Available online at www.sciencedirect.com
ScienceDirect
Soil & Tillage Research 92 (2007) 18–29
Soil & Tillage Research
www.elsevier.com/locate/still

Microbiological parameters as indicators of soil quality under various soil management and crop rotation systems in southern Brazil

J.C. Franchini, C.C. Crispino, R.A. Souza, E. Torres, M. Hungria *

Embrapa Soja, Cx. Postal 231, 86001-970 Londrina, Paraná, Brazil

Received 4 June 2005; received in revised form 15 December 2005; accepted 23 December 2005

Abstract

The objective of this work was to identify soil parameters potentially useful to monitor soil quality under different soil management and crop rotation systems. Microbiological and chemical parameters were evaluated in a field experiment in the State of Paraná, southern Brazil, in response to soil management [no-tillage (NT) and conventional tillage (CT)] and crop rotation [including grain (soybean, S; maize, M; wheat, W) and legume (lupin, L.) and non-legume (oat, O) covers] systems. Three crop rotation systems were evaluated: (1) (O/M/O/S/W/S/L/M/O/S), (2) (O/S/L/M/O/S/W/S/L/M), and (3) (O/S/W/S/L/M/O/M/W/M), and soil parameters were monitored after the fifth year. Before ploughing, CO_2-emission rates were similar in NT and CT soils, but plough increased it by an average of 57%. Carbon dioxide emission was 13% higher with lupin residues than with wheat straw; decomposition rates were rapid with both soil management systems. Amounts of microbial biomass carbon and nitrogen (MB-C and MB-N, respectively) were 80 and 104% higher in NT than in CT, respectively; however, in general these parameters were not affected by crop rotation. Efficiency of the microbial community was significantly higher in NT: metabolic quotient (qCO_2) was 55% lower than in CT. Soluble C and N levels were 37 and 24% greater in NT than in CT, respectively, with no effects of crop rotation. Furthermore, ratios of soluble C and N contents to MB-C and MB-N were consistently lower in NT, indicating higher immobilization of C and N per unit of MB. The decrease in qCO_2 and the increase in MB-C under NT allowed enhancements in soil C stocks, such that in the 0–40 cm profile, a gain of 2500 kg of C ha^{-1} was observed in relation to CT. Carbon stocks also varied with crop rotation, with net changes at 0–40 cm of 726, 1167 and −394 kg C ha^{-1} year, in rotations 1, 2 and 3, respectively. Similar results were obtained for the N stocks, with 410 kg N ha^{-1} gained in NT, while crop rotations 1, 2 and 3 accumulated 71, 137 and 37 kg of N ha^{-1} $year^{-1}$, respectively. On average, microbial biomass corresponded to 2.4 and 1.7% of the total soil C, and 5.2 and 3.2% of the N in NT and CT systems, respectively. Soil management was the main factor affecting soil C and N levels, but enhancement also resulted from the ratios of legumes and non-legumes in the rotations. The results emphasize the importance of microorganisms as reservoirs of C and N in tropical soils. Furthermore, the parameters associated with microbiological activity were more responsive to soil management and crop rotation effects than were total stocks of C and N, demonstrating their usefulness as indicators of soil quality in the tropics.

Keywords: Soil microbial biomass; Soil microbial respiration; Soil organic matter; Conventional tillage; No-tillage; Crop rotation

1. Introduction

The no-tillage (NT) system – sowing directly through the residue of the previous crop – has been widely adopted in many countries. In Brazil the area devoted to NT has increased from 2.02 Mha in 1992/1993 to almost

* Corresponding author. Tel.: +55 4333716206; fax: +55 4333716100.
E-mail addresses: hungria@cnpso.embrapa.br, hungria@sercomtel.com.br (M. Hungria).

0167-1987/$ – see front matter
doi:10.1016/j.still.2005.12.010

22 Mha today (FEBRAPDP, 2005). In comparison to conventional tillage (CT), NT enhances soil-moisture retention, decreases soil-temperature oscillation and soil erosion by water and wind, improves soil structure and, with time, increases soil organic matter (SOM), often resulting in higher yields (e.g., Castro Filho et al., 1991, 2002; Derpsch et al., 1991; Bayer et al., 2000, 2002; Amado et al., 2001; Sá et al., 2001). Furthermore, NT may play a key role in reducing global warming by providing a greater sink for CO_2 and saving up to 40% of human labor and fossil fuels, in comparison to the CT (e.g., Derpsch et al., 1991; Derpsch, 1998; Kladivko, 2001; Bayer et al., 2002; FAO, 2004).

Maintenance of crop residues on the soil surface is considered responsible for the beneficial effects of NT (Vieira, 1981; Sidiras et al., 1982, 1983). For example, in southern Brazil, when compared with CT, NT reduced the rates of decomposition of several crop residues by an average of 20% (Saraiva et al., 2003). However, differences in chemical composition of cover crops (especially legumes) also affect rate of residue decomposition (Franchini et al., 2002).

A few experiments performed in the tropics have indicated that both NT and appropriate crop rotation systems may increase soil microbial biomass and activity (Balota et al., 1998, 2003; Hungria, 2000), as well as the populations of agriculturally beneficial microorganisms, such as N_2-fixing rhizobia (Hungria and Stacey, 1997; Ferreira et al., 2000; Hungria and Vargas, 2000; Kaschuk et al., 2005) and mycorrhizal fungi (Hungria, 2000). A major effect of the enhancement of microbial biomass is increased immobilizations of C and N that are slowly released according to the plant's needs.

Quantitative and qualitative changes in the population of soil microorganisms were thought to reflect changes in soil quality. These changes are potentially useful as responsive indicators of the effects of crop and soil management. However, there is a lack of consistent information, especially for the tropics, about the long-term effects of addition of various crop residues and of soil management systems on SOM and microbial activity. Therefore, the objective of this work was to evaluate the usefulness of microbiological parameters to monitor soil changes in response to soil management and crop rotation systems in southern Brazil.

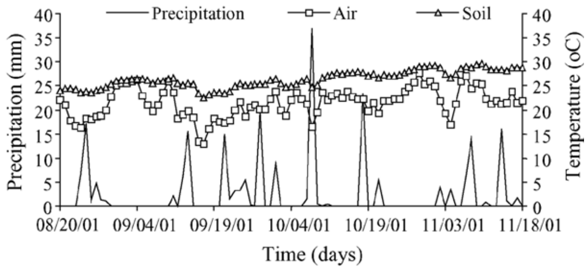

Fig. 1. Soil and air temperatures and precipitation during the experiment, from August to November of 2001.

2. Material and methods

2.1. Field area and experimental design

A field trial was established in 1997 at the experimental station of Embrapa Soja, in the city of Londrina (23°11′S, 51°11′W), State of Paraná, Brazil. The station is located at an altitude of 620 m and the trials were performed on an oxisol (Latossolo Vermelho Eutroférrico, Brazilian classification; Rhodic Eutrudox, USA classification). The climate is subtropical (Cfa, according to Koppen's classification) (IAPAR, 1994), with mean maximum and minimum temperatures of 28.5 °C in February and 13.3 °C in July, respectively, mean annual precipitation of 1651 mm $year^{-1}$, with January the wettest month (217 mm) and August the driest (60 mm). Soil and air temperatures as well as precipitation during the experiment are shown in Fig. 1. The experiment was designed to compare the effects of two soil management systems (NT, only a narrow channel is opened in the sowing row and CT, soil is ploughed and disked/harrowed), and three crop rotations [including grain (soybean, *Glycine max* L. Merr., maize, *Zea mays* L., and wheat, *Triticum aestivum* L.) and cover crops (lupin, *Lupinus angustifolius* L., and oat, *Avena strigosa* Schreb., rotations are shown in Table 1]. The trial had a completely randomized block

Table 1
Crop rotations under different soil management systems in the field experiment conducted at Embrapa Soja

Crop rotation	Winter 1997	Summer 1997/1998	Winter 1998	Summer 1998/1999	Winter 1999	Summer 1999/2000	Winter 2000	Summer 2000/2001	Winter 2001	Summer 2001/2002
1	Oat	Maize	Oat	Soybean	Wheat	Soybean	Lupin	Maize	Oat	Soybean
2	Oat	Soybean	Lupin	Maize	Oat	Soybean	Wheat	Soybean	Lupin	Maize
3	Oat	Soybean	Wheat	Soybean	Lupin	Maize	Oat	Maize	Wheat	Maize

In this study sampling took place after the winter 2001 crops and before the summer 2001/2002 crops as shown in Fig. 2.

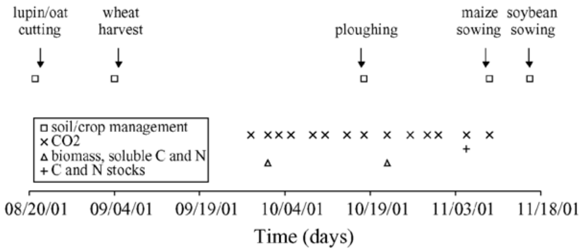

Fig. 2. Chronology of the activities and evaluations performed in the experiment from August to November of 2001.

design, with four replicates, thus with a total of 24 plots (each with 8 m width × 12 m length). Statistical analyses were performed for each sampling time using the SAS package (SAS, 1999).

2.2. Sampling procedures

Determinations of CO_2-emission rates, microbial biomass-C and -N, soluble C and N and C and N stocks were made in the fifth year of the experiment, from August to November 2001. This 4-month period comprised three main stages of the annual procedures: harvesting of the cover crop at full-flowering stage; wheat harvest; and soil preparation for sowing the summer crops, as shown in Fig. 2. The parameters were evaluated at sampling times before and after ploughing; C and N stocks were evaluated at the final sampling.

2.3. CO_2-emission rates

CO_2-emission rates from soil (respiration) were determined in 13 periods, six before and seven after plough, as shown in Fig. 2. Due to labor limiting conditions, CO_2 was assayed only in crop rotations 2 and 3, with a static chamber (PVC pipes, 10 cm × 20 cm, diameter × length) and the soda lime trap method, modified from Anderson (1982). A plastic receptacle containing 10 mL of 1N NaOH was attached inside each chamber, to trap the evolved CO_2; the chambers were buried at approximately 5-cm depth, six per replicate plot. If plant residues were present on the soil surface, they were kept inside the chambers. For each sampling time, three out of the six chambers were closed with a PVC cap and sealed with a rubber ring to prevent CO_2 losses. The three remaining chambers were kept opened to avoid sealing for long periods that might result in different conditions inside the chambers (temperature, moisture, gas composition, etc.). The plastic receptacles containing NaOH were replaced every Monday and Friday. The CO_2 captured was indirectly determined after the addition of saturated $BaCl_2$ to the NaOH solution, followed by the titration of the non-consumed NaOH with HCl, and values were expressed as g of CO_2–C m^{-2} day^{-1}. The three replicates obtained for each plot were averaged to represent one replicate (experiment with four replicates). Statistical analyses were performed for each sampling time, and combining the samples before and after ploughing of the CT plots, using the SAS package (SAS, 1999).

2.4. Microbial biomass analyses

Microbial biomass-C and -N (MB-C and MB-N) were determined before and after ploughing, in all treatments, as shown in Fig. 2. Five subsamples from each plot were collected from the 0–10 cm layer, homogenized and combined as one sample per replicate plot. Microbial biomass-C and MB-N were evaluated by the fumigation-extraction method using K_c values of 0.33 and 0.54, respectively (Brookes et al., 1985; Vance et al., 1987). Carbon content in the extracts was determined using a spectrophotometer, according to Bartlett and Ross (1988), while N content in the same fractions was evaluated by the Kjeldahl method followed by the spectrophotometric determination of NH_4–N using the indophenol blue method (Feije and Anger, 1972). Three soil samples were collected from the 0–10 cm layer and dried at 105 °C for the determination of soil bulk density (Blake, 1965). Values obtained for microbial biomass were corrected for soil bulk density and expressed in g m^{-2} of microbial biomass.

2.5. Soluble carbon and nitrogen

Soluble C and N were measured in non-fumigated samples used in the analysis of soil microbial biomass. 0.5 M K_2SO_4 was used as extractor and C and N contents were evaluated as described in the previous item. The values obtained were also corrected for soil bulk density and expressed as g m^{-2}. The ratios of soluble C and N in relation to soil microbial biomass were also obtained and expressed as g of soluble C or N g^{-1} of microbial C or N.

2.6. Metabolic quotient (qCO_2)

The qCO_2 was estimated using the mean values of CO_2–C emission (g m^{-2} day^{-1}) and of the MB-C obtained before and after ploughing. The qCO_2 results were expressed as mg of CO_2–C g^{-1} of microbial C day^{-1}.

2.7. Carbon and nitrogen stocks

The final sampling included undisturbed and disturbed soil samples obtained from the 0–10, 10–20 and 20–40 cm depth layers for the evaluation of C and N stocks (Fig. 2). In the central part of each plot (four replicates per treatment), a 20 cm × 50 cm × 50 cm (width × length × depth) trench was opened from which disturbed soil samples were collected with a spatula and undisturbed soil samples were taken by using a stainless steal cylinder (100 cm^3). The cylinder was oil-lubricated and introduced vertically into the soil layer (0–10 cm) and horizontally in the 10–20 and 20–40 cm layers. Three soil samples were collected from each layer and dried at 105 °C for the determination of soil bulk density (Blake, 1965). Organic-C was determined after Walkley-Black by the oxidation of $Cr_2H_2O_7$ 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 $CuSO_4$ with colorimetric determination of NH_4–N using the indophenol blue method (Feije and Anger, 1972). Soil bulk density was considered in the calculations of C and N contents and values were expressed as kg m^{-2} for each layer. Carbon and N stocks were also calculated considering soil mass, as suggested by Balesdent et al. (2000).

2.8. Estimation of plant biomass

In relation to the grain crops, plant residues were collected after the grain harvest, while for the cover crops plant residues were collected at full flowering stage; both materials were estimated considering a 0.5 m^2 area of each plot, with four replicates. Plant material was placed in a forced-air dryer at 65 °C until constant weight was obtained (approximately 72 h) and dry weight was then recorded. Estimated values were obtained considering the mean dry weight of each plant species during the 5-year period. Amounts of C added were estimated considering a 45% average content in the dry matter.

3. Results and discussion

The rates of CO_2-emission (soil respiration) were affected by variations in the sampling period, as well as in soil management and crop rotation (Table 2). Before ploughing of the CT, CO_2-emissions were similar in both soil management systems, however, after the sixth sampling, plough increased CO_2 losses in CT by an average of 57%, when compared to NT. Considering the 13 samples, CO_2-emission was 21% greater in CT (Tables 2 and 3). Decreases in CO_2-emission were mainly related with the decomposition of plant residues, as Fig. 1 shows that environmental conditions were satisfactory.

Table 2
CO_2-emission rates (g C m^{-2} day^{-1}) from soils after 5 years under different soil management (CT: conventional tillage; NT: no-tillage) and crop rotation (2 and 3 as described in Table 1 systems

	Sampling												
	1	2	3	4	5	6	7	8	9	10	11	12	13
Treatment													
CT2	1.16[a]	1.10	2.15	1.23[a]	1.83[a]	1.64[a]	1.74 a	1.16 a	1.08 a	1.33 a	1.47 a	0.87 a	0.98 a
CT3	0.94	0.97	1.86	1.03	1.36	1.48	1.50 a	1.03	0.93 a	1.23 a	1.33 a	0.86 a	0.98 a
NT2	1.11	1.23	2.19	1.18[a]	1.71[a]	1.60	1.10 b	0.98 b	0.51 b	0.79 b	0.85 b	0.62 b	0.60 b
NT3	1.07	1.06	1.92	1.03	1.41	1.53	1.23 b	0.98	0.36 b	0.72 b	0.81 b	0.49 b	0.49 b
Soil management													
CT	1.05	1.03	2.01	1.13	1.60	1.56	1.62[b]	1.09	1.01[b]	1.28[b]	1.40[b]	0.87[b]	0.98[b]
NT	1.09	1.15	2.06	1.10	1.56	1.57	1.17	0.98	0.44	0.76	0.83	0.55	0.54
Crop rotation													
2	1.13	1.17	2.17[c]	1.21[c]	1.77[c]	1.62[c]	1.42	1.07	0.80	1.06	1.16	0.74	0.79
3	1.00	1.02	1.89	1.03	1.39	1.51	1.37	1.01	0.65	0.97	1.07	0.68	0.73

Thirteen samples were taken August to November 2001, as shown in Fig. 2. First sampling: 28 September 2001; last sampling: 11 November 2001; in CT, soil ploughing was performed between the sixth and seventh samplings, on 17 October 2001, as shown in Fig. 2. Different lower case letters indicate difference at $P \leq 0.05$ in means between soil managements with the same crop.

[a] Difference at $P \leq 0.05$ in means between crops within the same soil management.

[b] Difference at $P \leq 0.05$ in means between soil managements.

[c] Difference at $P \leq 0.05$ in means between crop rotations.

Table 3
CO_2-emission rates and metabolic quotients (qCO_2) in soils after 5 years under different soil management (CT: conventional tillage; NT: no-tillage) and crop rotation (2 and 3 as described in Table 1 systems, considering the whole sampling period (1st to 13th samplings), before (1st to 6th samplings) and after ploughing (7th to 13th samplings), as shown in Fig. 2

	CO_2-emission (g C m^{-2} day^{-1})			Metabolic quotient (qCO_2) (mg C g^{-1} microbial-C day^{-1})		
	Whole period	Before ploughing	After ploughing	Whole period	Before ploughing	After ploughing
Treatment						
CT2	1.36 a[a]	1.52[a]	1.23 a[d]	43.5 a	51.1 a	35.9 a[d]
CT3	1.19 a	1.27	1.12 a	42.5 a	49.0 a	36.0 a[d]
NT2	1.11 b	1.50	0.78 b[d]	19.0 b	26.4 b	11.6 b[d]
NT3	1.01 b	1.34	0.73 b[d]	19.4 b	26.2 b	12.5 b[d]
Soil management						
CT	1.28[b]	1.40	1.18[b,d]	43.0[b]	50.1[b]	35.9[b,d]
NT	1.06	1.42	0.75[d]	19.2	26.3	12.0[d]
Crop rotation						
2	1.24[c]	1.51[c]	1.01[d]	31.2	38.7	23.7[d]
3	1.10	1.30	0.93[d]	30.9	37.6	24.2[d]

Different lower case letters indicate difference at $P \leq 0.05$ in means between soil managements with the same crop.
[a] Difference at $P \leq 0.05$ in means between crops within the same soil management.
[b] Difference at $P \leq 0.05$ in means between soil managements.
[c] Difference at $P \leq 0.05$ in means between crop rotations.
[d] Difference at $P \leq 0.05$ in means before and after ploughing.

Regarding crop rotation, the averaged total emission of CO_2 was 13% higher in the field previously growing lupin than in the field with wheat (Tables 2 and 3). Lupin was used as a cover crop and cut on the 21st of August, at full flowering, while wheat grain was harvested 2 weeks later, leaving only straw of high C/N ratio on the soil (Fig. 2). Differences between the two crop rotations were thus associated with the decomposition of lupin residues from the third to the sixth samplings after cutting, with similar values after that, indicating a rapid decomposition rate of the legume residues. Indeed, after the sixth harvest, CO_2-emission rates were 31% lower than in the previous samplings (Tables 2 and 3). In addition, significant differences related to the decomposition of the lupin residues were more prevalent in the CT (samplings 1, 4, 5, 6 and 7) than in the NT system (samplings 4, 5 and 12) (Table 2). In conclusion, differences in CO_2 were identified soon after the addition of crop residues to the soils, especially lupin, stimulating microbial activity and resulting in strong and rapid emission of CO_2 in both soil management systems (Table 2).

Soil microbial biomass (MB) was also influenced by sampling period and soil management, but not by crop rotation (Table 4). Microbial biomass-C and MB-N in the fifth year under NT were 80 and 104% higher than in CT, respectively. Furthermore, MB was consistently higher under NT, independently of crop rotation. Differences among samplings were observed only for MB-C in NT, and were associated with the crop rotation with lupin (Table 4). The lower MB in CT (Table 4), associated with the higher CO_2-emission (Table 3) implies little conversion of C from plant residues into MB. It is also noteworthy that the differences between NT and CT on the MB accumulated in the 5-year period were considerably higher than in studies performed in non-tropical regions. Differences in SOM levels between CT and NT soils in temperate regions are usually in the order of 10–20% and this is reflected in microbial biomass levels, such that the MB-C under NT is often less than 20% higher than in CT (Wardle, 1995).

Efficiency of the microbial community was significantly higher in NT soils; on average, metabolic quotient (qCO_2) was 55% lower than in CT (Table 3). Metabolic quotient was higher before ploughing of CT soil and, therefore, appears to be related to the decomposition of plant residues; however, no effects of crop rotation were observed (Table 3).

Under natural vegetation, SOM is, at least to some extent, chemically and physically protected from biological degradation. However, soil disturbance by tillage and replacement of natural vegetation by crops affect the accessibility of microbes to various compartments of SOM and thus the decomposition rate of plant residues (Balesdent et al., 2000). Furthermore, the presence or absence of plant residues on the soil surface also affects several soil properties and, in consequence, the microorganisms habitat, the microbial activity, and the SOM dynamics. Among the benefits observed in NT are more favorable soil temperatures in tropical soils,

Table 4
Microbial biomass of C and N in soils (surface layer) after the fifth year under different soil management (CT: conventional tillage; NT: no-tillage) and crop rotation (1, 2 and 3 as described in Table 1 systems

	Microbial biomass-C (g C m^{-2})			Microbial biomass-N (g N m^{-2})		
	Whole period	Before ploughing	After ploughing	Whole period	Before ploughing	After ploughing
Treatment						
CT1	34.7 b	35.0 b	34.5 b	4.76 b	5.21 b	4.32 b
CT2	32.1 b	29.7 b	34.4 b	4.16 b	4.71 b	3.61 b
CT3	28.6 b	26.0 b	31.3 b	4.90 b	5.33 b	4.48 b
NT1	55.1 a	51.9 a	58.3 a	10.09 a	10.98 a	9.19 a
NT2	62.1 a	57.0 a	67.2 a[b]	9.53 a	9.47 a	9.58 a
NT3	54.6 a	51.0 a	58.2 a	8.65 a	9.29 a	8,01 a
Soil management						
CT	31.8[a]	30.2[a]	33.4[a]	4.61[a]	5.08[a]	4.14[a]
NT	57.3	53.3	61.3[b]	9.42	9.92	8.93
Crop rotation						
1	44.9	43.5	46.4	7.43	8.09	6.76
2	47.1	43.3	50.8[b]	6.84	7.09	6.60
3	41.6	38.5	44.7	6.78	7.31	6.24

Means of samplings performed considering the whole sampling period (means of 1st and 2nd samplings), before (1st sampling) and after (2nd sampling) ploughing, as shown in Fig. 2; different lower case letters indicate difference at $P \leq 0.05$ in means between soil managements with the same crop.
[a] Difference at $P \leq 0.05$ in means between soil managements.
[b] Difference at $P \leq 0.05$ in means before and after ploughing.

e.g., decreases in temperature of about 10 °C at sowing time in southern Brazil (Derpsch et al., 1991), and increases of up to 45% in moisture availability (Sidiras et al., 1983), both providing conditions more favorable for the growth of soil microorganisms and plants. In this study, increases in MB due to improvements in soil conditions under NT were even greater than those obtained by the addition of plant residues of different C/N ratio. Furthermore, the reduction in the qCO_2 associated with the higher MB in NT resulted in greater SOM with time, consistent with data reported by Balota et al. (1998, 2003).

Soluble C and N values were 37 and 24% greater in NT than in CT soils, respectively (Table 5). Differences were observed in both sampling periods, but were higher after ploughing of the CT plots. No differences in soluble C and N were associated with crop rotation, and increases in soluble C were observed at the final sampling in all three rotations growing under NT (Table 5). Comparison of Tables 4 and 5 shows that greater MB-C and MB-N values were associated with higher contents of soluble C and N. Higher soluble fractions implies greater losses of C and N by leaching or run-off under NT conditions. However, ratios of soluble C and N contents to MB-C and MB-N were always lower in NT, and were not affected either by sampling period or by crop rotation (Table 6). The ratio was 24% lower in NT than in CT (Table 6), indicating higher immobilization of C and N per unit of microbial biomass, therefore tending to decrease losses of nutrients as they are released to the plants.

Soil management affected organic-C stocks, which also varied with soil depth (Table 7). The 0–10 and 20–40 cm layers showed the highest differences between soil management systems, with the former accumulating higher contents in NT and the second in CT. Organic-C in NT showed the following net changes in relation to CT: +0.476, −0.019 and −0.207 kg m^{-2}, in the 0–10, 10–20 and 20–40 cm layers, respectively. However, although NT accumulated more C on the surface and less at depth, considering the whole profile analyzed (0–40 cm), a balance of +0.250 kg C m^{-2} was obtained in NT, corresponding to 2500 kg C ha^{-1} accumulated in the 5-year period, or 500 kg ha^{-1} $year^{-1}$.

Carbon stocks also varied with the crop rotation system, and net changes in the 0–40 cm layer were +0.363, +0.584 and −0.197 kg C m^{-2} in crop rotations 1, 2 and 3 (Fig. 3A), equivalent to 726, 1167 and −394 kg ha^{-1} $year^{-1}$, respectively.

On average, microbial biomass corresponded to 2.4 and 1.7% of the total C in soil in the NT and CT systems, respectively (microbial quotient-C, represented by the ratio of MB-C/total organic-C) (Table 7). The increase of MB-C with time (Table 4) implied in an enhancement of the microbial quotient-C in NT (Table 7). In relation

Table 5
Soluble C and N after the fifth year in soils (surface layer) under different soil management (CT: conventional tillage; NT: no-tillage) and crop rotation (1, 2, and 3 as described in Table 1 systems

	Soluble C (g C m^{-2})			Soluble N (g N m^{-2})		
	Whole period	Before ploughing	After ploughing	Whole period	Before ploughing	After ploughing
Treatment						
CT1	22.6 b	21.4 b	23.7 b	3.50 b	3.64 b	3.36 b
CT2	22.8 b	22.0 b	23.6 b	3.59 b	3.91 b	3.27 b[b]
CT3	22.9 b	22.2 b	23.5 b	3.29 b	3.41 b	3.16 b
NT1	30.6 a	27.4 a	33.8 a[b]	4.10 a	4.09 a	4.10 a
NT2	31.2 a	28.3 a	34.0 a[b]	4.30 a	4.19 a	4.42 a
NT3	32.0 a	29.0 a	35.0 a[b]	4.48 a	4.48 a	4.47 a
Soil management						
CT	22.7[a]	21.9[a]	23.6[a]	3.46[a]	3.65[a]	3.26[a,b]
NT	31.2	28.2	34.3[b]	4.29	4.25	4.33
Crop rotation						
1	26.6	24.4	28.8[b]	3.80	3.86	3.73
2	27.0	25.1	28.8[b]	3.94	4.05	3.84
3	27.4	25.6	29.2[b]	3.88	3.95	3.81

Means of samplings performed considering the whole sampling period (means of 1st and 2nd samplings), before (1st sampling) and after (2nd sampling) ploughing, as shown in Fig. 2; different lower case letters indicate difference at $P \leq 0.05$ in means between soil managements with the same crop.
[a] Difference at $P \leq 0.05$ in means between soil managements.
[b] Difference at $P \leq 0.05$ in means before and after ploughing.

to crop rotation, a higher ratio of legumes:non-legumes in rotation 2 has also resulted in a higher microbial quotient-C (Table 7).

Similar results were observed for the organic-N stocks (Table 8). On average, the comparison of NT with CT resulted in the following net changes in N: +0.034, +0.010 and −0.003 kg m^{-2} in the 0–10, 10–20 and 20–40 cm layers, respectively. Therefore, major differences between NT and CT were also detected in the surface layer. Considering the whole profile (0–40 cm), an

Table 6
Ratio of soluble to microbial biomass-C and N (g g^{-1}) in soils (surface layer) after 5 years under different soil management (CT: conventional tillage; NT: no-tillage) and crop rotation (1, 2 and 3 as described in Table 1 systems

	Carbon			Nitrogen		
	Whole period	Before ploughing	After ploughing	Whole period	Before ploughing	After ploughing
Treatment						
CT1	0.65	0.61	0.69	0.74 a	0.70 a	0.78 a
CT2	0.71 a	0.74 a	0.69	0.87 a	0.83 a	0.91 a
CT3	0.80 a	0.86 a	0.75	0.67 a	0.64	0.71
NT1	0.55	0.53	0.58	0.41 b	0.37 b	0.45 b
NT2	0.50 b	0.50 b	0.51	0.45 b	0.44 b	0.46 b
NT3	0.58 b	0.57 b	0.60	0.52 b	0.48	0.56
Soil management						
CT	0.72[a]	0.74[a]	0.71[a]	0.76[a]	0.72[a]	0.80[a]
NT	0.55	0.53	0.56	0.46	0.43	0.49
Crop rotation						
1	0.60	0.57	0.63	0.57	0.54	0.61
2	0.61	0.62	0.60	0.66	0.64	0.68
3	0.69	0.71	0.68	0.60	0.56	0.63

Means of samplings performed considering the whole sampling period (means of 1st and 2nd samplings), before (1st sampling) and after (2nd sampling) ploughing, as shown in Fig. 2; different lower case letters indicate difference at $P \leq 0.05$ in means between soil managements with the same crop.
[a] Difference at $P \leq 0.05$ in means between soil managements.

Table 7
Organic-C stock in soil layers and ratio of microbial biomass-C to organic-C (g kg^{-1}) in the (surface layer) after 5 years under different soil management (CT: conventional tillage; NT: no-tillage) and crop rotation (1, 2 and 3 as described in Table 1 systems

	Organic-C (kg m^{-2})				Microbial biomass-C/organic-C		
	Layers (cm)				0–10 cm		
	0–10	10–20	20–40	0–40	Whole period	Before ploughing	After ploughing
Treatment							
CT1	1.89 b	2.16	3.06	7.11	18.4 b	18.5	18.3 b
CT2	1.80 b	2.12	2.89	6.80 b	17.8 b	16.5 b	19.1 b
CT3	1.97 b	2.16	3.09 a	7.22	14.5 b	13.2 b	15.9 b
NT1	2.46 a	2.17	2.84	7.47	22.4 a	21.1	23.7 Ba
NT2	2.31 a	2.18	2.90	7.39 a	26.9 a	24.7 a	29.1 Aa[b]
NT3	2.32 a	2.03	2.67 b	7.02	23.6 a	22.0 a	25.1 ABa
Soil management							
CT	1.89[a]	2.14	3.01[a]	7.04[a]	16.9[a]	16.1[a]	17.7[a]
NT	2.36	2.13	2.81	7.29	24.3	22.6	26.0[b]
Crop rotation							
1	2.17	2.17	2.95	7.29	20.4 AB	19.8	21.0
2	2.06	2.15	2.89	7.10	22.3 A	20.6	24.1[b]
3	2.14	2.09	2.88	7.12	19.1 B	17.6	20.5

For the microbial biomass-C/organic-C ratio, means of samplings performed considering the whole sampling period (means of 1st and 2nd samplings), before (1st sampling) and after (2nd sampling) ploughing, as shown in Fig. 2; different lower case letters indicate difference at $P \leq 0.05$ in means between soil managements with the same crop. Different upper case letters indicate difference at $P \leq 0.05$ in means between crops with the same soil management or between means of crops.

[a] Difference at $P \leq 0.05$ in means between soil managements.

[b] Difference at $P \leq 0.05$ in means before and after ploughing.

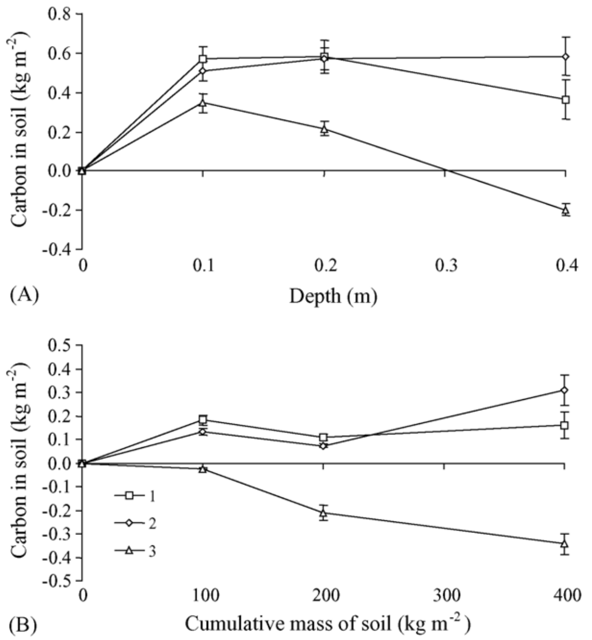

Fig. 3. Differences in C contents in soils under conventional and no-tillage systems with three crop rotations, as a function of soil depth (A) and cumulative mass of soil in the profile (B). 1, 2 and 3 represent crop rotation systems described in Table 1. Vertical bars indicate the standard deviation (S.D.) values.

increase of 0.041 kg N m^{-2} was obtained in NT, representing 410 kg of N ha^{-1}, or 82 kg of N ha^{-1} $year^{-1}$. Crop rotation also affected N accumulation in NT, and for the 0–40 cm layer as a whole, changes of +0.036, +0.069 and +0.018 kg N m^{-2} were observed in rotations 1, 2 and 3 (Fig. 4A), equivalent to 71, 137 and 37 kg of N ha^{-1} $year^{-1}$, respectively.

On average, microbial biomass represented 5.2 and 3.2% of the total N in NT and CT soils, respectively (microbial quotient-N, estimated by MB-N/total organic-N), and values were not statistically different between sampling times (Table 8). Similarly to the C data, the increase of MB-N with time (Table 4) also resulted in enhancement of the microbial quotient-N in NT (Table 8), emphasizing the importance of soil microbes as reservoirs for N in NT. However, contrary to the previous observation with C, crop rotation did not affect microbial quotient-N (Table 8).

The greater potential for immobilization of N under NT was, therefore, evidenced in this study by both the lower soluble N/MB-N ratio, and the lower microbial quotient-N in comparison to the CT system. Increased immobilization of N might result in N deficiency in crops such as maize and wheat under NT; however, with a legume included in the crop rotation, such a deficiency

Table 8
Total N stock in soil layers and ratio of microbial biomass-N to total N (g kg^{-1}) in the (surface layer) after 5 years under different soil management (CT: conventional tillage; NT: no-tillage) and crop rotation (1, 2 and 3 as described in Table 1 systems

	Total N (kg m^{-2})				Microbial biomass-N/total N		
	Layer				0–10 cm		
	0–10 cm	10–20 cm	20–40 cm	0–40 cm	Whole period	Before ploughing	After ploughing
Treatment							
CT1	0.148 b	0.164	0.269	0.582	32.2 b	35.2 b	29.2 b
CT2	0.137 b	0.161	0.249	0.547 b	30.3 b	34.3 b	26.3 b
CT3	0.143 b	0.162	0.253	0.558	34.4b	37.4 b	31.4 b
NT1	0.185 a	0.175	0.257	0.617	54.4 a	59.2 a	49.6 a
NT2	0.178 a	0.173	0.266	0.616 a	53.6 a	53.3 a	53.9 a
NT3	0.168 a	0.168	0.241	0.576	51.6 a	55.5 a	47.8 a
Soil management							
CT	0.143[a]	0.162	0.257	0.562[a]	32.3[a]	35.6	29.0
NT	0.177	0.172	0.254	0.603	53.2	56.0	50.4
Crop rotation							
1	0.167	0.170	0.263	0.600	43.3	47.2	39.4
2	0.157	0.167	0.257	0.582	42.0	43.8	40.1
3	0.155	0.165	0.247	0.567	43.0	46.4	39.6

For the microbial biomass-N/total N ratio, means of samplings performed considering the whole sampling period (means of 1st and 2nd samplings), before (1st sampling) and after (2nd sampling) ploughing, as shown in Fig. 2; different lower case letters indicate difference at $P \leq 0.05$ in means between soil managements with the same crop.

[a] Difference at $P \leq 0.05$ in means between soil managements.

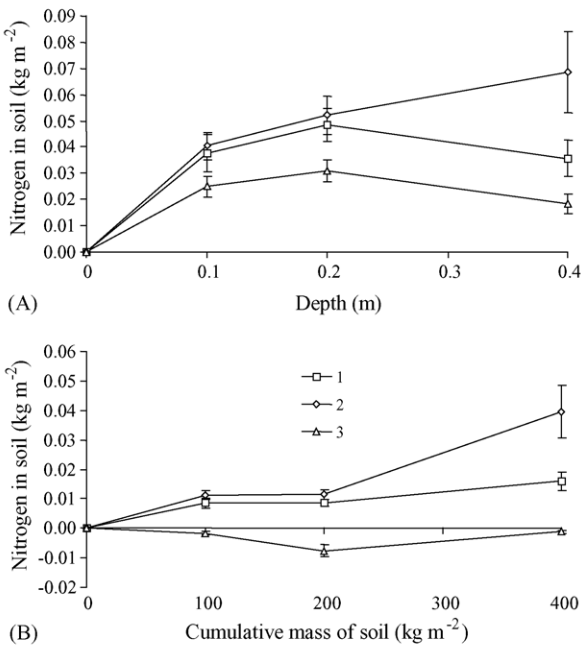

Fig. 4. Differences in N content in soils under conventional and no-tillage systems with three crop rotations, as a function of soil depth (A) and cumulative mass of soil in the profile (B). 1, 2 and 3 represent crop rotation systems described in Table 1. Vertical bars indicate the standard deviation (S.D.) values.

may be reduced (Muzilli, 1981; Muzilli et al., 1983; Castro Filho et al., 1991). Furthermore, after a certain period under NT, the slower rate of turnover of N will be compensated by the increased fraction of N immobilized, reducing the need for addition of N fertilizer in NT when compared to CT. Under conditions prevailing in Paraná State, Muzilli (1981) and Muzilli et al. (1983) have shown that 3–5 years may be needed for maize yields to be similar in NT and CT. However, recently it was shown that if a legume is used as a first crop – even a grain crop such as soybean – yield of the following non-legume crop may be similar from the first year of establishment of NT (Hungria et al., 2005).

In this study, C and N stocks were also estimated considering the mean values of soil mass (kg m^{-2}): 104, 117 and 120 for CT and 123, 127 and 114 for NT in the 0–10, 10–20 and 20–40 cm layers, respectively (data not shown). The results indicate that higher C and N stocks in NT may be at least partially related to soil bulk density, especially in the surface layers. By correcting C and N stock values for soil mass, as recommended by Balesdent et al. (2000), differences related to soil management were lower (Figs. 3B and 4B). However, considering 400 kg m^{-2} of soil, C balance was still positive in crop rotations 1 and 2, but decreased to 325 and 627 kg ha^{-1} year^{-1}, while in rotation 3, the loss was increased, and estimated at –687 kg ha^{-1} year^{-1}.

Table 9
Estimates of plant biomass and C added to the crop rotation systems, differences in stocks with soil management and transference rate of C from plants to soil.

Crop	Crop rotation								
	1			2			3		
	MS[a] (Mg ha^{-1})	C[b] (Mg ha^{-1})	%	MS (Mg ha^{-1})	C (Mg ha^{-1})	%	MS (Mg ha^{-1})	C (Mg ha^{-1})	%
Oat	24.0 (3)[c]	10.8	48	16.0 (2)	7.2	34	16.0 (2)	7.2	36
Lupin	7.4 (1)	3.3	15	14.8 (2)	6.6	31	7.4 (1)	3.3	17
Maize	10.6 (2)	4.8	21	5.3 (1)	2.4	11	10.6 (2)	4.8	24
Soybean	6.0 (2)	2.7	12	9.0 (3)	4.0	19	6.0 (2)	2.7	13
Wheat	2.3 (1)	10.6	5	2.3 (1)	1.1	5	4.7 (2)	2.1	11
Non-legumes	37.0 (6)	16.7	73	23.7 (4)	10.7	50	31.3 (6)	14.1	70
Legumes	13.4 (3)	6.0	27	23.7 (5)	10.7	50	13.4 (3)	6.0	30
Cover crop	31.4 (4)	14.1	62	30.8 (4)	13.9	65	23.4 (3)	10.5	52
Grain crop	18.9 (5)	8.5	38	16.6 (5)	7.5	35	21.3 (6)	9.6	48
Total	50.4	22.7		47.4	21.3		44.7	20.1	
Stock[d]		3.6	16		5.8	27		−2.0	−10
Stock[e]		1.6	7		3.1	14		−3.4	−17

[a] MS: total plant biomass (Mg ha^{-1}) considering the following mean values of periodical evaluations: 8.0 (oat); 7.4 (lupin); 5.3 (maize); 3.0 (soybean); 2.3 (wheat).
[b] Total C in plant biomass, calculated considering an average content of 45% of C in plant tissues.
[c] Number of times that the crop is repeated in the crop rotation system.
[d] Stock of C considering the layer 0 to 0.4 m. The percentage (%) relates the stock determined in soil to the total carbon added.
[e] Stock of C considering the cumulative mass of 400 kg m^{-2}. The percentage (%) relates the stock determined in soil in relation to the total C added.

For the N stocks, corrected values resulted in changes of 32, 79 and −2 kg ha^{-1} $year^{-1}$ in rotations 1, 2 and 3, respectively. These results emphasize the need to take into account variation in soil bulk density with depth when estimating C and N stocks.

The amounts of plant biomass added to the soil in each crop rotation were also estimated (Table 9). Regarding total C added by the crops, the following order was observed: crop rotation 1 > rotation 2 > rotation 3 (Tables 1 and 9). However, the addition of plant biomass was not the major factor affecting C accumulation in soil. The highest difference in C stock between CT and NT was observed in rotation 2 (Table 7), which included more legumes. However, differences were related to a decrease in C stock under CT rather than an increase in NT (Table 7). Therefore, N from the legumes might have increased the rate of decomposition of C under the CT conditions, which favored mineralization. On the other hand, rotations 1 and 3 were similar regarding the presence of the legume, whereas the non-legume cover crop was included three times in rotation 1 and twice in rotation 3, resulting in a higher input of C in the former, estimated at 2549 kg of C ha^{-1}. It is also important to emphasize that the last four crops in rotation 3 were non-legumes, with negative effects on the C stock in soil.

Carbon-conservation rates (ratio of organic-C in plant residues transformed to organic-C in soil) were generally low when compared to the amounts of plant residues added to the soil (Table 9); they were estimated at 16, 27 and −10% in rotations 1, 2 and 3, respectively. After correction for soil mass, the values were 7, 14 and −17% respectively.

The set of data obtained in this study has shown that a higher ratio of legumes:non-legumes in rotation 2 favored C accumulation in NT, whereas non-legume grains in rotation 3 favored C accumulation in CT. The benefits of including legumes in crop rotation to enhance C accumulation in soil has been reported before in other trials in southern Brazil (Bayer et al., 2000; Amado et al., 2001; Sisti et al., 2004) However, the results obtained in our study emphasize the particular importance of including legumes under NT to guarantee higher accumulation of C in soil.

4. Concluding remarks

Greater MB-C and MB-N, lower CO_2-emission rates, lower qCO_2, lower ratios of soluble-C/MB-C and soluble-N/MB-N and higher microbial quotients-C and -N emphasize the importance both of NT and of inclusion of legumes in crop rotations as efficient means of conserving SOM in the tropics. Such patterns of enhancement in C and N stocks in the soil after only 5 years are suggestive of achievement of agricultural sustainability. The parameters associated with microbiological activity were sensitive and rapid indicators of effects of soil management, demonstrating their usefulness as indicators of soil quality in the tropics.

Acknowledgments

The authors thank to Dr. Robert M. Boddey (Embrapa Agrobiologia) and Dr. Lincoln Zotarelli for the establishment of the experiment, to Luciano Santos for help with several aspects of the work and Dr. Allan Eaglesham and Glaciela Kaschuk for helpful suggestions on the article. The work was partially supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico, PQ), PRONEX and Fundação Araucaria (046/2003). MH gratefully acknowledges a research fellowship from CNPq (301241/2004-0).

References

Allison, L.E., 1965. Organic carbon. In: Black, C.A. (Ed.), Methods of Soil Analysis. Part II. American Society of Agronomy, Madison, WI, pp. 1367–1378.

Amado, T.J.C., Bayer, C., Eltz, F.L.F., Brum, A.C.R., 2001. Potencial de culturas de cobertura em acumular carbono e nitrogênio no solo no plantio direto e a melhoria da qualidade ambiental. R. Bras. Ci. Solo 25, 189–197.

Anderson, J.P.E., 1982. Soil respiration. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Agronomy Monograph Number 9. Part II. Chemical and Biological Properties. 2nd ed. American Society of Agronomy and Soil Science Society of America, Madison, WI, pp. 831–871.

Balesdent, J., Chenu, D., Malabane, M., 2000. Relationship of soil organic matter dynamics to physical protection and tillage. Soil Till. Res. 53, 215–230.

Balota, E.L., Colozzi-Filho, A., Andrade, D.S., Dick, R.P., 2003. Microbial biomass in soils under different tillage and crop rotation systems. Biol. Fertil. Soils 38, 15–20.

Balota, E.L., Colozzi-Filho, A., Andrade, D.S., Hungria, M., 1998. Biomassa microbiana e sua atividade em solos sob diferentes sistemas de preparo e sucessão de culturas. R. Bras. Ci. Solo 22, 641–649.

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., Mielniczuck, J., Amado, T.J.C., Martin-Neto, L., Fernandes, S.V., 2000. Organic matter storage in a sandy clay loam acrisol affected by tillage and cropping systems in southern Brazil. Soil Till. Res. 54, 101–109.

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.

Blake, G.R., 1965. Bulk density. In: Black, C.A. (Ed.), Methods of Soil Analysis. Part II. American Society of Agronomy, Madison, WI, pp. 374–390.

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.

Castro Filho, C., Henklain, J.C., Vieira, M.J., Casão Jr., R., 1991. Tillage methods and soil and water conservation in southern Brazil. Soil Till. Res. 20, 271–283.

Castro Filho, C., Lourenço, A., Guimaraes, 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.

Derpsch, R., 1998. Historical review of no-tillage cultivation of crops, in: Proceedings of the 1st JIRCAS Seminar on Soybean Research: No-Tillage Cultivation and Future Research Needs. JIRCAS, Working Report No. 13, pp. 1–18.

Derpsch, R., Roth, C.H., Sidiras, N., Kopke, U., 1991. Controle da Erosão no Paraná, Brasil: Sistemas de Cobertura do Solo, Plantio Direto e Preparo Conservacionista do Solo. GTZ-IAPAR, Eschborn, Germany-Londrina, Brazil, pp. 272.

FAO (Food and Agriculture Organization), 2004. What is Conservation Agriculture? Available from <http://www.fao.org/ag/AGS/AGSE/agse_e/1ero/INT1.htm>, Retrieved on the March 28, 2004.

FEBRAPDP (Federação Brasileira de Plantio Direto na Palha), 2005. Available from <http://www.febrapdp.org.br/historico.htm>, Retrieved on the March 28, 2005.

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

Ferreira, M.C., Andrade, D.S., Chueire, L.M.O., Takemura, S.M., Hungria, M., 2000. Effects of tillage method and crop rotation on the population sizes and diversity of bradyrhizobia nodulating soybean. Soil Biol. Biochem. 32, 627–637.

Franchini, J.C., Vila, F.J.G., Rodriguez, J., 2002. Transformations of plant residues used in the no-tillage management system as seen by flash pyrolysis. J. Anal. Appl. Pyrol. 62, 35–43.

Hungria, M., 2000. Características biológicas em solos manejados sob plantio direto, in: Memorias de la V Reunión Bienal de la Red Latinoamericana de Agricultura Conservacionista, 1999. Florianópolis, SC, Brazil, EPAGRI, Florianópolis, pp. 1–15 (CD Rom).

Hungria, M., Stacey, G., 1997. Molecular signals exchanged between host plants and rhizobia: basic aspects and potential application in agriculture. Soil Biol. Biochem. 29, 819–830.

Hungria, M., Vargas, M.A.T., 2000. Environmental factors affecting N_2 fixation in grain legumes in the tropics, with an emphasis on Brazil. Field Crops Res. 65, 151–164.

Hungria, M., Franchini, J.C., Campo, R.J., Crispino, C.C., Moraes, J.Z., Sibaldelli, R.N.R., Mendes, I.C., Arihara, J., 2005. Nitrogen nutrition of soybean in Brazil: contributions of biological N_2 fixation and of N fertilizer to grain yield. Can. J. Plant Sci., submitted for publication.

IAPAR (Instituto Agronômico do Paraná), 1994. Cartas climáticas do Estado do Paraná. IAPAR, Londrina, Paraná, Brazil, pp. 49 (IAPAR, Documento, 18).

Kaschuk, G., Hungria, M., Santos, J.C.P., Berton-Junior, J.F. 2005. Differences in common bean rhizobial populations associated with soil tillage management in Southern Brazil. Soil Till. Res. (available online May 10, 2005).

Kladivko, E.J., 2001. Tillage systems and soil ecology. Soil Till. Res. 61, 61–76.

Muzilli, O., 1981. Manejo da fertilidade do solo, in: Fundação Instituto Agronômico do Paraná (Ed.), Plantio Direto no Estado do Paraná. Fundação Instituto Agronômico do Paraná, Londrina, Paraná, Brazil, pp. 43–57.

Muzilli, O., Vieira, M.J., Almeida, F.L.S., Nazareno, N.R.X., Carvalho, A.O.R., Laurenti, A.E., Llanilo, R.F., 1983. Comportamento e possibilidades da cultura do milho em plantio direto no Estado do Paraná. Pesq. Agropec. Bras. 18, 41–47.

Sá, J.C.M., Cerri, C.C., Dick, A., Lal, R., Venske-Filho, S.P., Piccolo, M.C., Feigl, B.E., 2001. Organic matter dynamics and carbon sequestration rates for a tillage chronosequence in a Brazilian Oxisol. Soil Sci. Soc. Am. J. 65, 1486–1499.

Saraiva, O.F., Torres, E., Loni, D.A., Pires, M.S., 2003. Manejo dos resíduos da colheita condicionado por sistemas de preparo do solo, in: Embrapa Soja (Ed.), Resultados de Pesquisa da Embrapa Soja, 2002: Manejo do Solo, Plantas Daninhas e Agricultura de Precisão, Embrapa Soja, Londrina, Paraná, Brazil, pp. 29–37 (Embrapa Soja, Documentos 214).

SAS Institute Inc., 1999. Proprietary of Software, Version 6, 4th. ed. SAS Institute, Cary, NC, USA.

Sidiras, N., Derpsch, R., Mondardo, A., 1983. Influência de diferentes sistemas de preparo do solo na variação da umidade e rendimento da soja, em Latossolo Roxo distrófico (Oxisol). R. Bras. Ci. Solo 7, 103–106.

Sidiras, N., Henklain, J.C., Derpsch, R., 1982. Comparison of three different tillage systems with respect to aggregate stability, the soil and water conservation and the yields of soybean and wheat on an oxisol. J. Agron. Crop Sci. 151, 137–148.

Sisti, C.P.J., dos Santos, H.P., Kohhann, R., Alves, B.J.R., Urquiaga, S., Boddey, R.M., 2004. Change in carbon and nitrogen stocks in soil under 13 years of conventional or zero tillage in southern Brazil. Soil Till. Res. 76, 39–58.

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.

Vieira, M.J., 1981. Propriedades físicas do solo, in: Fundação Instituto Agronômico do Paraná (Ed.), Plantio Direto no Estado do Paraná. Fundação Instituto Agronômico do Paraná, Londrina, Paraná, Brazil, pp. 19–32.

Wardle, D.A., 1995. Linkages between the soil microbial biomass and climate and biotic factors. In: Hungria, M., Balota, E.L., Colozzi-Filho, A., Andrade, D.S. (Eds.), Microbiologia do Solo: Desafios para o Século XXI. IAPAR/EMBRAPA-CNPSo. Londrina, Paraná, Brazil, pp. 82–95.