

7-2015

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Elcio Liborio Balota

*Instituto Agronômico do Paraná, Brazil*

Ines Fumiko Ubukata Yada

*Instituto Agronômico do Paraná, Brazil*

Higo Furlan Amaral

*Instituto Agronômico do Paraná, Brazil*

Andre Shigueyoshi Nakatani

*Centro Nacional de Pesquisa em Soja, Brazil*

Mariangela Hungria

*Centro Nacional de Pesquisa em Soja, Brazil*

*See next page for additional authors*

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**Authors**

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**Soil Quality in Relation to Forest Conversion to Perennial or Annual Cropping in Southern Brazil****Notes/Citation Information**

Published in *Revista Brasileira de Ciência do Solo*, v. 39, no. 4, p. 1003-1014.

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**Digital Object Identifier (DOI)**

<https://doi.org/10.1590/01000683rbc20140675>

# SOIL QUALITY IN RELATION TO FOREST CONVERSION TO PERENNIAL OR ANNUAL CROPPING IN SOUTHERN BRAZIL

Elcio Liborio Balota<sup>(1)\*</sup>, Ines Fumiko Ubukata Yada<sup>(1)</sup>, Higo Furlan Amaral<sup>(1)</sup>, Andre Shigueyoshi Nakatani<sup>(2)</sup>, Mariangela Hungria<sup>(2)</sup>, Richard Peter Dick<sup>(3)</sup> and Mark Steven Coyne<sup>(4)</sup>

<sup>(1)</sup> Instituto Agronômico do Paraná, Londrina, Paraná, Brasil.

<sup>(2)</sup> Empresa Brasileira de Pesquisa Agropecuária, Centro Nacional de Pesquisa em Soja, Londrina, Paraná, Brasil.

<sup>(3)</sup> Ohio State University, School of Environment and Natural Resources, Columbus, Ohio, USA.

<sup>(4)</sup> University of Kentucky, Department of Plant and Soil Sciences, Lexington, Kentucky, USA.

\*Corresponding author.

E-mail: balota@iapar.br

## ABSTRACT

Many forested areas have been converted to intensive agricultural use to satisfy food, fiber, and forage production for a growing world population. There is great interest in evaluating forest conversion to cultivated land because this conversion adversely affects several soil properties. We examined soil microbial, physical, and chemical properties in an Oxisol (*Latosolo Vermelho distrófico*) of southern Brazil 24 years after forest conversion to a perennial crop with coffee or annual grain crops (maize and soybeans) in conventional tillage or no-tillage. One goal was to determine which soil quality parameters seemed most sensitive to change. A second goal was to test the hypothesis that no-tillage optimized preservation of soil quality indicators in annual cropping systems on converted land. Land use significantly affected microbial biomass and its activity, C and N mineralization, and aggregate stability by depth. Cultivated sites had lower microbial biomass and mineralizable C and N than a forest used as control. The forest and no-tillage sites had higher microbial biomass and mineralizable C and N than the conventional tillage site, and the metabolic quotient was 65 and 43 % lower, respectively. Multivariate analysis of soil microbial properties showed a clear separation among treatments, displaying a gradient from conventional tillage to forest. Although the soil at the coffee site was less disturbed and had a high organic C content, the microbial activity was low, probably due to greater soil acidity and Al toxicity. Under annual cropping, microbial activity in no-tillage was double that of the conventional tillage management. The greater microbial activity in forest and no-tillage sites may be attributed,

Received for publication on October 17, 2014 and approved on February 19, 2015.

DOI: 10.1590/01000683rbc20140675

at least partially, to lower soil disturbance. Reducing soil disturbance is important for soil C sequestration and microbial activity, although control of soil pH and Al toxicity are also essential to maintain the soil microbial activity high.

**Keywords:** land use, tillage, coffee, biological activity, potential mineralization, aggregate stability.

## RESUMO: QUALIDADE DO SOLO EM RAZÃO DA CONVERSÃO DE MATA NATIVA EM CULTURAS ANUAL E PERENE NO SUL DO BRASIL

Muitas áreas de mata nativas têm sido convertidas para o uso agrícola intensivo para satisfazer a produção de alimentos, fibras e forragem em razão do crescimento da população mundial. Assim, existe grande interesse em avaliar o efeito da conversão de florestas em áreas de cultivo agrícola, porque essa conversão interfere em várias propriedades do solo. Foram estudados indicadores microbiológicos, químicos e físicos em um Latossolo Vermelho distrófico (LVd) no sul do Brasil 24 anos após a conversão da mata nativa em área com cultura perene (cafeiro) ou culturas anuais de grãos (milho/soja) em preparo convencional (PC) ou plantio direto (PD). O tipo de uso do solo influenciou significativamente a biomassa microbiana e sua atividade, a mineralização do C e N e a estabilidade de agregados por causa da profundidade do solo. Áreas cultivadas apresentaram menor biomassa microbiana e mineralização do C e N do que a área de controle (Mata Nativa). Apesar de a mata nativa e de o PD terem apresentado maior biomassa microbiana e mineralização do C e N do que o PC, o quociente metabólico foi de 65 e 43 % inferiores, respectivamente. A análise multivariada das variáveis microbiológicas do solo evidenciou clara separação entre os tratamentos, apresentando um gradiente do tratamento PC para a mata nativa. Apesar de o solo no cafeiro não ser revolvido e apresentar alto teor de C orgânico demonstrou baixa atividade microbiana, provavelmente por causa da maior acidez e toxidez de Al do solo. A atividade microbiana no PD foi o dobro da observada no PC. A maior atividade microbiana na área da mata e de PD pode ser atribuída, pelo menos parcialmente, à maior estabilidade de agregados. A redução do revolvimento do solo é importante para o incremento do sequestro de C e da atividade microbiana; entretanto, o controle do pH e o da toxidez por Al do solo também são importantes para manter a atividade microbiana do solo elevada.

*Palavras-chave:* uso do solo, preparo do solo, cafeiro, atividade biológica, mineralização potencial, estabilidade de agregados.

## INTRODUCTION

An increasing world population demands more food, fiber, and forage production. To satisfy this need, many native vegetation areas have been converted to intensive cultivation. Of the more than 350 million hectares used for agriculture in Brazil, 50 % are pastures. There are also extensive areas, used for decades for perennial crops such as coffee (*Coffea arabica* L.) (about 2.4 million hectares) or annual crops such as maize (*Zea mays* L.) (9.0 million hectares) or soybean (*Glycine max* L.) (22.9 million hectares) (IBGE, 2014).

Forest conversion to cultivated land leads to several changes in soil properties. Intense management promotes soil erosion and depletes organic matter, which decreases soil quality. Conventional or plow tillage (CT), uses traditional practices of plowing and disking to prepare the land for sowing. Tillage alters the soil structural stability (Six et al., 2004; Caesar-Tonthat et al., 2011) resulting in losses of soil organic matter (Franzluebbers, 2009) and microbial biomass (Islam

and Weil, 2000; Hungria et al., 2009; Frazão et al., 2010; Balota and Auler, 2011).

There is great interest in agricultural practices that reduce soil degradation and increase agricultural sustainability and soil conservation. A key point in sustainable use of agricultural soil is increasing the soil C status and the processes relating to, or at least maintaining, levels close to the original ones. One of these practices is no-tillage (NT), which corresponds to sowing directly through mulch and litter with minimal soil disturbance. The area devoted to NT systems in Brazil (31 million hectares) has increased almost 10-fold in the last 10 years, corresponding to 40 % of the annual cropland (Febrapdp, 2014).

Soil and crop management directly influence physical, chemical and biological soil properties, which are used to measure soil quality, which means the capacity of soil to serve a desired function, such as sustainably provide food, fiber and forage (Doran and Parkin, 1994). Microbial parameters have been successfully used to evaluate soil quality (Frazão et al., 2010; Kaschuk et al., 2010). For example, soil microbial biomass is a source of labile nutrients and an agent for transformation

and cycling of organic matter and plant nutrients. Microbial biomass and activity can be used as sensitive indicators of agro-ecosystem stress or changes in soil quality. Microorganisms have a relatively high rate of turnover and quick response compared to physical/chemical properties (Brookes, 2001; Wonprasaid, 2003; Kaschuk et al., 2010). Aggregates function as a habitat for microorganisms (Six et al., 2004; Caesar-Tonthat et al., 2011; Guillou et al., 2012) and have a strong relationship to microbial population and activity.

This study evaluated the effect of land use and management on microbial (C and N microbial biomass and mineralization), physical (aggregate stability and mean weight diameter) and chemical (organic carbon content, pH and CEC) indicators of soil quality 24 years after forest conversion to intensive perennial or annual crops cultivation. Our hypothesis was that the beneficial aspects of no-tillage management would partially offset the transition from forest to cultivated soil in terms of the effect on measured soil quality properties. The organic C content, pH and CEC were measured because these parameters are affected by soil management and influence plant nutrition and microbial activity. Aggregate stability was measured because this physical parameter is affected by the degree of soil disturbance and microbial activity.

## MATERIAL AND METHODS

### Sites and soil description

The study was conducted at the Experimental Station of the Agronomic Institute of Paraná State (IAPAR), district of Londrina, State of Paraná, in southern Brazil (23° 22' S, 51° 10' W and 585 m altitude). The soil is an Oxisol, *Latossolo*

*Vermelho distrófico* according to the Brazilian Classification System (Bhering and Santos, 2008), with 760 g kg<sup>-1</sup> clay, 130 g kg<sup>-1</sup> silt, 110 g kg<sup>-1</sup> sand and slope of about 6 %.

The climate is a humid subtropical, of the Cfa type according to the Köppen classification, characterized by a hot summer with rain in all seasons, but with a tendency to rain mainly in summer. Mean annual precipitation was about 1,600 mm for the period 1976-2011. The rainfall in January (219 mm) is four times greater than in July (53 mm). The relative humidity is about 62 % in the winter and 77 % in the summer. Typical evaporation is from 81 mm in June to 156 mm in October. The mean air temperature is 24 °C in January (from 11 to 36 °C) and 17 °C in July (from -1 to 31 °C). Sunshine occurs for 191 h in February to 238 h in August (Iapar, 2014).

The agricultural areas adjacent to the forest were selected due to their similarity of soil physical and chemical characteristics according to soil profile description (Table 1) (Sidiras et al., 1983; Castro Filho, 1988). According to the records of the Experimental Station, the soil environment was homogeneous at the beginning of land use transition. So these agricultural sites are good representatives of long-term land use due to their initial homogeneity and proximity to undisturbed forest. The perennial crop, represented by coffee, is about 100 m from the forest site, while the conventional tillage and no-tillage sites with annual crops are about 400 m away.

Conventional Tillage (CT): area used in an experiment for 24 years (since 1976) with a 2-year crop-rotation with wheat (*Triticum aestivum* L.) during winter and soybean rotation with maize each year as summer crop. The experiment consisted of plots (8 × 25 m), separated by 2 m rows, with three replications. Tillage consisted of one disk plowing and two diskings with a light

**Table 1. Initial values of soil physical and chemical properties**

Horizon	Layer cm	Sand	Silt %	Clay	OC g kg <sup>-1</sup>	pH(H <sub>2</sub> O)	CEC cmol <sub>c</sub> dm <sup>-3</sup>	P mg kg <sup>-1</sup>
CT - NT <sup>(1)</sup>								
Ap1	0-8	11.0	13.0	76.0	16.0	5.9	14.2	3.2
Ap2	8-20	8.0	13.0	79.0	16.0	5.1	11.7	2.4
Coffee <sup>(2)</sup>								
Ap1	0-14	3.2	12.0	84.8	19.4	4.4	9.70	5.9
Ap2	14-24	2.5	11.0	86.5	8.2	4.4	9.01	1.6
Forest								
A1	0-16	5.2	11.0	83.8	21.3	4.2	12.82	2.3
A3	16-40	3.4	12.0	84.6	14.3	4.1	10.61	1.5

<sup>(1)</sup> Samples were collected after preparing the area for establishment of the experiment (1976). <sup>(2)</sup> Samples were collected after 10 years of coffee production (1986). CT: conventional tillage; NT: no-tillage; OC: organic carbon; CEC: cation exchange capacity.

harrow to level the soil and prepare the seedbed. Crop stubble is tilled conventionally (plowed to a depth of 20 cm) after harvest, in fall and spring every year. Fertilizers were regularly distributed in the tillage treatments before each crop cycle, consisting of: N 95, P 55, and K 42 kg ha<sup>-1</sup> yr<sup>-1</sup>, for the last 24 years. Nitrogen fertilizer was never applied to soybean.

No-Tillage (NT): same experimental area described for the CT site. No-tillage consisted of sowing on undisturbed soil into a narrow opened trench. Crop stubble was mostly left on the surface after harvest in fall and spring every year.

Coffee (CO): coffee plantation (about 4,500 m<sup>2</sup>) of cultivar "Mundo Novo", with 2200 trees ha<sup>-1</sup> (spacing 4.0 × 2.0 m), since 1976. Fertilization according to soil analysis before/during the growth season (Nov-Feb), on average 150 kg ha<sup>-1</sup> yr<sup>-1</sup> N, 40 kg ha<sup>-1</sup> yr<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, and 80 kg ha<sup>-1</sup> yr<sup>-1</sup> K<sub>2</sub>O for the last 24 years. Before planting and every three years, dolomitic lime was applied to the whole area.

Forest (FO): The forest site (4.5 ha) is a remnant of primary tropical semi-deciduous forest that used to cover the north of Parana State. Leguminosae, Myrtaceae, Euphorbiaceae and Lauraceae are the main families of flowering plants at the site and the forest canopy height can reach 15 m.

### Soil sampling

Composite soil samples (consisting of five sub-samples each) were taken from each replicate of the NT and CT sites from three layers (0-5, 5-10 and 10-20 cm) in March 2000, at the end of the summer growing season (maize). The FO and CO sites were divided into three parts according to slope (high, medium and low). From each part, a central area (50 × 70 m for FO and 20 × 60 m for CO) was considered a sampling unit from which 10 soil sub-samples (about 150 g each) were taken in a zigzag pattern, at 10 m intervals, to compose one replicate composite for each layer (0-5, 5-10 and 10-20 cm depth). In the CO site the sub-samples were evenly divided between samples taken near the edge of the tree canopy, in the planting row, and in the inter row.

The fresh soil samples were sieved (<4 mm mesh) and the large plant material removed. All determinations for each replicate were made in triplicate and expressed on a dry-weight basis. The moisture was adjusted to 55-60 % of water-holding capacity, for microbial and mineralization analysis. For physical analyses, undisturbed soil samples were taken with a shovel. Care was taken to avoid soil smearing or compaction during transport to the laboratory. At the laboratory, the soil was spread in a room at 22 °C to air-dry. During drying the larger clods/aggregates were gently broken into smaller pieces. Then soil samples were subjected to physical analysis.

### Soil chemical analyses

Chemical analyses were made as described for routine analyses adapted for IAPAR by Pavan et al. (1992). Organic C concentration (C<sub>org</sub>) was measured by the Walkley-Black potassium dichromate sulfuric acid oxidation procedure and total N (N<sub>tot</sub>) by Kjeldahl digestion with sulfuric acid.

### Soil physical analyses

Aggregate stability was determined by wet sieving to estimate size distribution and aggregate stability in water (Castro Filho et al., 2002). The aggregate indices: Mean Weight Diameter of soil particle (MWD) and Aggregate Stability Index (ASI) were calculated according to the following equations 1 and 2:

$$\text{MWD} = \sum x_i y_i, \quad \text{Eq. 1}$$

where  $y_i$  is the proportion of each size class with respect to the total sample and  $x_i$  is the mean diameter of the size classes (mm).

$$\text{ASI} = \frac{\text{aggregate weight} - \text{weight of } <0.25 \text{ size class sample} - \text{sand}}{\text{weight of the sample} - \text{sand}} \times 100$$

Eq. 2

### Soil microbial analyses

Microbial biomass-C (MB-C) was determined by fumigation-extraction (Vance et al., 1987) and calculated using a correction factor (kc) of 0.33. Microbial biomass-N (MB-N) was determined by the method of Brookes et al. (1985), with 0.54 as correction factor. Basal respiration (BR) was analyzed in 20 g of soil (fresh weight) incubated in gas-tight jars containing NaOH to trap the released CO<sub>2</sub>. The CO<sub>2</sub> released in 10 days was determined by Flow Injection Analysis for differences in conductivity of the solution. Carbon and N mineralization (C<sub>MIN</sub> and N<sub>MIN</sub>) were determined by incubating 30 g of soil sample as described by Balota et al. (2004). For C<sub>MIN</sub>, soil was incubated in a sealed jar with NaOH trap in a dark chamber. The concentration of CO<sub>2</sub> released in the period was determined by flow injection analysis of conductivity and N<sub>MIN</sub> was assessed by the nitrate concentration in soil before and after the 24-d incubation period.

### Statistical analyses

The data were subjected to tests of normality of the variables and of homogeneity of variances, according to Bartlett's test. After all assumptions required for the analysis of variance were verified. ANOVA was conducted for each experimental area and in the forest to check the equality of variance of each variable. Factorial was the procedure used to evaluate land use and sampling layer by F-test (p<0.05). The Minimum Significant Difference (MSD) by Tukey's test (p<0.05) was used to identify significant differences between the means. These

procedures were analyzed by SAS Statistical Package (SAS, 2009).

Multivariate statistical analyses were used to evaluate the effects of management type and soil depth on soil microbial quality as described by the simultaneous analysis of five variables (MB-C, MB-N, BR,  $C_{\text{MIN}}$ , and  $N_{\text{MIN}}$ ). For this test, the data were analyzed using multivariate ordination “non-metric multidimensional scaling” - NMS (Sokal, 1979), with Sorensen distances. Prior to analysis, data were standardized by totals within each variable, to eliminate the differences in the variable units. The ordination was performed in the autopilot mode, “slow and thorough” option in the program PC-ORD v. 6.0 (McCune and Mefford, 2011). The number of dimensions to be interpreted was selected considering the criteria of stress and stability of the graphical solutions. The effect of soil management type and soil depth on soil microbial quality was analyzed using PERMANOVA in the program PC-ORD v. 6.0 (McCune and Mefford, 2011). Significance of differences in soil microbial condition between treatments was also determined by PERMANOVA. The DistLM procedure was used to check for the existence of significant relationship between soil microbial data and soil physical and chemical properties by the program DISTLM v.5 (Anderson, 2004). In addition, this procedure was used to assess the contribution of the most

important variables (soil microbial, physical and chemical properties) accounting for the variability among the treatments with DISTLM *forward* (Anderson, 2003). Pearson’s correlation between soil microbial, physical and chemical properties were performed using the SAS Statistical Package (SAS, 2009).

## RESULTS

### Chemical properties

Chemical properties of soils under the different land uses are in table 2. Layer was always a significant factor except for Al content; Land Use was always a significant factor except for CEC. Soil pH values ranged from 4.10 to 4.63. Aluminum content in CO at each layer (1.59-2.09  $\text{cmol}_c \text{kg}^{-1}$ ) was routinely twice as high as Al content in any other treatment.

Compared to the forest (FO),  $C_{\text{org}}$  always decreased with transition to cultivation in all layers. Overall declines for FO were 20 % in CO, 33 % in NT and 48 % in CT. Total N ( $N_{\text{tot}}$ ) also declined with depth in all treatments. In the 0-5 cm layer  $N_{\text{tot}}$  values declined relative to the FO site in the same

**Table 2. Chemical properties of a southern Brazilian Oxisol 24 years after forest conversion to a perennial crop with coffee or annual grain crops (maize and soybeans) in conventional tillage or no-tillage**

Land use	pH(H <sub>2</sub> O)	$C_{\text{org}}$	$N_{\text{tot}}$	Al <sup>3+</sup>	CEC
0-5 cm					
Forest	4.20 <sup>(1)</sup>	30.4	3.40	0.83	17.65
Coffee	4.11	24.4	2.59	1.59	20.83
Conventional Tillage	4.33	15.8	1.76	0.30	13.51
No-Tillage	4.63	20.3	2.20	0.12	14.76
5-10 cm					
Forest	4.13	25.8	2.69	0.93	15.50
Coffee	4.15	19.6	1.92	1.88	19.24
Conventional Tillage	4.37	14.8	1.60	0.32	13.53
No-Tillage	4.57	17.2	1.81	0.17	13.41
10-20 cm					
Forest	4.10	20.9	2.28	1.04	14.19
Coffee	4.17	16.4	1.66	2.09	18.88
Conventional Tillage	4.40	13.6	1.25	0.24	12.92
No-Tillage	4.53	14.6	1.61	0.20	12.18
Factor	Probability levels of ANOVA analysis				
Depth	ns	≤0.01	≤0.01	ns	≤0.01
Land use	0.01	≤0.01	≤0.01	≤0.01	ns
CV (%)	7.82	6.75	27.01	30.07	9.9

<sup>(1)</sup> Average of three replications for each depth. ns: not significant; CV: coefficient of variation.

order and approximately the same amount as  $C_{org}$  (CO = 24 %, NT = 35 % and CT = 48 %).

### Physical properties

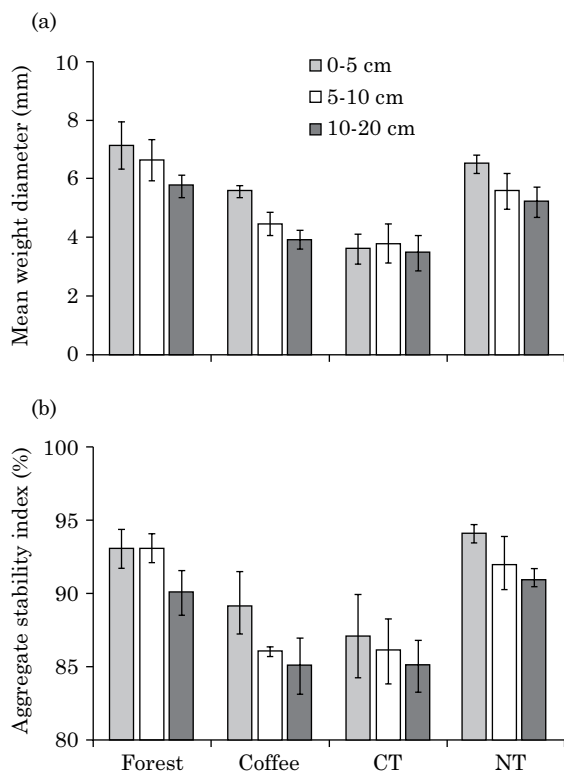
Land use and management had a significant effect on aggregate mean weight diameter (MWD) and aggregate stability index (ASI) ( $p \leq 0.001$ ), although depth within each treatment was not significant for either soil property ( $p \geq 0.05$ ). The MWD values were highest in FO followed by NT in all layers, even when not significant (Figure 1a). Likewise, ASI was routinely higher in FO and NT compared to CO and CT for each layer (Figure 1b).

### Microbial properties

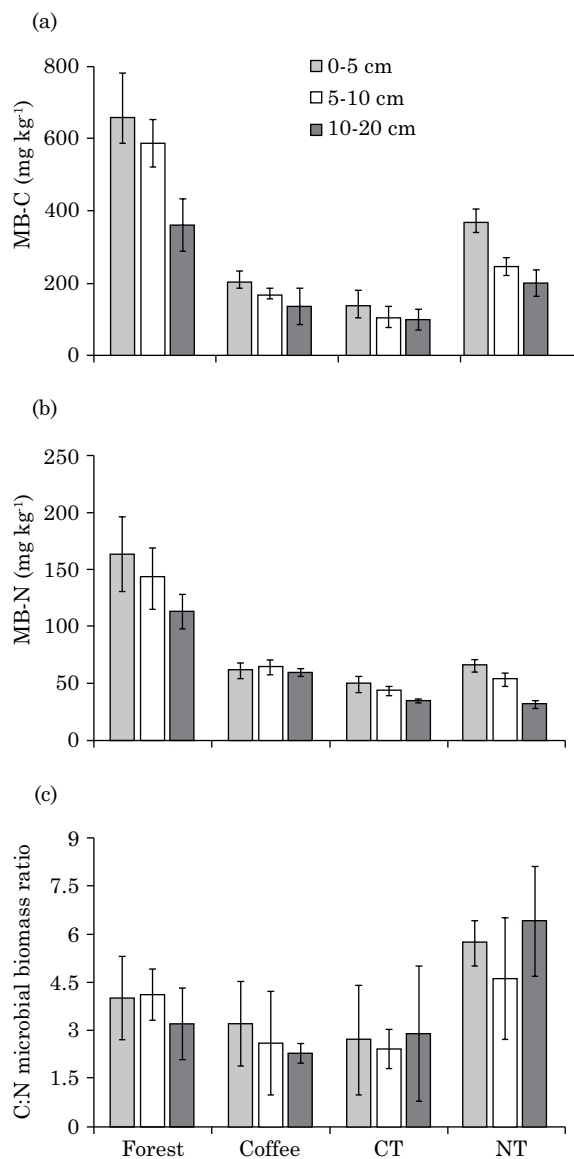
Microbial biomass-Carbon (MB-C) and microbial biomass-Nitrogen (MB-N) varied significantly for each land use ( $p \leq 0.001$ ) and layer ( $p \leq 0.05$ ). About 40 % of the total MB-C observed in the 0-20 layer for FO, CO and CT was concentrated in the 0-5 cm layer, and a similar proportion was observed in NT

(45 %). For the total MB-N in the 0-20 layer, about 39 % was concentrated in the 0-5 cm layer for FO and CT, 30 % for CO and 44 % for NT. The FO site had the highest MB-C in all layers evaluated, while the lowest values were observed in CT (Figure 2a). The same pattern was evident in terms of MB-N (Figure 2b).

Different land use/management had a significant effect on microbial biomass C:N ratio (C:N MB) ( $p \leq 0.004$ ), contrary to soil layer. The highest C:N



**Figure 1.** Mean weight diameter (a) and aggregate stability index (b) of a Brazilian Oxisol 24 years after forest conversion to a perennial crop with coffee or annual grain crops (maize and soybeans) in conventional tillage or no-tillage from three layers (0-5, 5-10 and 10-20 cm). Average of three replications. Bars indicate standard deviation.



**Figure 2.** Microbial biomass C (MB-C) (a), and N (MB-N) (b) and C:N microbial biomass ratio (c) of a Brazilian Oxisol 24 years after forest conversion to a perennial crop with coffee or annual grain crops (maize and soybeans) in conventional tillage or no-tillage from three layers (0-5, 5-10 and 10-20 cm). Average of three replications. Bars indicate standard deviation.



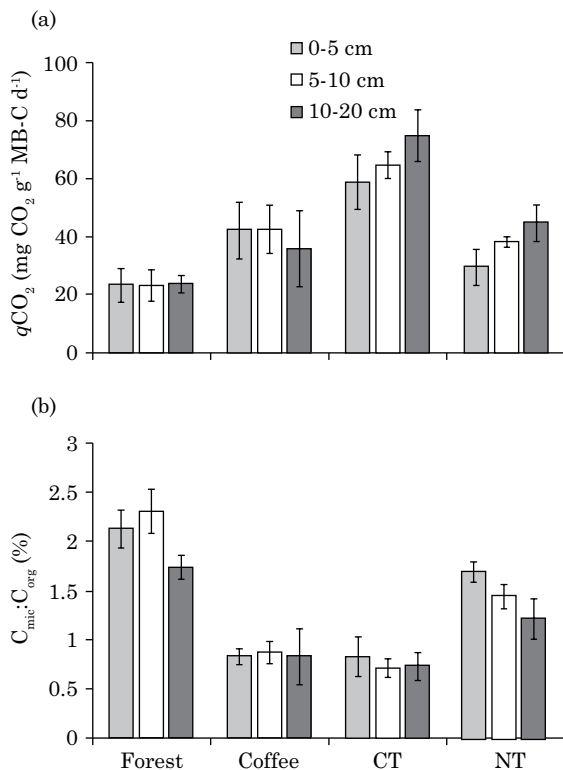
MB ratios occurred in NT (Figure 2c). The C:N MB ratio declined with depth (from 0-5 to 10-20 cm) in FO and CO about 20 and 28 %, respectively.

Metabolic quotient ( $qCO_2$ ), which represents the ratio of soil respiration per unit of MB-C, was only significantly affected by soil layer ( $p \leq 0.006$ ) (Figure 3a). It approached significance ( $p \leq 0.07$ ) with respect to land use. Metabolic quotient decreased (about 14 %) with depth in CO, but increased with depth in CT and NT (27 and 52 %, respectively). Averaged across depths,  $qCO_2$  increased 59 % for NT, 71 % for CO and 182 % for CT compared to FO.

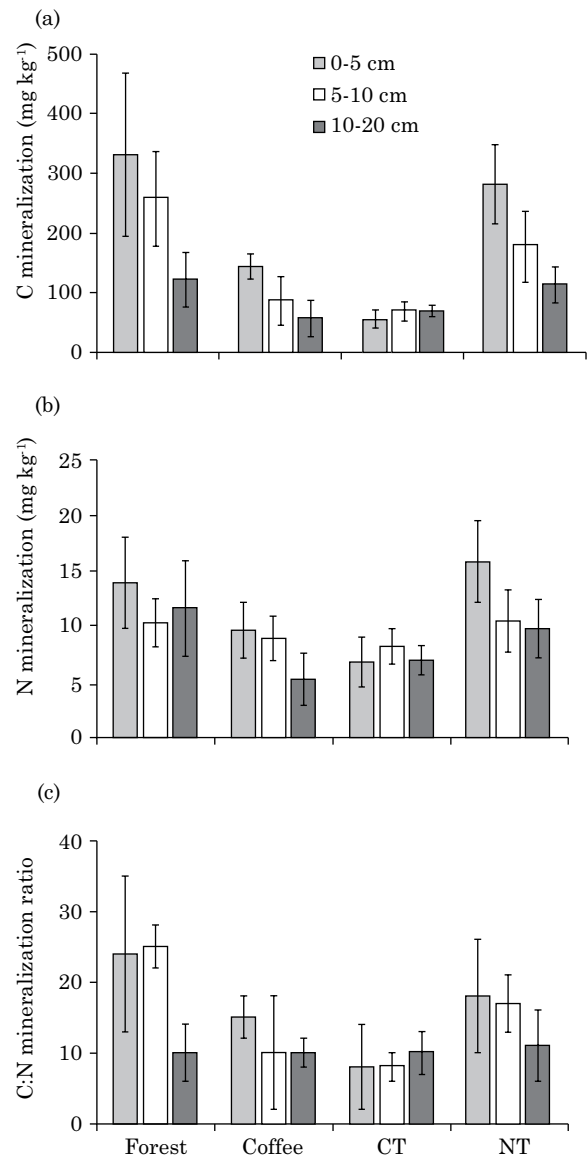
The ratio of microbial C to organic C ( $C_{mic}:C_{org}$ ) was affected by soil management ( $p \leq 0.001$ ) and layer ( $p \leq 0.002$ ) (Figure 3b). Forest soil had a higher  $C_{mic}:C_{org}$  ratio than other uses/managements, while the lowest values occurred in CO and CT. Averaged across land use/management treatment, the  $C_{mic}:C_{org}$  decreased with depth.

Carbon mineralization ( $C_{MIN}$ ) (Figure 4a) and nitrogen mineralization ( $N_{MIN}$ ) (Figure 4b) varied significantly at each site for land use ( $p < 0.001$ ) and

layer ( $p < 0.05$ ). About 48 % of the  $C_{MIN}$  observed in the 0-20 cm layer for FO, CO and NT was concentrated in the 0-5 cm layer, while about 28 % of the  $C_{MIN}$  in CT occurred in the 0-5 cm layer. For  $N_{MIN}$  in the 0-20 cm layer, about 40 % was concentrated in the 0-5 cm layer for FO, CO and NT, while about 31 % of  $N_{MIN}$  in CT occurred in the 0-5 cm layer. The C:N mineralization ratios varied from 8 to 25 (Figure 4c). Land use/management ( $p < 0.001$ ) but not layer ( $p < 0.825$ ) had a significant effect on this property.



**Figure 3.** Metabolic quotient ( $qCO_2$ ) (a) and microbial carbon to organic carbon ( $C_{mic}:C_{org}$ ) ratio (b) of a Brazilian Oxisol after forest conversion to a perennial crop with coffee or annual grain crops (maize and soybeans) in conventional tillage or no-tillage from three layers (0-5, 5-10 and 10-20 cm). Average of three replications. Bars indicate standard deviation.



**Figure 4.** Mineralization of carbon (a) and nitrogen (b) during 24 days, and C:N mineralization ratio (c) of a Brazilian Oxisol after forest conversion to a perennial crop with coffee or annual grain crops (maize and soybeans) in conventional tillage or no-tillage from three layers (0-5, 5-10 and 10-20 cm). Average of three replications. Bars indicate standard deviation.

## Multivariate analyses

The changes in soil microbial properties in response to different land use/management and soil layer, according to the NMS ordination method (Figure 5). Most of this variability was associated with Axis 1 (88.6 %), which was positively correlated with MB-C, MB-N, BR,  $C_{\text{MIN}}$ , and  $N_{\text{MIN}}$  (Table 3). Axis 2 accounted for about 9 % of the variability in the data from five microbial properties that correlated with only MBN and  $N_{\text{MIN}}$ . NMS Axis 1 of soil microbial properties shows a clear separation among the treatments, displaying a gradient from CT to FO (Figure 5a). The NMS Axis 1 representation of five variables related to soil microbiota also showed a gradient increase from deepest (10-20 cm) to shallowest (0-5 cm) layers.

When all treatments and depths were analyzed, PERMANOVA showed that soil microbial properties were significantly affected by land use/management ( $F = 25.6, p = 0.001$ ) and soil layer ( $F = 7.59, p = 0.003$ ). The pairwise comparisons between land use/management and soil layer were all significant ( $p < 0.05$ ) except for the comparisons between land use/managements CO vs. CT and 0-5 vs. 5-10 cm layers.

The DistLM analysis showed that soil microbial variables were significantly related to soil physical and chemical properties ( $F = 10.79, p = 0.001$ ). MB-C and MB-N together contributed with 77 % of the observed data variability among the treatments in terms of microbial variables (Table 4). Among the soil physical and chemical properties,  $C_{\text{org}}$ , MWD and  $N_{\text{tot}}$  together accounted for 85 % of the land use/management variability (Table 4). Overall, significant positive correlations were found between the most important soil microbial (MB-C and MB-N) and soil physical and chemical (MWD,  $C_{\text{org}}$ , MWD and  $N_{\text{tot}}$ ) variables (Table 5).

## DISCUSSION

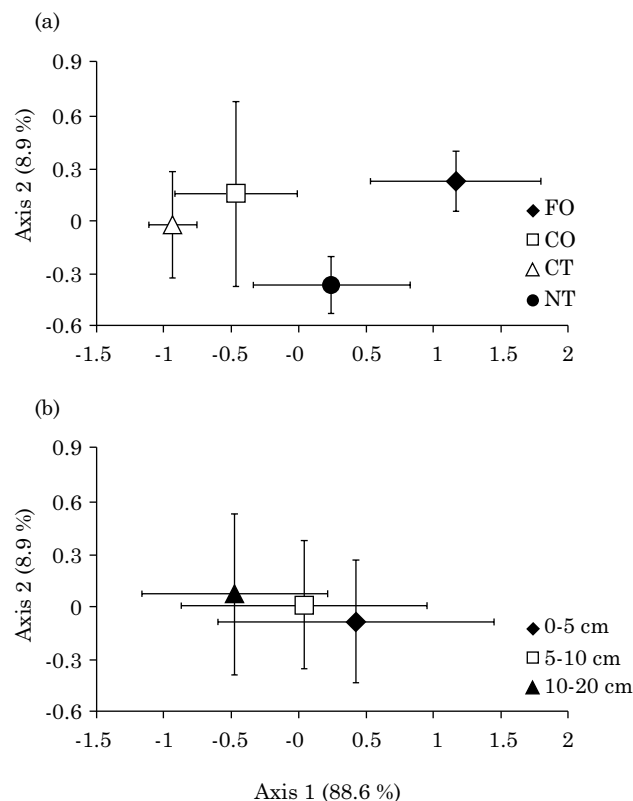
The soil pH at the FO site remained relatively unchanged throughout this period. Soil acidification in CO is greater than at the other cultivated sites, due to various factors: the high amount of fertilizer applied, leaching of base cations and coffee physiology, which absorbs more cations than anions. In addition, the soil was sampled near the edge of the tree canopy, where the fertilizers were broadcasted, most coffee roots are found and the acidification processes are more intense. Although the CEC of the CO site was initially lower than at the others, by the time of sampling it was numerically higher. This is most likely a consequence of continuous lime application to buffer the pH in relation to the FO site, as well as of higher  $C_{\text{org}}$  than at the other cultivated sites.

The higher values of MWD and ASI in FO observed in this study are consistent with those

**Table 3. Correlation coefficients (r) between soil microbial properties and NMS axis**

Variable <sup>(1)</sup>	NMS Axis 1	NMS Axis 2
MB-C	0.957***	0.097
MB-N	0.811***	0.519***
BR	0.841***	-0.089
$C_{\text{MIN}}$	0.878***	-0.180
$N_{\text{MIN}}$	0.695***	-0.362*

<sup>(1)</sup> MB-C: microbial biomass carbon; MB-N: microbial biomass nitrogen; BR: basal respiration;  $C_{\text{MIN}}$ : carbon mineralization;  $N_{\text{MIN}}$ : nitrogen mineralization. \*\*\* and \*:  $p < 0.001$  and  $p < 0.05$ , respectively.



**Figure 5. Changes in soil microbial properties after forest conversion to a perennial crop with coffee or annual grain crops (maize and soybeans) in conventional tillage or no-tillage (a) and soil layer (b), according to NMS ordination method. The symbols represent the geometric mean of soil microbial properties in each treatment, while the vertical and horizontal lines indicate  $\pm 1$ SD. 2D stress = 6.96. FO: Forest; CO: Coffee; CT: Conventional Tillage; NT: No-Tillage.**

observed by Castro Filho et al. (2002) for the same cultivated area. Greater MWD and ASI in FO and NT were expected because there are conditions to increase addition of  $C_{\text{org}}$  and, consequently,

**Table 4. Contribution of significant ( $p \leq 0.05$ ) soil microbial, physical and chemical variables to data variability according to DistLM analysis**

Variable <sup>(1)</sup>	Contribution	Cumulative
	%	
Microbial		
MB-C	67.51	67.51
MB-N	9.37	76.88
C <sub>MIN</sub>	5.82	82.71
N <sub>MIN</sub>	5.47	88.18
BR	1.60	88.78
Physical and chemical		
OC	46.18	46.18
Total N	14.33	84.64
pH(H <sub>2</sub> O)	7.43	92.07
CEC	3.95	96.02
MWD	24.14	70.31
ASI	2.51	98.53

<sup>(1)</sup> MB-C: microbial biomass carbon; MB-N: microbial biomass nitrogen; C<sub>MIN</sub>: carbon mineralization; N<sub>MIN</sub>: nitrogen mineralization; BR: basal respiration; OC: organic carbon; Total N: total nitrogen; pH in water; CEC: cation exchange capacity; MWD: mean weight diameter; ASI: aggregate stability index.

**Table 5. Pearson's correlation coefficients between soil microbial, physical and chemical properties 24 years after forest conversion to a perennial crop with coffee or annual grain crops (maize and soybeans) in conventional tillage or no-tillage**

Variable <sup>(1)</sup>	MWD	ASI	OC	Total N	CEC	pH(H <sub>2</sub> O)
MB-C	0.34*	0.60*	0.87*	0.61*	0.44*	-0.05
MB-N	0.18	0.44*	0.80*	0.55*	0.41*	-0.26
BR	0.28	0.51*	0.74*	0.59*	0.42*	0.14
C <sub>MIN</sub>	0.39*	0.49*	0.75*	0.57*	0.48*	0.05
N <sub>MIN</sub>	0.34*	0.37*	0.60*	0.42*	0.29	0.18

\*:  $p \leq 0.05$ . <sup>(1)</sup> MB-C: microbial biomass carbon; MB-N: microbial biomass nitrogen; BR: basal respiration; C<sub>MIN</sub>: carbon mineralization; N<sub>MIN</sub>: nitrogen mineralization; MWD: mean weight diameter; ASI: aggregate stability index; OC: organic carbon; Total N: total nitrogen; CEC: cation exchange capacity; pH in water.

increasing microbial activity in these land uses, which would produce more organic binding agents and microbial biomass that contribute to the formation and stability of soil aggregates (Six et al., 2004; Green et al., 2007; Caesar-TonThat et al., 2011; Guillou et al., 2012). The proportion of labile C<sub>org</sub> that is physically protected from microbial decomposition may be increased in macroaggregates, thus enhancing microhabitats for microorganisms (Caesar-TonThat et al., 2011; Guillou et al., 2012). Aggregate stability, which expresses the resistance of soil structural aggregates to dispersion when

wetted, is an important soil quality property, because it influences many soil functions and reflects an interrelationship among biological, chemical and physical soil properties (Islam and Weil, 2000).

Our results agree with previous studies that soil management affects significant soil properties such as microbial mass and activity (Islam and Weil, 2000; Hungria et al., 2009; Frazão et al., 2010; Balota and Auler, 2011). The microbial biomass measured in FO and NT indicated the same trend as other studies, which show a significant positive effect of decreasing soil disturbance on microbial biomass (Balota et al., 2004; Frazão et al., 2010; Kaschuk et al., 2010). The generally higher microbial biomass under FO and NT compared to CT, especially in tropical/subtropical climates, could be attributed to several factors, such as lower temperature, higher moisture content, greater aggregation and higher C content. The lack of a major disturbance event under FO likely favors the steady supply of C<sub>org</sub> to support the microbial community, while in CT, the temporary flush of microbial activity with each tillage event results in large losses of C as CO<sub>2</sub>.

Although CO had higher C<sub>org</sub> compared to NT and CT, the microbial biomass was low, as previously observed by Marchiori Junior and Melo (2002) in which a coffee site had half the MB-C of forest and maize sites and about 30 % of that present in sugarcane. The low value of MB-C under CO could be due to higher soil acidity and Al toxicity. Repeated applications of Cu fungicides for a long period cause Cu accumulation in soil (Nachtigall et al., 2007) which may decrease microbial activity. In CO, long-term fertilizer use can improve soil nutrient status, but can also increase soil acidification, by repeated N fertilizer applications (Aciego Pietri and Brookes, 2008). According to the same authors, soil acidity combined with high Al content may also limit microbial growth, in a mechanism similar to that caused by heavy metals, which decrease the efficiency of C residue conversion into microbial biomass C.

A change in the microbial biomass C/N ratio due to different land use/management may reflect a change in the pattern of microbial nutrient immobilization. Soil microbial biomass is a composite of several groups of organisms. Each microbial group has a different C/N ratio and the predominance of one group may result in the prevalence of a particular ratio, e.g. a range of 4.5-15 for fungi *vs.* three to six for bacteria. Therefore, a change in microbial biomass C/N ratio may occur as a result of changes in microbial populations during the decomposition of crop residue.

Litter incorporation due to tillage may reduce the microbial biomass C/N ratio as observed in the present study in CT. On the other hand, the wider microbial biomass C/N ratio in FO, and particularly NT, suggests that these sites might have a greater proportion of fungi than bacteria compared to CO

and CT (Frey et al., 1999). Reduced soil disturbance may also favor establishment and preservation of fungal hyphal networks with higher C/N ratio. Although the fungi to bacteria dominance under NT system is broadly accepted by the scientific community, Strickland and Rousk (2010) have suggested research addressing the mechanism by which the eventual shift may occur, because recent studies have shown that NT enhances both fungal and bacterial biomass. For example, a metagenomic study comparing NT and CT in a similar soil of Londrina, the dominance of bacteria in CT was not confirmed (Souza et al., 2013). Independent of shifts in the fungi-to-bacteria ratio, the changes in the microbial biomass C/N ratio may also affect the rate of immobilization and mineralization of soil nutrients, as more nutrients are stored in microbial biomass.

Although FO and NT had higher microbial biomass than CO and CT, the  $q\text{CO}_2$  was lower. The inverse relationship between MB-C and  $q\text{CO}_2$  is commonly observed in reduced tillage systems. For example, Balota et al. (2004), studying different crop rotations and sampling seasons in the same area, observed that no-tillage had 56 % higher MB-C and 68 % lower  $q\text{CO}_2$  than conventional tillage. These differences may be due to differences of microbial accessibility to C substrates, changes in the metabolic rates, changes in microbial community composition and proportion of recalcitrant material (Islam and Weil, 2000). Environmental variation determines changes in microbial composition and physiological status. No tillage may also have a greater proportion of larger aggregates, which was observed in our study, which protect microorganisms from adverse conditions (Green et al., 2007; Caesar-TonThat et al., 2011) and permit more efficient C use.

Generally,  $q\text{CO}_2$  decreases in more stable systems, suggesting more efficient C use by the microbial community (Anderson and Domsch, 2010). The lower  $q\text{CO}_2$  values may indicate that FO and NT are more stable systems, encouraging a shift toward fungal-dominated microbial communities. Therefore,  $q\text{CO}_2$  may be expected to decline, as more C-efficient fungi come to dominate soil communities in certain soil conservation managements. In this context, the high value of  $q\text{CO}_2$  for CT suggests that soil practices such as tillage decrease the efficiency of C substrate use by the soil microbial community. The same effect under CO may be due to low pH and higher Al content. Greater acidity and potential Al toxicity requires greater effort by the microbial community to repair damage due to stress (Anderson and Domsch, 2010). Under field conditions, when liming reduces acidity, a more diverse and efficient microbial community can be established (Aciego Pietri and Brookes, 2008). The increase of  $q\text{CO}_2$  with depth (at both CT and NT sites) may also be due to an increasing proportion

of recalcitrant organic substrate, as suggested by Islam and Weil (2000).

Microbial C pools typically decline at a faster rate than soil organic matter, and the  $C_{\text{mic}}:C_{\text{org}}$  percentage will decrease during soil degradation (Islam and Weil, 2000; Anderson and Domsch, 2010; Balota and Auler, 2011), a trend observed in our study. This may allow for a calibrated early soil quality indicator to predict whether soils are accumulating or losing soil C. This relationship also allows comparisons across soils with different organic matter contents. The  $C_{\text{mic}}:C_{\text{org}}$  ratio could be an indicator of conversion efficiency of  $C_{\text{org}}$  into  $C_{\text{mic}}$  and losses of soil C during decomposition. This indicator may reveal the microbial community capacity to enrich the microbial C pool under conservation management (Islam and Weil, 2000; Balota et al., 2004; Frazão et al., 2010; Kaschuk et al., 2010; Balota and Auler, 2011; Pellegrino et al., 2011). The  $C_{\text{mic}}:C_{\text{org}}$  ratio was higher in FO followed by NT presumably because it maintained more labile organic substrates in the soil, which allowed higher MB-C per unit of soil  $C_{\text{org}}$ . The result observed in NT indicates a shift in  $C_{\text{org}}$  equilibrium toward less loss of labile soil organic C than in CO and CT. As a result, this indicates higher quality soil in NT, closer to that of FO, which is more biologically active than in CO and CT, which are therefore considered inferior soils.

In FO and NT,  $C_{\text{MIN}}$  and  $N_{\text{MIN}}$  were, respectively, more than 200 % and about 64 % higher than at the CT site. The great difference in  $C_{\text{MIN}}$  and  $N_{\text{MIN}}$  among sites suggested differences in labile soil  $C_{\text{org}}$  and N accumulated, due to soil management and crop species, as previously observed (Franzluebbbers and Stuedemann, 2008; Balota and Chaves, 2010). A broad range of  $C_{\text{MIN}}$  are reported in the literature, ranging from 2 to 70  $\mu\text{g C g}^{-1} \text{d}^{-1}$  according to Franzluebbbers (2009) who compiled data for 33 published studies. Although in some studies CT has higher  $C_{\text{MIN}}$  than NT, the conservation system has, on average, 50 % higher  $C_{\text{MIN}}$ . These results suggest that soil use/management causes different decomposition rates of organic matter and variation in the microbial biomass and activity, with consequent differences in substrate availability.

The C:N mineralization ratio showed significant effects due to land use. There was a wider C:N mineralization ratio in FO and NT than CO and CT, which may have been due to microbial populations using organic matter with wide C:N ratios. A low soil mineralizable C:N ratio may suggest that the flow of C and N through the mineralizable fraction has become less stable - more dependent on the microbial biomass turnover - which may decrease conservation of C and N in the long term. The C:N mineralization ratio can be used as an index of labile substrate availability. Our results are in agreement with previous study (Balota and Chaves, 2010), that demonstrate the sensitivity

of soil organic quality (potential mineralization) to changes in soil use/management.

Five microbial properties were selected for multivariate analysis. Each was highly correlated with the principle NMS ordination axis and in sum they clearly distinguished the different land uses. Soil depth had apparent but much less separation, probably because the depth increments were small. Despite being a master variable, soil pH was poorly correlated with the microbial soil quality parameters, probably because the pH range at the sites was relatively small. Other physical and chemical parameters were better correlated with the microbial measures.

The results of the DistLM analysis in the partitioning of microbial, chemical and physical soil quality parameters to separate land use types were extraordinary. Three physical and chemical properties ( $C_{org}$ , MWD and total N) contributed with 85 % of the variability while only four microbial variables (microbial biomass C and N and mineralizable C and N) contributed with 88 % of the variability. The implication of the results, in agreement with the evidence from  $qCO_2$  microbial biomass C/N ratios, and distribution of  $C_{org}$  in soil aggregates suggests that improvements of the physical environment for microbial population are critical to enhance soil quality. Conservation soil management practices such as no-tillage enhance each of these properties for annual crop production in conventional tillage systems. Consequently, C and N are more labile and have higher contents, are contained in larger more stable aggregates, and ultimately make the soil more similar to the original forest soil environment.

## CONCLUSIONS

Different land use/management had significant effects on soil biological, physical and chemical properties.

The reduction of soil disturbance is important for soil carbon content and microbial activity, but the control of soil pH and aluminum toxicity is also important to maintain the soil microbial activity high.

Multivariate analysis showed that land use/management could be separated based on five readily measured soil quality properties (microbial biomass C and N, mineralizable C and N and basal respiration).

These microbial measures showed no-tillage management more closely associated with the original forest soil than more intensive cropping systems such as perennial coffee cultivation or annual crop managed in conventional tillage.

## ACKNOWLEDGEMENTS

The authors are grateful to Dr. M. A. P. for his helpful suggestions. E. L. B. acknowledges CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico (National Counsel of Technological and Scientific Development) and the Program Science without Borders, for a postdoctoral fellowship at the University of Kentucky, United States (Process 201930/2012-9).

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