microbial biomass under various soil- and crop-management systems in short- and long-term experiments in Brazil

Adriana Pereira Silva[a,b], Letícia Carlos Babujia[a,b], Julio Cezar Franchini[a], Rosinei Aparecida Souza[a], Mariangela Hungria[a,b,*]

[a] Embrapa Soja, Cx. Postal 231, 86001-970, Londrina, PR, Brazil
[b] Universidade Estadual de Londrina, Depto. Biotecnologia, Londrina, PR, Brazil

ARTICLE INFO

Article history:
Received 24 February 2010
Received in revised form 9 June 2010
Accepted 11 June 2010

Keywords:
Microbial biomass
Soil tillage
Crop rotation
Crop sequence
Maize
Soil quality
Soybean

ABSTRACT

Management and cropping systems varying in soil mobilization rates and plant-residue inputs may have profound effects on the biological properties of soil. Therefore, the objective of this study was to quantify soil microbial biomass carbon and nitrogen (MB-C and MB-N)—by means of the fumigation-extraction method—under varied soil-management and crop-rotation/succession systems in southern Brazil, correlating the results with yields of soybean and maize crops. The microbial biomass and grain yields were examined at the 0–10 cm layer in four short- to long-term field experiments. Experiment 1 was a 26-year trial consisting of four soil-management systems: (1) no-tillage (NT), (2) conventional tillage [(CT) with disc plough], (3) field cultivator (FC) or (4) heavy-disc harrow (DH), each with a crop succession (CS) of soybean (summer) and wheat (winter). Experiment 2 was a 21-year trial consisting of one CS, soybean/wheat every year) and seven crop rotations (CRs) comprising soybean, maize, wheat and green manures (lupine, radish and black oat), under the NT system. Experiment 3 comprised a 14-year CT trial, and 4-year and 14-year NT trials, with both one CS and two CRs. Experiment 4, a 10-year trial consisted of CT and NT and three CRs. Analyses were performed during the summer and winter croppings. Differences in microbial parameters, as a function of crop succession and rotation, were not easily detected as they varied as a function of a complex combination of plant species and time of implementation of the experiment. In contrast, MB-C and MB-N values were consistently higher—up to more than 100%—under NT in comparison to CT and were associated with higher grain yields. Our results—from this wide range of experiments—suggest that MB-C and, particularly, MB-N are sensitive indicators of the effects of soil- and crop-management regimens.

1. Introduction

In Brazil, about 300 million ha, in varied ecosystems, have been converted to agricultural use (MAPA, 2008), with impacts on soil properties differing as a function of diverse management systems. Adoption of practices that minimize these impacts is fundamental to agricultural sustainability. For example, the soil environment is affected by return, or not, of plant residues, in terms of soil structure, temperature, moisture and aeration, which, in turn, affect soil microbial biomass. Microbes function as agents of transformation of organic matter, nutrient cycling, energy flow, among other functions (e.g., Wardle and Giller, 1996; Six et al., 2004) that impinge on sustainability.

According to Sparling (1992), changes in soil organic matter (SOM) are difficult to detect in the short term, necessitating long-term monitoring. In contrast, changes in microbial biomass carbon (MB-C) and nitrogen (MB-N) were found to rapidly reflect impacts of agricultural management (Carter and Rennie, 1982), before any changes in chemical or physical parameters are detectable (Franchini et al., 2007; Kaschuk et al., 2010). Accordingly, parameters associated with microbial biomass have been proposed as biological indicators of soil quality (e.g., Doran and Parkin, 1994; Hungria et al., 2009).

The no-tillage (NT) cropping system was introduced in Brazil in the 1970s, in the State of Paraná, for soil-erosion control, and has been increasingly adopted such that, according to estimates of the Brazilian Federation of No-Tillage, in 2005/2006 25.5 million ha were devoted to grain production under NT (FEBRAPDP, 2010). Among other benefits, NT increases water infiltration and retention

* Corresponding author at: Embrapa Soja, Soil Biotechnology, Cx. Postal 231, 86001-970, Londrina, PR, Brazil. Tel.: +55 4333716206; fax: +55 4333716100.
E-mail addresses: drikapera@yahoo.com.br (A.P. Silva), leticiacb_@hotmail.com (L.C. Babujia), franchin@cnpso.embrapa.br (J.C. Franchini), rosyquimica@yahoo.com.br (R.A. Souza), hungria@cnpso.embrapa.br (M. Hungria).

0378-4290/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.fcr.2010.06.012

and reduces soil-temperature oscillation (Castro-Filho et al., 2002). In addition, NT increases soil organic C content in comparison with conventional tillage (CT), which may contribute to sequestration of atmospheric C (Bayer et al., 2002; Pacala and Socolow, 2004).

It has been demonstrated that, in tropical soils, the inclusion in crop rotations under NT of nitrogen-fixing legumes, such as lupine (*Lupinus* spp.), and of green-manure crops such as black oat (*Avena strigosa* Schreb.) and radish (*Raphanus sativus* L.), increases soil organic matter and improves soil-chemical, -physical, and -biological properties (Franchini et al., 2007; Hungria et al., 2009). However, differential effects have been observed on the rate of residue decomposition and on microbial community composition according to the quantity and quality of plant residues added to the soil (Franchini et al., 2002).

Studies of microbial biomass may be valuable as an adjunct to research on the effects of agricultural practices on soil quality and crop productivity. In this context, the objective of the present study was to evaluate MB-C and MB-N in four short- to long-term field experiments under varied soil- and crop-management systems, correlating microbial parameters with soil productivity capacity, assessed as grain yield.

2. Materials and methods

2.1. Geographic location and general description of the field sites

The experiments were carried out at the experimental area of Embrapa Soja in Londrina, Paraná, Brazil, with an altitude of 620 m, latitude 23°11′S, longitude 51°11′W. Average annual temperature in Londrina is 21 °C, with an average high at 28.5 °C in February and an average low at 13.3 °C in July. Average annual rainfall is approximately 1651 mm, with 123 days of rainfall per year; maximum rainfall occurs in the summer (January–March) and the minimum in the winter (June–August). According to Köeppen's classification, the climate in Londrina is subtropical humid—*Cfa* type (humid, temperate, with hot summer). The soil (Latossolo Vermelho Eutroférrico, Brazilian classification; Rhodic Eutrudox, USA classification) is very clayey, averaging 71% clay, 16% silt, and 12% sand. The four experimental areas described below were selected for sampling.

2.1.1. Experiment 1—26-year trial

The area where the experiment was established had been cropped with coffee trees (*Coffea arabica* L.) for about 40 years. The experiment was set up in the summer of 1981, with a crop succession of soybean (*Glycine max* L. Merr.)/summer and wheat (*Triticum aestivum* L.)/winter every year. The experiment had a randomized complete block design with four blocks as replicates (Cochran and Cox, 1957), and four types of soil management as treatments: (1) no-tillage (NT) (sowing directly through the residue of the previous crop, with the opening of only a narrow furrow in the sowing row); (2) conventional tillage (CT) [soil is prepared with disc plough and heavy-disc harrow followed by light-disc harrow (DH)]; (3) field cultivation (FC) (with the subsoil scarified to a depth of 15–20 cm followed by light-disc harrow); and (4) DH. Field plots were 50 m in length x 8 m in width. Soil preparation of the winter crop in the CT and FC treatments was accomplished by DH, followed by light-disc harrow. Previously, other cultivars had been used, but in the summer of 2006/2007 soybean cultivar BRS 232 was planted, and in the winter of 2007 the wheat cultivar planted was BRS 208. In this experiment and in the other three (described below) herbicides were applied after the grain harvest, to desiccate crop residues. With NT, the crop residues were left on the soil surface, whereas, in the other soil-management systems, the residues were incorporated into the soil.

2.1.2. Experiment 2—21-year trial

This area had also been cropped to coffee for about 40 years. The experiment began in the winter of 1986, and included one crop succession (CS) and seven crop-rotation (CR) systems under the NT system. The experiment had a randomized complete block design with four blocks as replicates (Cochran and Cox, 1957), and the field plots were 14 m in length × 5 m in width. Cropping effects were studied including grain crops [soybean, maize (*Zea mays* L.), and wheat)] and cover and green-manure crops [lupine (*Lupinus albus* L.), radish (*R. sativus* L.), and black oat (*A. strigosa* Schreb.)]. In southern Brazil more than 90% of the farmers plant exclusively the soybean (summer)/wheat (winter) crop succession and it has been a technical challenge to introduce other species into crop rotations. Few adapted species, with seeds commercially available, can usefully be included in crop rotations relevant to Brazil; the species were chosen accordingly. The sequence, shown in Table 1, represents various strategies for rotating legumes, grasses and cover crops. In the early years the cultivars were chosen according to annual recommendations, and in the 2006/2007 season soybean cultivar BRS 184 and maize cultivar BRS 10-30 were grown in the summer, whereas in the winter the wheat cultivar was BRS 208, black oat was BRS 139, and radish was IPR 116.

2.1.3. Experiment 3—14-year trial

Again the area had been cropped to coffee for about 40 years. The experiment was set up in the winter of 1993, in a randomized complete block design in a factorial scheme with three soil-management and three cropping systems, each with four replicates (Cochran and Cox, 1957). Field plots were 38 m in length × 8 m in width. The following soil-management systems were compared: (1) CT, with disc plough in the summer and DH in the winter; it should be noted that this CT was slightly different from that of Experiment 1; in view of the benefits accruing from NT, the farmers started to reduce the soil-management operations also with CT; (2) old NT (NTo—named "old" because it was established in 1993); and (3) new NT (NTn) (where part of the area previously treated to CT was converted to NT at the beginning of October 2003). The experiment consisted of two crop rotations (CRs) and one crop succession (CS), which included grain crops [short-season maize ("safrinha") (the summer crop sown and harvested early followed by a "late" short-season maize) and cover and green-manure crops (lupine and black oat)]. Nine treatments were compared: CT (CR 1, CR 2, and CS), old NT (CR 1, CR 2, and CS), and new NT (CR 1, CR 2, and CS). The sequences of the crops in the soil-management systems are described in Table 1. In previous years, the cultivars were chosen according to annual recommendations, and in the 2006/2007 summer crop, both the CR and CS had soybean cultivar BRS 232, whereas, in the winter of 2007, wheat cultivar BRS 208 was used in CR 1 and CS, and the "short-season" maize cultivar BRS 10-10 in CR 2.

2.1.4. Experiment 4—10-year trial

This site had been farmed with conventional tillage (CT), with the traditional practices of plowing and disking, and planted to soybean in the summer and to wheat in the winter over the previous 6 years. The experiment was set up in the summer of 1997/1998, with a randomized complete block design in a factorial scheme, with two soil-management systems [NT and CT (as described in Experiment 3)] and three CR systems (CR 1, CR 2, and CR 3), including soybean, maize, wheat, lupine, and black oat, each with four replicates. Field plots were 15 m in length × 8 m in width. Crop rotations are shown in Table 1. In the early years, the cultivars were chosen according to annual recommendations, and in the 2006/2007 summer, hybrid maize Pioneer 30 F 33 was planted, and in the winter of 2007 black oat cultivar Iapar 61 and wheat cultivar BRS 239 were used.

Table 1
Crop-management systems (CS, crop succession; CR, crop rotation) adopted for the last 5 years in Experiments 2–4.

Crop management	S[a] 2002/2003	W 2003	S 2003/2004	W 2004	S 2004/2005	W 2005	S 2005/2006	W 2006	S 2006/2007	W 2007
Experiment 2										
Crop succession (CS)	S[b]	W	S	W	S	W	S	W	S	W
Crop rotation										
CR 1	S	W	S	O	S	W	S	O	S	W
CR 2	M	O	S	W	S	W	S	R	M	O
CR 3	M	W	S	W	S	W	S	R	M	W
CR 4	M	W	S	R	M	W	S	R	M	W
CR 5	S	O	S	O	S	W	S	O	S	O
CR 6	M	O	S	O	S	W	S	R	M	O
CR 7	M	R	M	O	S	W	S	R	M	R
Experiment 3										
CS	S	W	S	W	S	W	S	W	S	W
CR 1	S	W	S	L	M	O	S	W	S	W
CR 2	S	W	S	M(s)	S	M(s)	S	M(s)	S	M(s)
Experiment 4										
CR 1	S	W	S	L	M	O	S	F	M	W
CR 2	M	W	S	O	S	L	M	F	M	O
CR 3	M	W	M	O	M	O	M	F	M	W

[a] S, summer; W, winter.
[b] S, soybean; M, maize; W, wheat; O, black oat; R, radish; M(s), "short-season" maize; L, lupine; F, fallow.

Experimental procedures relative to soil preparation, sowing, fertilization, liming, weed and insect control were uniformly performed for each experiment, according to the technical recommendations for each crop.

2.2. Soil sampling

Microbial biomass carbon (MB-C) and nitrogen (MB-N) were determined in the summer in samples collected on January of 2007, corresponding to full flowering of soybean and maize, and in the winter, on July of 2007, during maturation of wheat and black oat maturation and flowering of "short-season" maize.

For the soil sampling, in each plot an area of 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; a previously labeled pot marked with a soil volume corresponding to 150 g was used to guide the collection. The procedure was repeated eight times in each plot, in points spatially distributed to represent the whole area. Eight subsamples of soil were combined such that each replicate consisted of a composite of approximately 1 kg. Samples were homogenized, placed in plastic bags, and carried to the laboratory. Before the beginning of the analysis, in the laboratory, samples were homogenized again, and plant residues removed. Samples were passed through a 4-mm (5 mesh) sieve, and kept in plastic bags in the refrigerator, at 4 °C, for no more than 10 days.

2.3. Determination of soil moisture

Soil samples of 10 g were transferred to 100-mL-capacity flasks and the assemblies were weighed and then placed in an oven at 105 °C overnight. On the following day, the assemblies were weighed again to determine soil dry matter and, by difference, soil moisture. Microbial biomass values were corrected for soil moisture and expressed in μg microbial C or N g^{-1} dry soil. In addition, on the basis of the actual dry weight, the samples were corrected to reach a moisture level of 40% of water-holding capacity (WHC) (Vance et al., 1987) by adding distilled water.

2.4. Microbial evaluations

2.4.1. Microbial biomass-C and -N

Microbial biomass-C (MB-C) and microbial biomass-N (MB-N) were determined by the modified fumigation-extraction method of Vance et al. (1987), with some modifications. Eight subsamples (20 g) of each composite soil sample were weighed and stored in glass receptacles (300 mL), four of which were submitted to fumigation as described by Vance et al. (1987), and the other four were not fumigated. Fumigated and non-fumigated samples were kept in the dark at 25 ± 2 °C for 16 h. After the incubation, 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 as described by Franchini et al. (2007). Carbon contents of the extracts were determined by oxidation with Mn^{3+} and evaluation on a spectrophotometer (Bartlett and Ross, 1988). MB-N was evaluated by adding to the extract 1.5 mL of H_2SO_4 concentrated and 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 the spectrophotometric determination of NH_4 using the indophenols-blue method (Feije and Anger, 1972). The MB-C and MB-N values were estimated from the differences between the fumigated and non-fumigated samples, employing a K_{CE} value of 0.38 for C (Vance et al., 1987) and a K_{NE} value of 0.54 for N (Brookes et al., 1985).

2.5. Grain yield

Grain yield was evaluated by means of mechanical harvest in all experiments. Seeds were cleaned, weighed, and values were corrected for 13% moisture, after determination of grain moisture in a Vurroughf 700 grain-moisture analyzer.

2.6. Statistical analysis

The data were analyzed using the SAS (Statistical Analysis System) for PC statistical package (SAS Institute, 2001). All assumptions required for the analysis of variance (ANOVA) were verified. The error normality was evaluated according to Shapiro and Wilk (1965), the variance of homogeneity according to Burr and Foster (1972), and the non-additivity after Tukey's model (1949). Coefficients of skewness and kurtosis were also checked. Data from all experiments were first submitted to the tests of normality of the variables and of homogeneity of variances, and then to ANOVA. When confirming a statistically significant P value, a post hoc test was applied, and Duncan's multiple-range test ($P \leq 0.05$) was used as a multiple comparison procedure (SAS Institute, 2001). In the comparison of means, the treatment considered as the main effect (either soil or crop management) was taken as the fixed effect and the blocks and the other treatments were considered as random effects.

3. Results

3.1. Microbial biomass-C and -N

Starting with the oldest trial (Experiment 1), after 26 years average values of MB-C and MB-N under NT were 20% and 78% higher, respectively, in the summer, and 71% and 90% higher in the winter, when compared with CT; all differences except for the first one (20%) were statistically significant (Table 2). In comparison to CT and DH treatments, soil management with the field cultivator (FC) had significantly higher values of MB-N in the summer; in the winter, MB-C and MB-N of DH and FC were equivalent and significantly higher than the values recorded under CT (Table 2).

In Experiment 2, after 21 years, the CS (CS, yearly cropping of soybean in the summer and wheat in the winter) and seven CRs (with species other than soybean and wheat included) systems (Table 1) revealed a significantly higher value for MB-C in CR 1 at the summer sampling (Table 2). At the same sampling time, CR 3, CR 5 and CR 6 (Table 1) had similar values of MB-C and were higher than for CS, CR 2 and CR 7 (Table 2). Superior values of MB-N were observed in CR 3, CR 5, CR 6 and CR 7 (Table 2) and, except for CR 5, where black oat had been introduced, all the rotation systems had radish in the winter prior to maize as the summer crop. In the winter, significantly higher values of MB-C were observed in the CS and CR 1, CR 5 and CR 7; except for the latter, in which maize was introduced, all of the rotation systems had soybean as the summer crop. CR 3, CR 4, CR 6, and CR 7, all cropped with maize in the summer, had the lowest values of MB-N in the winter; in addition, the low MB-N values in CR 2 were associated with maize cropping in the summer (Table 2).

In the third trial, after 14 years, when NT systems of different ages (old NT with 14 years, and new NT with 4 years) were compared to the CT, MB-C and MB-N values of the old NT were significantly higher at both samplings and with all crop-management regimens (Table 3). No differences in MB-C were observed between the CT and the new NT in summer, but differences were seen at the winter sampling. In comparison with CT, MB-C with old NT was 83% higher in the summer and 68% higher in the winter (Table 3). In the summer, values of MB-N were 122% and 152% higher with the new NT and the old NT, respectively, than with CT; in the winter, they were 35% and 82% higher, respectively (Table 3). In relation to the CS and CRs (Table 1), the lowest values for MB-C were seen with CR 1 in the summer and with CS in the winter, whereas the lowest MB-N values were obtained with CS in both summer and winter, and with CR 1 in the winter (Table 3).

In the summer evaluation in Experiment 4, the 10-year-NT soil had MB-C and MB-N values of 18% and 100% higher than with CT, respectively, and of 61% and 97% higher in the winter, respectively (Table 4). There were no statistically significant differences in MB-C values between crop rotations within each soil-management regimen (CT or NT), but considering the averaged values of each crop rotation under CT and NT, higher values were observed with CR 1 in the summer and with CR 3 in the winter (Table 4). For the MB-N,

Table 2
Microbial biomass MB-C and MB-N (μg C or N g^{-1} dry soil) in soils (0–10 cm) in two experiments: the first with 26 years and four types of soil management and the second with 21 years and on crop succession and seven different crop rotation systems under the no-tillage system. *Experiments 1 and 2*.

	Summer		Winter	
	MB-C	MB-N	MB-C	MB-N
Soil management[a]				
Conventional tillage	343[b] AB	37 C	264 C	30 C
Heavy-disc harrow	332 B	33 C	329 B	43 B
Field cultivator	365 AB	51 B	310 B	43 B
No-tillage	410 A	66 A	451 A	57 A
Crop management[c]				
Crop succession (CS)	343 C	42 BC	558 A	62 AB
Crop rotation				
CR 1	487 A	40 BC	547 AB	65 A
CR 2	336 C	44 B	452 C	56 C
CR 3	422 B	55 A	434 C	46 D
CR 4	284 D	35 C	460 C	44 D
CR 5	441 B	55 A	564 A	60 B
CR 6	435 B	53 A	515 B	45 D
CR 7	357 C	57 A	558 A	45 D

[a] *Experiment 1*: soil management.
[b] Means followed by different letters, within the same column, are statistically different ($P \leq 0.05$, Duncan's test).
[c] *Experiment 2*: crop succession (CS) and crop rotation (CR) as described in Table 1.

Table 3
Microbial biomass MB-C and MB-N (μg C or N g^{-1} dry soil) in soils (0–10 cm) after the 14th year in a field experiment under different soil[a] and crop[b] management systems. *Experiment 3*.

Soil management[a]	Summer											Winter										
	CR 1[b]			CR 2			CS			Mean		CR 1			CR 2			CS			Mean	
MB-C																						
Conventional tillage	157[c]	B	a	172	B	a	168	B	a	166	B	365	C	a	322	C	b	383	C	a	357	C
New no-tillage	160	B	a	169	B	a	190	B	a	173	B	477	B	a	454	B	a	447	B	a	459	B
Old no-tillage	220	A	c	421	A	a	270	A	b	304	A	612	A	a	635	A	a	551	A	b	599	A
Mean	179		c	254		a	209		b			485		a	470		ab	460		b		
MB-N																						
Conventional tillage	27	C	a	26	B	a	27	C	a	27	C	34	C	a	36	C	a	32	C	b	34	C
New no-tillage	57	B	b	68	A	a	54	B	b	60	B	41	B	c	46	B	b	50	B	a	46	B
Old no-tillage	75	A	a	66	A	b	62	A	b	68	A	61	A	b	70	A	a	56	A	c	62	A
Mean	53		a	53		a	48		b			45		b	51		a	46		b		

[a] New no-tillage, 4 years old; old no-tillage, 14 years old.
[b] Crop rotations (CRs) and crop succession (CS) as described in Table 1.
[c] Means of four replicates followed by the same capital letters within each column and by the same small letters within each line, for each parameter and sampling time are not statistically different ($P \leq 0.05$, Duncan's test). The same applies for the comparison of the mean values (12 replicates each) of the three soil and three cropping managements.

Table 4
Microbial biomass MB-C and MB-N (μg C or N g^{-1} dry soil) in soils (0–10 cm) after the 10th year in a field experiment under different soil and crop[a] managements. *Experiment 4.*

Soil management	Summer											Winter										
	CR 1[a]			CR 2			CR 3			Mean		CR 1			CR 2			CR 3			Mean	
MB-C																						
Conventional tillage	264[b]	A	a	198	A	a	227	A	a	230	B	361	A	a	322	A	a	420	A	a	368	B
No-tillage	296	A	a	277	A	a	239	A	a	271	A	529	A	a	621	A	a	626	A	a	592	A
Mean	280		a	238		b	233		b			445		c	471		b	523		a		
MB-N																						
Conventional tillage	36	B	a	32	B	a	39	B	a	36	B	37	B	a	37	B	a	38	B	a	37	B
No-tillage	62	A	b	76	A	a	78	A	a	72	A	74	A	b	70	A	c	76	A	a	73	A
Mean	49		b	54		ab	58		a			56		a	53		c	57		b		

[a] Crop rotations (CRs) as described in Table 1.
[b] Means of four replicates followed by the same capital letters within each column and by the same small letters within each line, for each parameter and sampling time are not statistically different ($P \leq 0.05$, Duncan's test). The same applies for the comparison of the mean values (8 replicates each) of soil to the three cropping managements.

the lower values were observed in CR 1 in the summer and in CR 2 in the winter (Table 4). NT always resulted in higher MB-C and MB-N (Table 4).

3.2. Grain yield

In Experiment 1, grain yields of the summer crops (soybean and maize) (2006/2007) were influenced by the soil-management systems. Statistically significant higher yields of soybean (3472 kg ha^{-1}) were observed in the NT system in comparison to the three other soil-management strategies that had varying degrees of soil tillage (CT, 2058 kg ha^{-1}; DH, 1873 kg ha^{-1}; FC, 1756 kg ha^{-1}). These values were associated with higher values of MB-C and MB-N (Table 2), also highlighted in Table 5.

In the second experiment, when CRs were compared in the NT soil-management system, there were no statistically significant differences among the rotation schemes in terms of yields of soybean (CS, CR 1 and CR 5; 2724, 2971 and 2840 kg ha^{-1}, respectively) or maize (CR 2, CR 3, CR 4, CR 6, CR 7; 8597, 8748, 8595, 8893 and 8697 kg ha^{-1}, respectively). However, although not statistically significant, soybean yields were higher in CR 1 and CR 5, crop rotations associated with higher values of MB-C in the summer, and similar results were observed for maize yield in CR 6.

In the third experiment, considering each soil-management system (CT, old NT, new NT), differences in soybean yield could not be attributed to CS succession or CR, and the averages for each crop management—CR 1, CR 2 and CS—were also similar, 3243, 3126 and 3208 kg ha^{-1}, respectively. However, considerable differences in soybean yield were related to soil management, averaging 2988 kg ha^{-1} with CT, 3173 kg ha^{-1} with new NT and 3399 kg ha^{-1} with old NT. Again, MB-C and MB-N were correlated with grain yield (Tables 3 and 5).

Only in the 10-year fourth experiment were significant effects on maize yield caused by CR, but just as an interaction with soil-management system. Yield with CR 2 under CT (5951 kg ha^{-1}) was significantly less than with the other treatments, as follows (in parentheses, kg ha^{-1}): CR 1/CT (7295); CR 1/NT (7727); CR 2/NT (7343); CR 3/CT (7709), CR 3/NT (7816). On average, treatments with CR 2 gave statistically significantly less maize yield (6647 kg ha^{-1}) than those with CR 1 or CR 3 (7510 and 7762 kg ha^{-1}, respectively). Once more, statistically significant higher yields were associated with NT, on average 7628 kg ha^{-1}, than with CT, on average 6985 kg ha^{-1}. The values of MB-C and MB-N as a function of soil management were related to yield, as highlighted in Table 5.

4. Discussion

In all of the experiments, when NT was compared to soil managements showing some degree of soil disturbance—CT, FC or DH, values of MB-C and MB-N were significantly higher with NT at both summer and winter harvests, demonstrating the influence of soil management on microbial parameters. Comparison of the NT and CT systems clearly shows that NT improved MB-C and MB-N from the first evaluation at 4 years after establishment, with differences increasing with time, such that after 26 years these two parameters were on average 42% and 95% higher, respectively, under NT (Table 5). Although MB-C and MB-N values were relatively stable after the 10th year, yields continued to increase (Table 5). The explanation may reside with the MB-C/MB-N ratio, with much higher values under CT than in NT; the proportionally higher values of MB-N may be the key component contributing to increased grain yields.

Several reports from tropical and subtropical areas have shown benefits related to NT, including improvements in soil-chemical and -physical properties (Bayer et al., 2002; Boddey et al., 2010).

Table 5
Effects of conventional tillage (CT) and no-tillage (NT) on microbial biomass carbon and nitrogen (MB-C and MB-N), ratio MB-C/MB-N and grain yield in field experiments with different times of establishment. For MB, values represent the means of evaluations performed in the summer and in the winter.

Time (years)	MB-C (μg C/N g^{-1} dry soil)		MB-N (μg C/N g^{-1} dry soil)		MB-C/MB-N		Yield[a] (kg ha^{-1})	
	Soil management							
	CT	NT	CT	NT	CT	NT	CT	NT
4	261[b] B	316 A	30 B	53 A	8.7 A	6.0 B	2988 A	3173 A
10	299 B	431 A	36 B	72 A	8.3 A	6.0 B	6985 B	7628 A
14	261 B	451 A	30 B	65 A	8.7 A	6.9 B	2988 B	3399 A
26	303 B	430 A	33 B	61 A	9.2 A	7.0 B	2058 B	3472 A

CT, conventional tillage; NT, no-tillage.
[a] Soybean at the 4-, 14- and 26-year-old experiments and maize at the 10-year-old experiment.
[b] Comparison of means for each parameter and year (24 replicates for the 4-, 10- and 14-year-old experiments, eight replicates for the 26-year-old experiment), and values followed by the same letter are not statistically different ($P \leq 0.05$, Duncan's test).

Also, an increasing number of reports are showing that NT favors soil microbial communities (e.g. Balota et al., 2004; Franchini et al., 2007; Hungria et al., 2009). Greater immobilization of C by the MB in the NT system has been attributed to increased organic matter added to the soil as plant residues (Bayer et al., 2002), whereas lower MB-C with CT may be related to reduced plant coverage and more intense soil disturbance, among other factors (Reganold et al., 2000). In addition, positive effects of NT on the soil microbial community may be attributed to a variety of benefits related to less soil disturbance, including less disruption of fungal hyphae, protection of microbial habitat, increased soil-moisture content and less extreme temperature conditions (Rhoton, 2000). Altogether, improved chemical and physical conditions, fostering growth and activity of microorganisms, may help to explain the impressive increases on MB-C and MB-N reported in this paper.

The differences reported here in MB-C were much larger than those from temperate areas, emphasizing that microbial turnover is considerably more rapid in tropical and subtropical than in temperate regions (Kaschuk et al., 2010). The MB-N was more sensitive than MB-C in its responses to changes in soil management, being favored by the absence of soil tillage; these results reinforce previous observations of the importance of MB-N in explaining benefits from NT in southern Brazil (Franchini et al., 2007; Hungria et al., 2009). Also important are our observations that higher MB-N and MB-C values under NT were associated with higher grain yields. Increases in soybean yields under NT, in comparison to CT, were of 6%, 14% and 69% after 4, 14 and 26 years, respectively; for maize, an increase of 9% was obtained after 10 years.

Also, less disturbance of the soil with HD and FC treatments, in comparison to CT, contributed to increases in both MB-C and MB-N. There have been suggestions that some soil disturbance, i.e. less than with plowing and disking associated with CT, has milder effects on soil organic matter and the microbial community while allowing incorporation of plant residues and increasing soil aeration, thus stimulating—at least temporarily—growth and activity of microorganisms (Salinas-Garcia et al., 1997). The MB-N data from Experiment 3 show that this parameter is strongly affected not only by the type of soil management, but also by the longevity of its application. This study also showed that even slight soil disturbance can considerably decrease MB-C and, especially, MB-N, i.e. advantages may be short-lived with deleterious effects in the long term from acceleration of mineralization and loss of microbial equilibrium, resulting in lower yields. Decreases in yields were seen even with relatively slight soil disturbance; for example, in the 26-year experiment, soybean grain yield under NT was 85% and 98% higher than with HD and FC treatments, respectively.

Crop rotations efficiently cycle nutrients from season to season and promote microbial diversity (Anderson, 2003; Pereira et al., 2007). However, a complicated picture emerged from the analyses of crop-rotation and -succession systems in this and from other studies (Franchini et al., 2007). Different sequences and rotations with plants showing varied C:N ratios affect qualitatively and quantitatively the immobilization and mineralization processes, making the picture unclear in terms of MB parameters. In some cases, reducing N availability in the soil may even result in N deficiency for subsequent crops in non-stabilized systems (Nicolardot et al., 2001). In relation to the plant species used in crop rotations, it has been suggested that radish has the capacity to recycle N from deep in the soil (Kristensen and Thorup-Kristensen, 2004), which may have contributed to superior values of N immobilized by microbial biomass in some rotation systems. However, in the rotation system in which radish inclusion was more frequent (CR 5), MB-C and MB-N values were lower at both sampling times, possibly due to the fact that radish is not associated with mycorrhizal fungi (Gijsman et al., 1997) and, apparently, has some anti-microbial activity (Tirranen et al., 2001). Effects of radish may be more drastic when followed by a summer crop of maize, which is highly dependent on association with mycorrhiza.

With maize as the summer crop, the consumption of N increased and thus the MB-N level was generally reduced in the winter. Addition of maize residues to the soil promotes consumption of mineral N by the microbial biomass during decomposition of the high C:N-ratio residues, reducing N availability which, in turn, negatively affects soil microbial biomass (Nicolardot et al., 2001; De Nobili et al., 2001). Additionally, immobilization of N may be greater in the association of grasses and NT, resulting in N deficiency for crops such as maize and wheat (Castro-Filho et al., 2002). In contrast, when soybean was used as the summer crop, it contributed to increased MB-N and MB-C. The benefits of Brazilian soybean cultivars selected for higher rates of N_2 fixation persist after harvest of the legume and residual N (usually 20–30 kg of N ha^{-1}) may be available to the following crop (Hungria et al., 2006a, 2006b). The contributions of plant species in CR and CS systems are far from being completely understood, and thus should not be considered exclusively as a function of legumes × grasses or inclusion of cover or green-manure species. There are large differences in the quality and quantity of residues added to the soil and, consequently, in (i) the release of compounds that may inhibit specific components of, or whole, microbial communities, and (ii) the speed of microbial decomposition of the residues (Borkert et al., 2003). This may explain why it has been difficult to relate MB-C and MB-N in CR and CS systems with plant biomass production and grain yields (e.g., Yusuf et al., 1999; Franchini et al., 2007). In contrast, it has been much easier to relate higher MB-C and MB-N with yield in the NT system (e.g., Ismail et al., 1994; this study).

In conclusion, greater soil microbial biomass with NT was confirmed in all of our experiments, with important implications especially in relation to C and N cycling in agricultural systems. Rapid changes in C and N in the microbial biomass were detected in this study, reinforcing the need to reduce soil disturbance in tropical conditions. The values of MB-C and MB-N were highly correlated with grain yield. MB-N was the parameter more readily affected by change in soil management. Most importantly, the results from this study—covering a wide range of experiments—suggest that MB-C and MB-N are among the most valuable and sensitive indicators of soil quality as a function of soil-management regimen in the tropics.

Acknowledgments

This work was partially funded by project Probio II/Embrapa. The authors thank Dr. Allan R. J. Eaglesham for English review and Dr. Maria Cristina N. Oliveira for statistical support. A.P. Silva acknowledges an MSc fellowship from CNPq (National Council for Scientific and Technological Development) and L.C. Babujia an MSc fellowship from Probio II/Embrapa. M. Hungria is also a researcher fellow from CNPq.

References

Anderson, T.H., 2003. Microbial eco-physiological indicators to assess soil quality. Agric. Ecosyst. Environ. 98, 285–293.
Balota, E.L., Colozzi-Filho, A., Andrade, D.S., Dick, R.P., 2004. Long-term tillage and crop rotation effects on microbial biomass and C and N mineralization in a Brazilian Oxisol. Soil Till. Res. 77, 137–145.
Bartlett, R.J., Ross, D.N., 1988. Colorimetric determination of oxidizable carbon in acid soil solutions. Soil Sci. Soc. Am. J. 52, 1191–1192.
Bayer, C., Mielniczuk, J., Martin-Neto, L., Ernani, P.R., 2002. Stocks and humification degree of organic matter fractions as affected by no-tillage on a subtropical soil. Plant Soil 238, 133–140.
Boddey, R.M., Jantalia, C.P., Conceição, P.C., Zanatta, J.A., Bayer, C., Mielniczuk, J., Dieckow, J., Santos, H.P., Denardin, J.E., Aita, C., Giacomini, S.J., Alves, B.J.R., Urquiaga, S., 2010. Carbon accumulation at depth in Ferralsols under zero-till subtropical agriculture. Glob. Change Biol. 16, 784–795.
Borkert, C.M., Gaudêncio, C.A., Pereira, J.E., Pereira, L.R., Oliveira-Júnior, A., 2003. Nutrientes minerais na biomassa da parte aérea em culturas de cobertura de solo. Pesq. Agropec. Bras. 38, 143–153.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biol. Biochem. 17, 837–842.

Burr, I.W., Foster, L.A., 1972. A Test for Equality of Variances. University of Purdue, West Lafayette, USA, 26 pp. (Mimeo series, 282).

Carter, M.R., Rennie, D.A., 1982. Changes in soil quality under zero tillage farming systems: distribution of microbial biomass and mineralizable C and N potentials. Can. J. Soil Sci. 62, 587–597.

Castro-Filho, C., Lourenço, A., Guimarães, M.F., Fonseca, I.C.B., 2002. Aggregate stability under different soil management systems in a red latosol in the State of Parana, Brazil. Soil Till. Res. 65, 45–51.

Cochran, W.G., Cox, G., 1957. Experimental Designs. John Wiley, New York, NY, USA, 611 pp.

De Nobili, M., Contin, M., Mondini, C., Brookes, P.C., 2001. Soil microbial biomass is triggered into activity by trace amounts of substrate. Soil Biol. Biochem. 33, 1163–1170.

Doran, J.W., Parkin, T.B., 1994. Defining and assessing soil quality. In: Doran, J.W., Coleman, D.C., Bezdicek, D.F., Stewart, B.A. (Eds.), Defining Soil Quality for Sustainable Environment. Soil Science Society of America, Madison, pp. 3–35, Special Publication Number 35.

FEBRAPDP (Federação Brasileira de Plantio Direto na Palha), 2010. Available in: <http://www.febrapdp.org.br/arquivos/BREvolucaoPD2002a2006.pdf> (accessed 28.01.10).

Feije, F., Anger, V., 1972. Spot test in inorganic analysis. Anal. Chem. Acta 149, 363–367.

Franchini, J.C., Crispino, C.C., Souza, R.A., Torres, E., Hungria, M., 2007. Microbiological parameters as indicators of soil quality under various tillage and crop-rotation systems in southern Brazil. Soil Till. Res. 92, 18–29.

Franchini, J.C., Gonzalez-Vila, F.J., Rodriguez, J., 2002. Decomposition of plant residues used in no-tillage systems as revealed by flash pyrolysis. J. Anal. Appl. Pyrol. 62, 35–43.

Gijsman, A.J., Oberson, A., Friesen, D.K., Sanz, J.I., Thomas, R.J., 1997. Nutrient cycling through microbial biomass under rice-pasture rotations replacing native savanna. Soil Biol. Biochem. 29, 1433–1441.

Hungria, M., Campo, R.J., Mendes, I.C., Graham, P.H., 2006a. Contribution of biological nitrogen fixation to the N nutrition of grain crops in the tropics: the success of soybean (*Glycine max* L. Merr.) in South America. In: Singh, R.P., Shankar, N., Jaiwal, P.K. (Eds.), Nitrogen Nutrition and Sustainable Plant Productivity. Studium Press, LLC, Houston, TX, pp. 43–93.

Hungria, M., Franchini, J.C., Brandão-Junior, O., Kaschuk, G., Souza, R.A., 2009. Soil microbial activity and crop sustainability in a long-term experiment with three soil-tillage and two crop-rotation systems. Appl. Soil Ecol. 42, 288–296.

Hungria, M., Franchini, J.C., Campo, R.J., Crispino, C.C., Moraes, J.Z., Sibaldelli, R.N.R., Mendes, I.C., Arihara, J., 2006b. Nitrogen nutrition of soybean in Brazil: contributions of biological N_2 fixation and of N fertilizer to grain yield. Can. J. Plant Sci. 86, 927–939.

Ismail, I., Blevins, R.L., Frye, W.W., 1994. Long-term no-tillage effects on soil properties and continuous corn yields. Soil Sci. Soc. Am. J. 58, 193–198.

Kaschuk, G., Alberton, O., Hungria, M., 2010. Three decades of soil microbial biomass studies in Brazilian ecosystems: lessons learned about soil quality and indications for improving sustainability. Soil Biol. Biochem. 42, 1–13.

Kristensen, H.L., Thorup-Kristensen, K., 2004. Root growth and nitrate uptake of three different catch crops in deep soil layers. Soil Sci. Soc. Am. J. 68, 529–537.

MAPA (Ministério da Agricultura Pecuária e Abastecimento), 2008. Available in: <http://www.agricultura.gov.br/portal/page?_pageid=33,968707and_dad=porta-land_schema=PORTAL> (accessed 15.04.08).

Nicolardot, B., Recous, S., Mary, B., 2001. Simulation of C and N mineralization during crop residue decomposition: a simple dynamic model based on the C/N ratio of the residues. Plant Soil 228, 83–103.

Pacala, S., Socolow, R., 2004. Stabilization wedges: solving the climate problem for the next 50 years with current technologies. Science 305, 968–972.

Pereira, J.O., De'fossez, P., Richard, G., 2007. Soil susceptibility to compaction by wheeling as a function of some properties of a silty soil as affected by the tillage system. Eur. J. Soil Sci. 58, 34–44.

Reganold, J.P., Glover, J.D., Andrews, P.K., 2000. Systematic method for rating soil quality of conventional, organic and integrated apple orchards in Washington State. Agric. Ecosyst. Environ. 80, 29–45.

Rhoton, F.E., 2000. Influence of time on soil response to no-till practices. Soil Sci. Soc. Am. J. 64, 700–709.

Salinas-Garcia, J.R., Hons, F.M., Matocha, J.E., 1997. Long-term effects of tillage and fertilization on soil organic matter dynamics. Soil Sci. Soc. Am. J. 61, 152–159.

SAS Institute, 2001. Procedure Guide for Personal Computers: Version 8.2. SAS Institute, Cary, NC, USA.

Shapiro, S.S., Wilk, M.B., 1965. An analysis of variance test for normality. Biometrika 52, 591–611.

Six, J., Ogle, S., Breidt, F., Conant, R., Mosier, A., Paustian, K., 2004. The potential to mitigate global warming with no-tillage management is only realized when practiced in the long term. Glob. Change Biol. 10, 155–160.

Sparling, G.P., 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. Aust. J. Soil Res. 30, 195–207.

Tirranen, L.S., Borodina, E.V., Ushakova, S.A., Rygalov, V.Y., Gitelson, J.I., 2001. Effect of volatile metabolites of dill, radish and garlic on growth of bacteria. Acta Astron. 49, 105–108.

Tukey, J.W., 1949. One degree of freedom for non-additivity. Biometrics 5, 232–242.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.

Wardle, D.A., Giller, K.E., 1996. The quest for a contemporary ecological dimension to soil biology. Soil Biol. Biochem. 28, 1549–1554.

Yusuf, R.I., Siemens, J.C., Bullock, D.G., 1999. Growth analysis of soybean under no-tillage and conventional tillage systems. Agron. J. 91, 928–933.