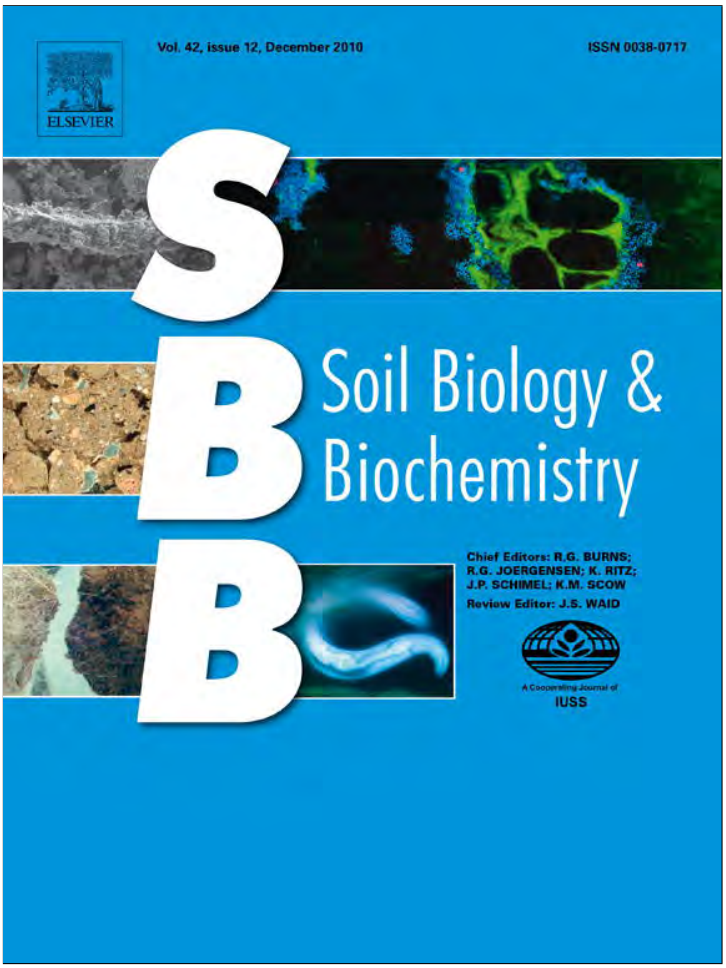

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Microbial biomass and activity at various soil depths in a Brazilian oxisol after two decades of no-tillage and conventional tillage

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ABSTRACT

The advantages of no-tillage (NT) over conventional tillage (CT) systems in improving soil quality are generally accepted, resulting from benefits in soil physical, chemical and biological properties. However, most evaluations have only considered surface soil layers (maximum 0–30 cm depth), and values have not been corrected to account for changes in soil bulk density. The objective of this study was to estimate a more realistic contribution of the NT to soil fertility, by evaluating C- and N-related soil parameters at the 0–60 cm depth in a 20-year experiment established on an oxisol in southern Brazil, with a soybean (summer)/wheat (winter) crop succession under NT and CT. At full flowering of the soybean crop, soil samples were collected at depths of 0–5, 5–10, 10–20, 20–30, 30–40, 40–50 and 50–60 cm. For the overall 0–60 cm layer, correcting the values for soil bulk density, NT significantly increased the stocks of C (18%) and N (16%) and microbial biomass C (35%) and N (23%) (MB-C and -N) in comparison to CT. Microbial basal respiration and microbial quotient (*q*Mic) were also significantly increased under NT. When compared with CT, NT resulted in gains of 0.8 Mg C ha^{-1} yr^{-1} (67% of which was in the 0–30 cm layer) and 70 kg N ha^{-1} yr^{-1} (73% in the 0–30 cm layer). In the 0–5-cm layer, MB-C was 82% higher with NT than with CT; in addition, the 0–30 cm layer accumulated 70% of the MB-C with NT, and 58% with CT. In comparison to CT, the NT system resulted in total inputs of microbial C and N estimated at 38 kg C ha^{-1} yr^{-1} and 1.5 kg N ha^{-1} yr^{-1}, respectively. Apparently, N was the key nutrient limiting C and N stocks, and since adoption of NT resulted in a significant increase of N in soils which were deficient in N, efforts should be focused on increasing N inputs on NT systems.

1. Introduction

The management of soils by reduced or no-tillage (NT) is many centuries old, with examples dating from the Egyptian and Inca's civilizations, and in modern agriculture, advantages of NT systems have been stressed since the 1940s (Derpsch, 1998; Lal et al., 2007). A good example was the publication of *Plowman's Folly*, stating the advantages of reduced tillage (Faulkner, 1942). However, despite an increasing number of reports in subsequent years, with NT there are usually problems to control weed growing, thus the adoption of NT started to be seriously considered only after the development of herbicides, such as paraquat, in 1955 (Derpsch, 1998). Nowadays, NT is practiced on over 100 million ha worldwide, mostly in North and South America, but also in Australia and in Europe, Asia and Africa (Derpsch, 1998; Lal, 2007; FEBRAPDP, 2010). In South America, the first NT trial was established in 1971 in the State of Paraná, Brazil (Derpsch et al., 1991; Derpsch, 1998). Brazil is now the best example of the broad adoption of NT, with 26 million ha cultivated on a continuous basis, representing 70% of the area cropped to annual legumes (Lal et al., 2007; FEBRAPDP, 2010). The main challenge is now to transpose this positive experience to resource-poor farmers, especially in Africa (Lal et al., 2007).

Since the 1970s, numerous studies have shown significant ecological, economical, environmental and social advantages of NT in comparison to conventional tillage (CT). Among the advantages are the control of soil erosion by wind and water, moisture conservation, lower pollution, more favorable soil temperatures, increased efficiency in nutrient cycling, improvement in soil structure, less consumption of fuel, machinery conservation and saving of time in terms of human and animal labor (e.g., Derpsch et al., 1991; Derpsch, 1998; Lal et al., 2007). Also important are

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reports of NT increasing soil organic C (SOC), highlighting the possibility of trading carbon credits (Pacala and Socolow, 2004; Lal et al., 2007). Finally, benefits from NT over CT by increasing soil microbial activity and diversity have been reported in Brazil (Balota et al., 1998, 2003, 2004; Mendes et al., 2003; Franchini et al., 2007; Pereira et al., 2007a,b; Hungria et al., 2009), including increases of up to 100% in microbial biomass C (MB-C) in as little as five years (Franchini et al., 2007). However, the real benefits of NT in increasing SOC and C-sequestration have been recently questioned, as most of the studies performed so far have only concerned the superficial layers, of 30 cm or less (Baker et al., 2007). There may be no C gains if deeper layers of soil are evaluated, a criticism that is pertinent to most studies performed in Brazil. In addition, concerns exist over estimates of increases in SOC from NT determined in studies in which bulk densities were not considered.

To obtain realistic estimates of the contribution of NT over CT on C-sequestration, SOC and microbial enhancement, we evaluated C- and N-related parameters, with an emphasis on microbial indicators, in a mature, 20-year-old experiment on a Brazilian oxisol, by considering soil depths to 60 cm and by correcting values with respect to bulk density.

2. Material and methods

2.1. Geographic location and general description of the field sites

The study was performed in a field trial established in the summer of 1988/89 at the experimental station of Embrapa Soja, located at an altitude of 620 m, in Londrina (23°11′S, 51°11′W), state of Paraná, southern Brazil. The climate is humid subtropical, with an annual average temperature of 21 °C, and mean maximum and minimum temperatures of 28.5 °C in February and 13.3 °C in July, respectively. Mean annual precipitation is 1651 mm, with January being the wettest month (217 mm) and August the driest (60 mm). The trial is established on an oxisol (Latossolo Vermelho Eutroférrico, Brazilian classification; Rhodic Eutrudox, USA classification), with the following physical composition: 710 g clay kg^{-1} soil, 82 g silt kg^{-1} soil and 208 g sand kg^{-1} soil.

Before the establishment of the experiment the area had been cropped with coffee trees for about 40 years, receiving the same management and inputs, therefore the soil was considerably uniform in terms of fertility. The experiment consists of plots, 7.5 m in width × 30.0 m in length (225 m^2) with four replicates (four plots) per treatment, distributed in a completely randomized block design. For this study we used the treatments under two soil-management practices: (1) no-till—NT, sowing directly through the residue of the previous crop, with the opening of only a narrow channel (~1.5–4 cm; ranging from 1.5–2 cm for wheat to 3–4 cm for soybean) in the sowing row; and (2) conventional till—CT, where the soil is prepared yearly with one pass with a disc plough (~20–25 cm) and disc harrow (~15 cm). A crop succession of soybean (*Glycine max* (L.) Merr.) in the summer and wheat (*Triticum aestivum* L.) in the winter was sown for both NT and CT.

Soil pH was corrected with lime applied as 2 ton ha^{-1} at the beginning of the experiment to reach a saturation of bases of 60% and to increase the pH to approximately 5.5 every three years. Fertilizers were applied at sowing, in equal amounts for the NT and CT treatments. For wheat, fertilizers consisted of no fertilizers in the first six years, and after that, 12.8–20 kg ha^{-1} of N (supplied as urea), 44.8–70 kg ha^{-1} of P (supplied as super triple phosphate), and 24–32 kg ha^{-1} of K (supplied as potassium chloride). For the soybean, every year, zero N, and 40–60 kg ha^{-1} of P (super triple phosphate) and K (potassium chloride) were added. After the 10th year soybean started to receive 20 g ha^{-1} of Mo (sodium molybdate) and 2 g ha^{-1} of Co (cobalt chloride). Other macro- and micronutrients were rarely needed and when a need was detected according to the leaf analysis in the previous crop and to the soil analysis before sowing, they were added equally to all treatments. Insects were controlled equally in all treatments with biological and chemical insecticide and fungicides which were also applied equally to all treatments when necessary throughout the 20 years of experiment. After the harvest crop residues were desiccated with glyphosate in both NT and CT treatments and after sowing other herbicides were applied to the CT, according to an analysis of infestation and the recommendation of herbicide efficacy for the dominant weeds.

2.2. Soil sampling

At the time of sampling, the experiment was 20 years old. Soil sampling was performed between cropped lines, in January of the 20th year, when soybeans were at full flowering stage (R2). The sampling included undisturbed and disturbed soil samples taken at six depths: 0–5, 5–10, 10–20, 20–30, 30–40, 40–50 and 50–60 cm. For disturbed samples, in the central part of each plot (four replicates per treatment), a trench, of 20 cm wide × 50 cm long × 60 cm deep was dug, from which soil samples were collected with a spatula, from the middle point of each layer and from the four sides of the trench. Subsamples were bulked to make a composite soil sample (approximately 1 kg). At the laboratory samples were mixed and sieved (<4 mm, 5 mesh), then stored in plastic bags, at 4 °C, for no more than 10 days.

Undisturbed soil samples used for evaluation of bulk density were taken with a stainless steel cylinder (100 cm^3) at each depth and the procedure was repeated five times in each plot and approximately 150 g were taken from each subsample.

2.3. Soil physical and chemical properties

For each layer sampled, bulk density was measured by the core method (Blake and Hartage, 1986).

Before being analyzed for chemical parameters, soil samples were dried (60 °C for 48 h), sieved through a 2 mm sieve and evaluated using standard procedures. Total soil organic C (TSOC) and total soil-N (TSN) were determined by combustion on a FLASH 2000 NC Analyzer (Thermo Scientific).

Soil pH was determined by using 0.01 M $CaCl_2$ (8 cm^3 soil: 20 mL of $CaCl_2$) (Embrapa, 1979). Exchangeable Ca, Mg and Al were extracted with 1 M KCl. Aluminium was determined by titration with 0.015 M standard NaOH, with bromothymol blue as indicator. Concentrations of Ca and Mg were determined with an atomic absorption spectrophotometer (Varian, Spectr AA-800). Available soil P and K contents were evaluated using the Mehlich-1 (0.05 M HCl + 0.0125 M H_2SO_4) reagent. Available P was determined by colorimetry, using the molybdenum-blue method and ascorbic acid as reducing agent, and exchangeable K by flame photometry (Micronal B 462) (Embrapa, 1979).

2.4. Microbial analyses

All measurements were made on moist soil, previously adjusted to 40% Water Holding Capacity (WHC) and soil weights, and all results were expressed on an oven-dry basis (105 °C overnight).

Microbial biomass C (MB-C) was evaluated by the fumigation–extraction (FE) method (Vance et al., 1987a), and microbial biomass N (MB-N) by the method of Brookes et al. (1985), both slightly modified as previously described (Hungria et al., 2009).

Four replicates of each soil sample (20 g for each sample) were used (Section 2.2). Two replicates were fumigated as described by Vance et al. (1987a), and the other two represented the non-

fumigated treatments. After extraction (in 0.5 M K_2SO_4), the C concentrations in the extracts were determined by oxidation with Mn^{3+}, and estimated on a spectrophotometer (Bartlett and Ross, 1988). The MB-C contents in the extracts were calculated from the difference between fumigated and non-fumigated samples, and although the correction factor of 0.38 was proposed by Vance et al. (1987a,b), we used a K_{CE} of 0.41, as recommended for tropical soils to avoid overestimation (Feigl et al., 1995; Oliveira et al., 2001).

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

Microbial quotient (qMic) was estimated as follows: (MB-C/TSOC) × 100 (considering the values corrected to m^3 of soil).

An adaptation of the method of fumigation–incubation (FI) of Jenkinson and Powlson (1976a,b) was also used to evaluate MB-C, as previously described (Hungria et al., 2009). Six replicates (100 g with 40% of WHC) of each composite sample (section 2.2) were placed in glass jars (300 mL). Two of those jars were subjected to fumigation (with alcohol-free chloroform for 16 h, in the dark, at 25 ± 2 °C) followed by reinoculation (1 g of soil) and incubation (7 days); two others were non-fumigated (held for 16 h followed by incubation for 7 days), and two were non-fumigated and, after the 16 h period at room temperature, 1 g of glucose was added and the soils were incubated for further 7 days. More details about the incubation procedures were given elsewhere (Hungria et al., 2009).

The MB-C was estimated from the difference between fumigated and non-fumigated samples not amended with glucose using a correction factor ($K_C = 0.41$) (Anderson and Domsch, 1978). The basal respiration (BR) was estimated in non-fumigated soil samples. Glucose-enhanced respiration, defined here as BR(i) was evaluated in non-fumigated soil samples receiving 1 g of glucose.

The metabolic quotient (qCO_2) (Anderson and Domsch, 1993) was obtained from the relation between BR per unit of MB-C. The $qCO_{2(i)}$ was estimated by the relation between BR(i) and MB-C.

2.5. Estimates of C and N stocks

As described in section 2.4, soil moisture levels were determined, so that the microbial biomass values were corrected for soil moisture and expressed as mg C or N kg^{-1} dry soil. In addition, soil bulk densities (section 2.3) were used in the calculations of C and N contents and values were expressed as g C or N m^{-3} soil for each layer. The same procedures were used for evaluation of TSOC and TSN stocks. To compare with results from the literature, values were also estimated considering the equation of: C or N values (kg Mg^{-1}) × soil bulk density (Mg m^{-3}) × soil volume (m^3) × 10^4 m^2 (ha). Values were also divided by the time period of the experiment (20 yr).

2.6. Statistical analysis

The experimental data were analyzed as a complete randomized block design with four blocks (Cochran and Cox, 1957). The data were analyzed using the SAS for PC statistical package (SAS Institute, 2001), using PROC GLM. All assumptions required by the analysis of variance (ANOVA) were verified. The error normality, according to the experimental model design, was evaluated by Shapiro–Wilk's test (Shapiro and Wilk, 1965), the variance of homogeneity by Burr–Foster's test (Burr and Foster, 1972), and the non-additivity of the model by Tukey's method (Tukey, 1949). Coefficients of skewness and kurtosis were also determined.

All results are expressed on an oven-dry soil basis (105 °C, overnight).

3. Results

3.1. Soil physical and chemical properties

Soil compaction was greater with NT than with CT at 5–20 cm (Table 1). Within NT, soil compaction was observed at 5–30 cm, and at 10–30 cm for CT (Table 1). No differences in pH were observed throughout the profile under CT, whereas higher pH values were observed in the two superficial layers with NT (Table 1). Differences in pH between soil-tillage regimes occurred in the 5–10 cm layer, with higher values for NT, and in the 30–40-cm layer, with higher values for CT. The two superficial layers of the NT soil also contained higher contents of P, K and Mg, whereas CT at 0–20 cm contained more P and K. The P concentration in the 0–10-cm layer under NT was 3.8 times higher than under CT. The Ca concentration did not show any significant difference between depth or the tillage treatments (Table 1).

3.2. Carbon and nitrogen stocks

When estimated per unit of soil dry weight (g C kg^{-1} soil) (data not shown), or corrected for bulk density (kg C m^{-3} soil) (Table 2), TSOC values were always higher in the three superficial layers in both NT and CT. In addition, values were higher under NT when compared to the CT system (Table 2). The same pattern occurred with TSN, and highest values of TSN were associated with NT at the 0–5 cm and 5–10 cm layers (Table 2).

When corrected for soil bulk density, TSOC accumulated at the 0–30 cm depth with NT and CT was estimated at 70.6 and 54.6 Mg C ha^{-1}, increasing to total values of 105.2 and 89.2 Mg C ha^{-1} if the 0–60-cm layer is considered, respectively. For TSN, estimates were 6.6 and 5.1 Mg N ha^{-1} for NT and CT at 0–30 cm, and 10.0 and 8.6 Mg N ha^{-1} at 0–60 cm, respectively (Table 5).

3.3. Microbial parameters

After correction for bulk density, under NT the microbial biomass (MB-C) was higher in the first 30 cm, turning to not significant differences in deeper layers in relation to CT (Table 3). Microbial quotient (qMic) values ranged from 1.96 to 4.23, and qMic was 33% higher in the 0–5-cm layer under NT than in the CT, and statistically higher also in the 20–30 and 30–40 cm layers. Except for the 40–50 cm layer, MB-N values were higher in the NT profile but showed only significant differences in 0–5 cm layer (Table 3).

The NT system accumulated 2.08 Mg C ha^{-1} in MB in the top 30 cm, increasing to 2.95 for the 0–60 cm layer, whereas for CT these values were 1.29 and 2.19 Mg C ha^{-1}, respectively. Therefore considering the 0–60 cm layer, MB-C values were 35% higher in NT in comparison to CT. The NT accumulated 127 kg N ha^{-1} at 0–30 cm, and 160 kg N ha^{-1} at the 0–60 cm layer, whereas, for CT, these values were of 91 and 130 kg N ha^{-1}, respectively. Therefore the MB-N values corrected for bulk density were 23% higher in NT in comparison to CT (0–60 cm layer). The increases in MB-C and MB-N values were statistically significant (Table 5).

Basal respiration (BR) values were higher in the two superficial layers and decreased by an average of three times from the top layer to 60 cm in both tillage systems. The same pattern was verified for the glucose-enhanced respiration (BRi) (Table 4). On average, values of BR enhanced by 4.1 times with the addition of glucose in the NT system, and by 5.4 times under CT; considering the two

Table 1
Soil physical and chemical properties after 20 years under no-till (NT) or conventional tillage (CT) systems with the soybean (summer)/wheat (winter) crop succession. Means of four field replicates for each soil depth.

Depth (cm)	Density				pH				P				K				Ca				Mg			
	($g\,cm^{-3}$)				($CaCl_2$)				($mg\,dm^{-3}$)				($cmol_c\,dm^{-3}$)				($cmol_c\,dm^{-3}$)				($cmol_c\,dm^{-3}$)			
	NT		CT		NT		CT		NT		CT		NT		CT		NT		CT		NT		CT	
0–5	1.05	c[a]	A[a]	1.05	d	A	5.59	ab	A	5.43	ns	A	64.25	a	A	16.88	a	B	0.73	a	A	0.34	a	B	7.60	ns	A	7.46	ns	A	2.48	a	A	1.33	ns	B
5–10	1.27	a	A	1.09	cd	B	5.70	a	A	5.24		B	44.51	b	A	17.87	a	B	0.76	a	A	0.20	abc	B	7.44		A	6.85		A	1.78	ab	A	1.25		A
10–20	1.30	a	A	1.21	a	B	5.14	cd	A	5.41		A	10.87	c	A	15.68	a	A	0.53	bc	A	0.30	ab	B	7.07		A	6.45		A	1.13	b	A	1.20		A
20–30	1.25	a	A	1.22	a	A	5.50	abc	A	5.26		A	2.77	c	A	5.52	b	A	0.63	ab	A	0.15	bc	B	9.53		A	7.23		A	1.50	b	A	1.43		A
30–40	1.16	b	A	1.17	ab	A	5.08	d	B	5.45		A	2.66	c	A	3.62	b	A	0.38	cd	A	0.23	abc	B	7.42		A	6.41		A	1.21	b	A	1.35		A
40–50	1.12	bc	A	1.13	bc	A	5.31	abcd	A	5.21		A	2.36	c	A	2.84	b	A	0.49	bc	A	0.11	c	B	7.66		A	6.37		A	1.75	ab	A	1.49		A
50–60	1.12	bc	A	1.13	bc	A	5.21	bcd	A	5.38		A	1.96	c	A	2.06	b	A	0.22	d	A	0.16	bc	A	6.42		A	5.42		A	1.18	b	A	0.92		A
P tillage	0.158				0.729				≤0.001				≤0.001				0.185				0.219			
CV_t (%)	2.48				1.69				8.05				7.78				11.60				18.76			
P depth	≤0.001				0.034				≤0.001				≤0.001				0.209				0.008			
P tillage × depth	≤0.001				≤0.001				≤0.001				≤0.001				0.925				0.086			
CV_{txd} (%)	2.74				3.40				19.67				20.18				24.48				30.72			

CV indicates coefficient of variation and n.s. statistically non-significant.
[a] Means followed by different lower case letters indicate differences (ANOVA) at $P \leq 0.05$ between depths and differences in upper case letters indicate difference between tillage systems.

surface layers, induction of glucose increased BR by 4.3-fold under NT and by 7.0-fold under CT (Table 4).

Lower qCO_2 values were observed in the two surface layers for both NT and CT, but the tillage system did not have a statistically significant effect at any depth (Table 4). However, when induced by glucose, qCO_2 was lower with NT than with CT (Table 4).

4. Discussion

In the last two decades, several studies have demonstrated the benefits of NT over CT, including improvements in physical, chemical and biological properties of the soil (e.g. Derpsch et al., 1991; Balota et al., 1998; Sá et al., 2001; Franchini et al., 2007; Calegari et al., 2008; Hungria et al., 2009; Boddey et al., 2010). Difficulties may still exist in relation to weed control, but with adequate management and new herbicides this problem is less relevant. However, concerns about the actual contributions of NT to increase SOC- and C-sequestration have been recently raised, as most work, mainly performed in North America has not considered soil depths below 30 cm (Baker et al., 2007). Our study was conducted not only to help to clarify this issue, but also to supply a rather unique data set of microbial biomass parameters in relation to soil depth beyond 20 cm. A depth of 60 cm was chosen because previous studies in similar conditions have shown that, for soybean, about 50–65% of the root system is distributed in the first 10 cm, 95% in the 0–40-cm layer and practically none below 60 cm (Cardoso et al., 2006). For wheat, roots are concentrated in the first 10 cm, and, in general, 90–95% are present at less than 40 cm (Pires et al., 1991).

In our study, NT resulted in higher soil density in the 5–20-cm layer, consistent with other reports (e.g., Fernandes et al., 1983), although the results were below the critical level of 1.33 $g\,cm^{-3}$ established by Torres and Saraiva (1999). In general, machine traffic may cause compaction in the soil which might be reduced by tillage (Tormena et al., 1998). In extreme conditions, compaction may represent the only physical disadvantage of NT, as other physical properties are greatly favored by NT, particularly the stability of soil aggregates and improvements in physical-chemical properties that result in maintenance of soil moisture (e.g., Sidiras et al., 1982; Blevins et al., 1983; Fernandes et al., 1983; Derpsch et al., 1991; Castro Filho et al., 2002; Barreto et al., 2009). Aggregation is also important because NT accumulates SOC by protecting it within macroaggregates (Barreto et al., 2009). To avoid miscalculations due to compaction, we estimated C- and N-related parameters after correction for bulk density to provide more precise data.

As in other reports (e.g. Almeida et al., 2005), NT had higher pH values in the 5–10 cm layer, possibly due to the application of lime to the soil surface without incorporation. Acidification at the soil surface may increase when high rates of N-fertilizer are applied (Blevins et al., 1983); however, this is not the case of our experiment, as very low levels of N-fertilizer were applied in the 20-year period, i.e. zero application to the soybean and zero application to the wheat for the first six years, then 12.8–20.0 kg N (urea) ha^{-1} subsequently. Soil pH directly affects the availability of several nutrients and, in addition to the increase in the CEC related to the accumulation of SOM, it helped to explain the increases in exchangeable cations (K^+, Ca^{2+} and Mg^{2+}) in our study. Important also are the higher values of P at the surface under NT, consistent with previous reports in Brazil (Sisti et al., 2004).

In our study, performed in a transitional zone between the tropics and subtropics, when compared to CT, NT resulted in a gain of 16 Mg C ha^{-1} if the 20-year period is considered (0.8 Mg C ha^{-1} yr^{-1}); 67% of the C accumulated in 0–30 cm with NT and 61% under CT. Elsewhere in Brazil, in Rio Grande do Sul, with a cooler, drier climate than at Londrina, values ranged from 0.48 to

Table 2
Total soil organic carbon (TSOC) and total soil-N (TSN) in an oxisol after 20 years under no-till (NT) and conventional tillage (CT) systems with the soybean (summer)/wheat (winter) crop succession. Means of four field replicates for each soil depth.

Depth (cm)	TSOC						TSN					
	($kg\ C\ m^{-3}$ soil)											
	NT			CT			NT			CT		
0–5	25.74	a[a]	A[a]	18.23	a	B	2.54	a	A	1.68	b	B
5–10	25.53	a	A	18.87	a	B	2.41	a	A	1.72	ab	B
10–20	27.92	a	A	19.38	a	B	2.65	a	A	1.90	a	B
20–30	17.01	b	A	16.68	ab	A	1.48	b	A	1.56	b	A
30–40	12.61	bc	A	13.49	bc	A	1.24	bc	A	1.32	c	A
40–50	11.78	c	A	11.40	c	A	1.11	c	A	1.10	d	A
50–60	10.19	c	A	9.68	c	A	1.00	c	A	1.03	d	A
P tillage	0.062						0.030					
CV_t (%)	5.97						5.89					
P depth	≤0.001						≤0.001					
P tillage × depth	≤0.001						≤0.001					
CV_{txd} (%)	3.90						3.82					

[a] Means followed by different lower case letters indicate differences (ANOVA) at $P \leq 0.05$ between depths and differences in upper case letters indicate difference between tillage systems. CV indicates coefficient of variation.

1.53 Mg C ha^{-1} yr^{-1} in the 0–105-cm layer, and C accumulations were up to 68% greater when estimated at 0–85 to 108 cm depths than when compared to the 0–30-cm layer (Sisti et al., 2004; Diekow et al., 2005; Boddey et al., 2010). In another experiment performed in Londrina, gains under NT were highly concentrated in the 0–10 cm layer, representing 58 and 45% of the C stock of the 0–40 cm layer in the NT and CT treatments, respectively; the increase in the 0–40-cm profile in the NT was estimated at 0.36 Mg C ha^{-1} yr^{-1} (Calegari et al., 2008).

Six et al. (2001) estimated that, in both tropical and temperate soils, a general increase in C levels (approximately 0.325 ± 0.113 Mg C ha^{-1} yr^{-1}) occurred with NT compared to CT. West and Post (2002), based mainly on experiments performed in the North Hemisphere estimated the average global C sequestered by NT at 0.57 ± 0.14 Mg C ha^{-1} yr^{-1} to a depth of approximately 30 cm. If we increase the values reported by West and Post (2002) by 40%, corresponding approximately to the percentage of C accumulated in deeper layers found in our study, we obtain a similar value of that from our study. This is a strong indication of consistency of estimates of potential global C-sequestration values under NT.

As highlighted in previous studies (Franchini et al., 2007; Hungria et al., 2009), the challenge in understanding N limitation and soil-N balance is far greater than with C. In our study, N accumulation at 0–60 cm under NT was 1.4 Mg N ha^{-1} higher than under CT, resulting in an estimated enrichment of 70 kg N ha^{-1} yr^{-1}. However, NT was not richer in N in all soil layers, emphasizing the need for further studies to understand limitations to N accumulation at soil deeper layers. For now, a strong hypothesis could rely on the benefits of NT over the CT on the nitrogen fixation with the soybean crop, as pointed before (Hungria and Vargas, 2000; Hungria et al., 2006).

It is noteworthy that the soybean/wheat crop succession provides lower inputs of C and N compared to other crop rotations, e.g., cover crops such as lupin (*Lupinus angustifolius* L.), oat (*Avena strigosa* Schreb.), oilseed radish (*Raphanus sativa* L.) and hairy vetch (*Vicia pillosa* Roth) (Franchini et al., 2007; Calegari et al., 2008), where C-sequestration can be far greater. For example, in long-term experiments in the state of Rio Grande do Sul, C-sequestration of up to 1.53 Mg C ha^{-1} yr^{-1} was obtained with NT, but only when legumes were included as intercrops or cover crops (Sisti et al., 2004; Diekow et al., 2005; Boddey et al., 2010). Nevertheless,

Table 3
Microbial biomass C and N (MB-C and MB-N) and microbial quotient (*q*Mic) in an oxisol after 20 years under no-till (NT) and conventional tillage (CT) systems with the soybean (summer)/wheat (winter) crop succession. Means of four field replicates for each soil depth.

Depth (cm)	MB-C						MB-N						*q*Mic					
	($g\ C\ m^{-3}$ soil)												%					
	NT			CT			NT			CT			NT			CT		
0–5	1090	a[a]	A[a]	582	a	B	66.54	a	A	36.48	a	B	4.23	a	A	3.19	a	B
5–10	800	b	A	570	ab	B	43.64	b	A	33.62	ab	A	3.13	b	A	3.02	ab	A
10–20	638	bc	A	390	bc	B	37.66	bc	A	31.28	ab	A	2.28	c	A	2.01	e	A
20–30	500	cd	A	327	c	B	34.53	bc	A	25.76	ab	A	2.94	b	A	1.96	e	B
30–40	382	de	A	336	c	A	22.72	cd	A	20.06	abc	A	3.03	b	A	2.49	d	B
40–50	270	e	A	296	c	A	10.96	de	A	16.80	bc	A	2.29	c	A	2.60	cd	A
50–60	221	e	A	272	c	A	0.01	e	A	5.25	c	A	2.17	c	A	2.81	bc	A
P tillage	0.003						0.026						0.033					
CV_t (%)	5.50						8.30						8.43					
P depth	≤0.001						≤0.001						≤0.001					
P tillage × depth	≤0.001						≤0.001						0.004					
CV_{txd} (%)	17.60						28.85						26.60					

[a] Means followed by different lower case letters indicate differences (ANOVA) at $P \leq 0.05$ between depths and differences in upper case letters indicate difference between tillage systems. CV indicates coefficient of variation.

Table 4
Basal respiration (BR), glucose-enhanced respiration BR(i), metabolic quotient non-induced (qCO_2) and induced by glucose [qCO_2 (i)] in an oxisol after 20 years under no-till (NT) and conventional tillage (CT) systems with the soybean (summer)/wheat (winter) crop succession. Means of four field replicates for each soil depth.

Depth (cm)	BR						BR(i)						qCO_2						qCO_2(i)					
	(mg C m^{-3} soil day^{-1})												(mg C m^{-3} soil day^{-1})											
	NT			CT			NT			CT			NT			CT			NT			CT		
0–5	751	a[a]	A[a]	467	a	B	2974	a	A	2571	a	B	0.72	b	A	0.85	b	A	2.85	c	B	5.40	bc	A
5–10	689	ab	A	429	ab	B	3256	a	A	2411	a	B	1.10	ab	A	0.82	b	A	5.19	a	A	6.26	b	A
10–20	605	bc	A	436	ab	B	2556	b	A	2294	a	B	1.23	a	A	1.36	ab	A	5.22	a	B	7.96	a	A
20–30	498	cd	A	389	ab	B	2056	c	A	1456	b	B	1.24	a	A	1.43	a	A	5.15	a	B	7.53	a	A
30–40	384	de	A	351	ab	A	1463	d	A	1262	b	A	1.17	ab	A	1.22	ab	A	4.45	b	A	5.08	c	A
40–50	295	e	A	266	b	A	1203	de	A	1181	bc	A	1.22	ab	A	1.02	ab	A	4.99	ab	A	4.59	c	A
50–60	266	e	A	254	b	A	1019	e	A	833	c	A	1.34	a	A	1.10	ab	A	5.14	ab	A	4.39	c	A
P tillage	0.009						≤0.001						0.205						0.353					
CV (%)	7.73						1.36						7.26						7.50					
P depth	≤0.001						≤0.001						≤0.001						≤0.001					
P tillage × depth	≤0.001						≤0.001						0.066						0.003					
CV (%)	14.14						9.24						19.78						16.50					

[a] Means followed by different lower case letters indicate differences (ANOVA) at $P \leq 0.05$ between depths and differences in upper case letters indicate difference between tillage systems. CV indicates coefficient of variation.

although farmers understand the benefits of cover crops, they cannot afford to lose a cash-crop season, resulting in negligible adoption of such crop rotations; but if, in the future, they receive cash for C credits they may adopt rotations that will significantly increase C-sequestration.

Our major objective was to determine soil microbial contributions with depth, as microbial parameters are useful bioindicators of soil quality (e.g. Doran and Parkin, 1994; Sparling, 1997; Kaschuk et al., 2010). In assessing the BR in the two tillage systems, NT resulted in respiratory rates higher than those with CT in the 0–40-cm layers. Similar results were observed for the glucose-enhanced respiration (BRi), confirming higher microbial activity. The ratio of BR to BR(i) shows that glucose was very effective in increasing basal respiration under CT, particularly in the 0–30-cm layer, corresponding to a 7-fold increase under CT, whereas there was a 4.3-fold increase under NT. Similar results have been reported previously (Hungria et al., 2009), suggesting that microorganisms are more C limited under CT. In addition, although BR(i) values were still higher under NT, as in the CT system, microorganisms responded vigorously to the addition of glucose, indicating that the microbial community could easily recover if conditions are more favorable. This "starving index" could thus represent a useful index to evaluate soil disturbance and resilience.

In general, no differences in qCO_2 and $qCO_{2(i)}$ were observed between the NT and CT systems. However, values were lower in the two surface layers in both systems, indicating higher efficiency of C utilization by the microbial communities, which may be important in maintaining or increasing stocks of SOC (Insam, 1990; Balota et al., 1998; Franchini et al., 2007; Hungria et al., 2009). Possibly, differences in qCO_2 with depth reflect the environmental conditions to which the microbial biomass is subjected, differences in the genetic structure of populations, or their normal functionality, lower organic matter contents, but they may also imply stress (Nielsen and Winding, 2002).

The MB-C concentrations were always higher in the superficial layers. In addition, at 0–5 cm, MB-C was 82% higher with NT than with CT, in accordance to previous reports (e.g. Balota et al., 1998, 2003, 2004; Franchini et al., 2007; Hungria et al., 2009). However, we showed that the benefits to MB-C go beyond that, as higher values were associated with NT to a depth of 60 cm. Overall, C associated with microorganisms under NT represented an input of 0.76 Mg C ha^{-1} over the 20-year period. But our results also show that the superficial layers (0–30 cm) of the NT treatment concentrated a higher proportion of the total MB-C (70%) than with CT (59%), suggesting that disturbance of the superficial layer may adversely affect the soil microbial population. Indeed, in a previous study we showed that using a field cultivator only every three years in an NT system resulted in significant decreases in microbial biomass and activity (Hungria et al., 2009). Our study thus adds support for the benefits of NT to MB-C in Brazilian soils (Kaschuk et al., 2010), and highlights the role of soil microorganisms in C cycling in the tropics, where soil-C turnover can be twice as fast as in temperate regions (Six et al., 2001). In support of this, Franchini et al. (2007) and Hungria et al. (2009) showed that *q*Mic is an outstanding bioindicator of soil quality in oxisols and in this study, *q*Mic clearly differentiated NT and CT in three out of the five superficial layers, with higher values under NT.

The limiting role of N in the tropics was confirmed in the MB-N analysis. Overall, MB-N with NT was 30 kg N ha^{-1} higher than with CT. However, values below 40 cm were higher under CT. In Londrina, estimates of nitrogen fixation by soybean crops are up to 300 kg N ha^{-1} and crop residues can contain 20–30 kg N ha^{-1}, potentially available to the subsequent wheat crop (Hungria et al., 2006). In addition, the amounts of N-fertilizer applied to the wheat plots were relatively small to support a mean grain yield of 2500 kg ha^{-1}. Therefore lower MB-N in some soil layers might indicate depletion in soil-N, accelerated by mineralization of MB-N to supply crop needs.

Table 5
Stocks[a] of total soil organic carbon (TSOC), total soil-N (TSN), microbial biomass C and N (MB-C and MB-N) at the 0–30-cm and 0–60-cm depths, and differences of stocks between no-till (NT) and conventional tillage (CT) (NT-CT) and % of increase of stocks with NT in comparison to CT in an oxisol after 20 years under NT and CT with the soybean (summer)/wheat (winter) crop succession. The data represent the mean of four field replicates.

Parameter	Depth	NT	CT	NT-CT	Increase in NT
	(cm)	(Mg ha^{-1})			(%)
TSOC	0–30	70.6	54.6		
(Mg C ha^{-1})	0–60	105.2	89.2	16	17.9[b]
TSN	0–30	6.6	5.1		
(Mg N ha^{-1})	0–60	10.0	8.6	1.4	16.3[b]
MB-C	0–30	2.08	1.29		
(Mg C ha^{-1})	0–60	2.95	2.19	0.76	34.7[b]
MB-N	0–30	0.127	0.091		
(Mg N ha^{-1})	0–60	0.160	0.130	0.03	23.1[b]

[a] Stocks were estimated as C or N values (kg Mg^{-1}) × soil bulk density (Mg m^{-3}) × soil volume (m^3) × 10^4 m^2 (ha).
[b] Increases were statistically significant ($P \leq 0.05$, ANOVA).

Previous work has shown that microbiological parameters are more sensitive indicators of soil disturbance than chemical or physical parameters and also that NT may greatly increase microbial activity (Balota et al., 1998; Franchini et al., 2007; Pereira et al., 2007a,b; Hungria et al., 2009). A potential limitation of these studies is that the assessments were made only in the superficial layers of the soil and, with the exception of the study by Franchini et al. (2007), without correction for soil bulk density. However, the results confirm the advantages of NT when the soil profile to a depth of 60 cm was analyzed and values were corrected for bulk density. Estimates of gains in the soybean/wheat succession under NT were of 0.8 Mg C ha^{-1} yr^{-1} of TSOC and 38 kg C ha^{-1} yr^{-1} in the MB-C, in addition to 70 kg N ha^{-1} yr^{-1} of TSN and 1.5 kg N ha^{-1} yr^{-1} in the MB-N. As adoption of NT is impressively increasing, efforts should now be focused on obtaining better understanding of N balance to increase inputs of N into systems under NT, especially in the deeper soil layers. Although the introduction of other cover crops in the soybean/wheat crop succession may increase C-sequestration, it represents a challenge to improving reserves of C and N in soil.

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