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Scientific Article

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Microbial soil quality indicators under different crop rotations and tillage management[1]

Indicadores microbianos da qualidade do solo sob diferentes rotações de culturas e manejo do solo

Enderson Petrônio de Brito Ferreira[2]*, Henrique Pereira dos Santos[3], Janaina Ribeiro Costa[4], Helvécio De-Polli[5] e Norma Gouvêa Rumjanek[4]

Abstract - An experiment was carried out under field conditions to assess the effects of soil management (no-tillage- NT and conventional tillage- CT) and crop rotation systems on microbial biomass-C (C_{mic}), basal soil respiration (BSR), metabolic quotient (qCO_2), soil organic carbon content (C_{org}) and microbial carbon to organic carbon ratio (C_{mic}/C_{org}). Soil samples were collected on an area cultivated with wheat as winter crop and soybean as summer crop, both in rotation with vetch, maize and oats. Samples were also collected in a secondary forest used as reference. Data of each management system (NT and CT) were compared to forest area by "t" test ($p<0.05$) and crop rotations were compared by Tukey test ($p<0.05$). All data were submitted to multivariate analysis (Principal Component Analysis - PCA). There were observed significant differences ("t" test; $p<0.05$) for C_{mic}, BSR, qCO_2 and C_{mic}/C_{org} between NT and CT, by which NT values resemble those for forest area. For crop rotations significant differences (Tukey test; $p<0.05$) were found only for BSR and qCO_2. The sum of the two first principal components on the PCA explained about 75% of the data variation. PCA showed NT closest to forest area than CT, especially treatments with soybean and vetch as consecutive crops. The forest area-NT clustering was mostly due to C_{mic} and C_{mic}/C_{org} relationship. Results indicate that the NT system is more sustainable than the CT system and can contribute for the accumulation a greater quantity of carbon in soil.

Key words - Microbial biomass. Microbial respiration. Organic matter. Soil management.

Resumo - Foi conduzido um experimento sob condições de campo para avaliar o efeito da forma de manejo do solo (Plantio direto - PD e plantio convencional - PC) e dos sistemas de rotação de culturas sobre o carbono da biomassa microbiana (C_{mic}), respiração basal do solo (RBS), quociente metabólico (qCO_2), carbono orgânico total (C_{org}) e relação carbono da biomassa microbiana: carbono orgânico total (C_{mic}/C_{org}). As amostras de solo foram coletadas em uma área cultivada com trigo como cultura de inverno e soja como cultura de verão, ambas em rotação com ervilhaca, milho e aveia preta. Amostras de solo também foram coletadas em uma floresta secundária usada com referência. Os dados de cada sistema de manejo do solo (PD e PC) foram comparados com a floresta secundária pelo teste "t" ($p<0.05$) e as rotações de culturas foram comparadas pelo teste de Tukey ($p<0.05$). Todos os dados foram submetidos a uma análise multivariada (Análise de Componentes Principais - ACP). Foram observadas diferenças significativas (teste "t"; $p<0.05$) para C_{mic}, RBS, qCO_2 e C_{mic}/C_{org} entre PD e PC, em que estes valores foram semelhantes àqueles encontrados na floresta. Entre as rotações de culturas foram observadas diferenças significativas (teste de Tukey; $p<0.05$) apenas para RBS e qCO_2. A análise de componentes principais mostrou que o PD aproximou-se mais da floresta do que o PC, especialmente nos tratamentos em que soja e ervilhaca faziam parte da rotação de culturas antes do trigo. A formação do agrupamento entre floresta e PD ocorreu principalmente devido ao C_{mic} e à relação C_{mic}/C_{org}. Os resultados indicam que o PD apresenta maior sustentabilidade que o PC, podendo contribuir para o acúmulo de uma grande quantidade de carbono no solo.

Palavras-chave - Biomassa microbiana. Respiração microbiana. Matéria orgânica. Manejo do solo.

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* Corresponding author

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[2] Pesquisador da Embrapa Arroz e Feijão, Rodovia GO-462, km 12, Caixa Postal 179, Santo Antônio de Goiás-GO, Brasil, 74375-000, enderson@cnpaf.embrapa.br

[3] Pesquisador da Embrapa Trigo, Rodovia BR 285, km 294, Passo Fundo-RS, Brasil, 99001-970, hpsantos@cnpt.embrapa.br

[4] Pesquisadora da Embrapa Agrobiologia, Rodovia BR 465, km 7, Seropédica-RJ, Brasil, 23890-000, janaina@cnpab.embrapa.br, norma@cnpab.embrapa.br

[5] Pesquisador da Embrapa Agrobiologia, Rodovia BR 465, km 7, Seropédica-RJ, Brasil, 23890-000, depolli@cnpab.embrapa.br

Introduction

The use of conventional system combined with excessive use of fertilizers and pesticides leads to loss in health and soil quality. Besides, depletion of soil organic carbon and associated nutrients in soil organic matter and intensive tillage causes severe negative impacts to the environment, by subjecting bare soil to erosive processes. Soil degradation and contamination of underground water, may also occur from residues of pesticides and fertilizers widely employed under this agricultural model. Oldeman (1994) has pointed out that this model of soil exploitation results in environmental degradation that goes far beyond its simple ecological aspects, which leads to irreversible environmental damage with destruction of fauna and flora and loss of agricultural soil (LEONARDOS et al., 2000), and greenhouse gases emission (ELDER; LAL, 2008).

According to Batjes (1998), the global average of C liberation to the atmosphere in the 1980s was 7.1 ± 1.1 Gt C year^{-1}, from which, 1.6 ± 1.0 Gt C year^{-1} was due to agriculture in tropical regions and 5.5 ± 0.5 Gt year^{-1} due to the burning of fossil fuels. Similar amounts were described for the 1990s where the total emission was estimated to be 7.0 Gt year^{-1} of C, from which, 5.5 Gt year^{-1} was due to fossil fuel burning and 1.2 Gt year^{-1} was due to agricultural soil use and deforestation (International..., 1996 cited by RESCK, 2001).

Joint scientific efforts have been focused on the development of systems capable of maintaining high yields while promoting greater sustainability to the agroecosystem through the use of alternative soil management practices, improved varieties and crop rotations. However, irrespectively of the adopted system, soil management will always result in changes in some soil characteristics. These variations have an important effect on the soil microbial community (GUNDALE et al., 2005) which in turn affects agroecosystem function and consequently crop yield.

Besides the influence of soil management on total microbial biomass, it also affects differentially the biomass activity resulting in greater or smaller CO_2 efflux. Nowadays the trend is towards soil management practices capable of increasing the immobilization of atmospheric CO_2. According to Hermle et al. (2008), it may be possible to manipulate paddy soil through conservational tillage and crop practices, and thereby maintain adequate soil organic matter concentrations, and mitigate soil organic carbon loss from soil to atmosphere, which increases C content and microbial activity (FRANCHINI et al., 2007; MOSCATELLI et al., 2005; ROLDÁN et al., 2005).

Soil microbial communities are influenced by many factors as soil management and cover crops (CARRERA et al., 2007); kind of fertilizer and its applying way (CARRERA et al., 2007); plant development stage and cultivars (FERREIRA et al., 2008) as well as pesticides (FERREIRA et al., 2009). Thus, microbial properties allied to the total organic C content can be used to evaluate the sustainability of agricultural production. These properties are described as biological indicators capable of detecting changes in soil quality and its biological properties (NOGUEIRA et al., 2006). High contents of organic C on the soil surface is an important factor contributing to the C_{mic} content in soil (VARGAS; SCHOLLES, 2000) which has been reported as a key factor for microbial community growth (MOSCATELLI et al., 2005).

This work aimed to determine the effects of soil management and crop rotation systems on soil microbial biomass and organic carbon and to verify the use of these parameters as indicators of soil quality.

Material and methods

This study was carried out in a long-term experiment conducted since 1996 at the Experimental Station at the National Wheat Research Center (Embrapa) in Passo Fundo, Rio Grande do Sul State, Brazil (28º12’56” S and 52º23’43” W, altitude 684m). The soil at the site is an Haplic ferralsol (10 g kg^{-1} of soil of coarse sand; 230 g kg^{-1} of soil of fine sand; 130 g kg^{-1} of soil of silt; 630 g kg^{-1} of soil of clay).

Crop rotations were evaluated in 2 management systems: no-tillage (NT) and conventional tillage (CT) where a disc plough was used. Crop rotations consisted of wheat (*Triticum aestivum* L.) and soybean (*Glycine max* (L.) Merrill) as the main crops for winter and summer, respectively, and vetch (*Vicia sativa* L.), maize (*Zea mays* L.) and oats (*Avena sativa* L.) as secondary crops for the rotation system. Three different rotation were used: 1- wheat/soybean (W/S); 2- wheat/soybean/vetch/maize (W/S/V/M); 3- wheat/soybean/vetch/maize/oats/soybean (W/S/V/M/O/S), in a way that all possible combinations had been cultivated at the same cropping season, resulting in 6 subplots per management system for each replicate. The experiment was carried out in a split-plot randomized block design with 3 replicates. The management systems (NT and CT) were the main plots and the subplots were the crop rotation treatments.

Composite samples consisting of 6 sub-samples per plot were taken at 0-10 cm depth. Soil sampling was also carried out in a neighboring secondary forest area (Araucaria woodland).

Microbial biomass-C (C_{mic}) and basal soil respiration (BSR) were performed by the use of three 20 g sub-samples derived from composite soil samples at 60% of field capacity. They were then homogenized and sieved through a 2 mm sieve. Microbial biomass-C (C_{mic}) evaluation was carried out by fumigation-extraction method (VANCE et al., 1987) using soil:extract solution ratio of 1:2.5 and a correction factor (kc) of 0.33 (TATE et al,. 1988). For the fumigation step 1 mL of ethanol-free chloroform was added to each sub-sample flask of 100 mL, kept tight closed, and after incubation of 24 hour the chloroform was allowed to evaporate in the fume hood for 60 min. After that, the extraction and titration were performed according to Vance et al. (1987). Basal soil respiration (BSR) was determined according to Jenkinson and Powlson (1976) where three 20 g sub-samples of soil were placed in a 3 L flask and incubated for 5 days at 28 ^{o}C. CO_2 was trapped in a 100 mL flask containing 10 mL of NaOH (1 M). After this period, 2 mL of $BaCl_2$ (10%) was used to precipitate the CO_2 and the excess NaOH was titrated using HCl (0.5 M).

The specific microbial respiration, or metabolic quotient (qCO_2), expresses the C-CO_2 evolved per unit of microbial biomass and time. Metabolic quotient was calculated according to the procedures described by Anderson and Domsch (1990) and expressed as mg C g C_{mic}^{-1} h^{-1}.

Organic carbon content (%C_{org}) was determined by potassium dichromate oxidation. The ratio of microbial biomass-C to organic carbon (C_{mic}/C_{org}) was calculated according to Jenkinson and Ladd (1981).

Data were submitted to analysis of variance and significant differences were determined by Tukey's test at $p<0.05$. To compare data from NT, CT and the forest area, a 't' test was used at $p<0.05$ and values found for forest area were used as a reference. Previously to variance analysis, data were submitted to Lilliefors and CoChran-Bartlett tests to verify its normality and homogeneity of variances using the software SAEG v. 7 (EUCLYDES, 1982). Comparisons between forest area and both NT and CT treatments should be done with care as the forest area was not part of the experimental design. A principal component analysis (PCA) was performed using the software CANOCO v. 4.5 (ter BRAAK; SMILAUER, 2002), for data obtained for all the parameters studied involving a matrix of 13 lines by 5 columns, representing treatments and parameters, respectively. The determination of the number of principal components used for the treatments clustering was defined according to Jollife (2002), since the sum of the first 2 principal components (PC1 and PC2) was greater than 70%.

Results and discussion

Table 1 shows C_{mic}, BSR, qCO_2, C_{org} and C_{mic}/C_{org} values obtained for NT, CT and forest area. Mean test between NT and CT was performed by Tukey´s test, and forest area was compared separately with each management systems (NT and CT) by "t" test. Significant differences between NT and CT were observed to C_{mic}, BSR, qCO_2 and C_{mic}/C_{org}. Values of C_{mic} and C_{org} of forest area were greater than NT and CT, and the C_{mic}/C_{org} of forest area was greater than CT but not statistically different of NT (Table 1). Fialho et al. (2006) did not found significant difference for C_{mic} between cultivated and forest area, however forest area showed a tendency to present greater values of C_{mic} than cultivated area.

C_{mic} value for NT was greater than CT and closer to forest area, suggesting that this system could promote a greater sustainability level compared to CT. Furthermore NT increases the litter accumulation rate on the soil surface which improves both the C content and microbial activity (MOSCATELLI et al., 2005; ROLDÁN et al., 2005), indicating a close association between these results and the low impacts to the environment caused by the NT system (HERMLE et al., 2008).

BSR did not show significant difference among soil management systems (NT and CT) and forest area, however NT was statistically different of CT (Table 1). The greatest values of BSR were expected in the treatments with the greatest C_{mic} values, such as NT and forest area, that compared to CT promoted greater CO_2 efflux. According to Zornoza et al. (2007), BSR shows a close relation to abiotic soil conditions such as temperature and humidity. Besides, the greatest quantity of organic material in the forest area

Table 1 - C_{mic} (mg C kg^{-1} soil), BSR (mg C-CO_2 kg^{-1} soil $hour^{-1}$), qCO_2 (mg C-CO_2 g^{-1} C_{mic} $hour^{-1}$), C_{org} (%) and C_{mic}/C_{org} ratio on a long-term experiment under NT, CT and forest area within 0-10cm depth

Treatments	Cmic	BSR	qCO2	Corg	Cmic/Cor
CT	97.22 b*	0.10 b	1.07 a	1.21 a*	0.86 b*
NT	153.39 a*	0.15 a	0.99 b	1.15 a*	1.34 a
Forest area	263.79	0.23	0.99	1.40	1.92

Same letters means no significant differences among treatments in the same column (Tukey's test *$p<0.05$*) and asterisk means significant difference between each treatment and forest area ("t" test *$p<0.05$*)

and NT reflects upon the decomposing microbial activity and, consequently on the BSR growth, as stated by Vargas and Scholles (2000) that found a correlation between the soil warming and substrate availability with BSR.

Significant differences in qCO_2 values were observed to NT system when compared to CT. However, both treatments were not different to forest area (Table 1). Compared to CT, NT showed lowest qCO_2 value and, consequently, greater sustainability because the soil microbial population under NT was less stressed, indicating the lowest relative loss of CO_2, which, in the long-term can translate into a greater accumulation of C in the soil (FRANCHINI et al., 2007). Besides, a low qCO_2 indicates a high quality of the substrate used by microorganisms or a low microbial maintenance requirement (SARMIENTO; BOTTNER, 2002). In addition, even with the lowest content of C_{mic}, CT can promote greater qCO_2 values due to differences in the substrate accessibility by the microorganisms, metabolic patterns changing or by the alteration of the soil microbial composition (ALVAREZ et al., 1995). According to Dilly et al. (2001), lowest values of qCO_2 are normally found under forest vegetation compared with cultivated areas because forest systems show greater stability and, therefore lower disturbance on the soil microbial community.

C_{org} content under NT and CT showed a significant difference compared with the forest area, however, under the present study conditions it was not possible to verify significant difference in C_{org} content between NT and CT management practices (Table 1). This result may be possible because C_{org} assessment was only performed on the soil surface (0-10 cm depth), and differences on C_{org} contents may occur on the whole soil profile, as stated by Sisti et al. (2004) that found significant differences for C stocks through the soil profile under crop rotations in this same experimental area.

Data for C_{mic}/C_{org} ratio show that there were significant differences only for CT, when the tillage treatments were compared to the forest area. In addition, between management systems the lowest value was found under CT management, indicating that the quantity of C_{mic} as a proportion of the total soil C_{org} is greater under the forest area and NT than under CT.

As reported by Jenkinson and Ladd (1981), the C_{mic}/C_{org} ratio is related to the changes in the quantities of soil C. In both the forest area and NT the greatest values of C_{mic}/C_{org} ratio suggest that a greater quantity of biomass is supported per organic carbon unit derived from the inputs of C in the system.

Data on Table 1 indicate a more evident impact of the CT on the soil quality indicators, which may be resulted as a consequence of the degradation of the natural soil conditions (OLDEMAN, 1994) and/or by a strong impact on the fauna and flora and loss of agricultural soil (LEONARDOS et al., 2000).

Table 2 shows the effects of crop rotations within each management system. Significant differences were found only for BSR and qCO_2 within each crop rotation (Table 2). Under NT BSR was higher for crop rotation IIb, while the highest value for CT was observed in the crop rotation IIIc. In both rotations soybean was included as the summer crop but during the sampling period, vetch, a leguminous plant, was under cultivation. On the other hand, the lowest values of BSR were found in the crop rotations IIIb and I for NT and CT, respectively, when wheat was under cultivation. These data suggests that a succession of legume plants (soybean and vetch), as presented for NT and CT, in the crop rotations IIb and IIIc, respectively , may increase microbial activity represented by the CO_2-C evolved.

Some factors such as C/N ratio (RAUT et al., 2008) and N content (WANG et al., 2008) have a great influence on the decomposition and mineralization of the organic matter. Compared with wheat, the organic residues of vetch had a smaller C/N ratio and a greater N content, which contributed to the faster decomposition of its residues results in a greater CO_2 efflux rate.

The metabolic quotient (qCO_2) was lower for the crop rotation IIIb under NT, and for the crop rotation I under CT (Table 2). In both crop rotations soybean was followed by wheat, which possibly had been influenced the low values of BSR observed under these crop rotations. Greater values of qCO_2 were found in the crop rotations IIb and IIIa under NT and IIIc under CT (Table 2). Except for crop rotation IIIa under NT, the other crop rotations had soybean/vetch in succession, and as stated above this situation may had influenced the high values of BSR under these treatments, resulting on high values of qCO_2.

Compared to data displayed on tables of mean tests, principal component analysis (PCA), provides more details of the interactions between the studied variables once it mitigate the data variation into several components and allows a multidimensional view of the data, which complements the information of the mean tests, helping the interpretation of the data. The difference between the X and Y axes with our data was about 20%, indicating that X axis was the most important for data interpretation because it had been explained most of the data variation.

The PCA was performed with basis on the data of C_{mic}, BSR, qCO_2, C_{org} and C_{mic}/C_{org}. The first three principal components discriminated 94.9% of all information. However, the sum of the first two principal components

Table 2 - C_{mic} (mg C kg^{-1} soil), BSR (mg C-CO$_2$ kg^{-1} soil hour^{-1}), qCO_2 (mg C-CO$_2$ g^{-1} C_{mic} hour^{-1}), C_{org} (%) and C_{mic}/C_{org} ratio on a long-term experiment under NT and CT management for the 0-10cm depth interval

Soil tillage	Crop rotation*	C_{mic}	BSR	qCO_2	C_{org}	C_{mic}/C_{org}
No-Tillage	I	146.13	0.14 bc	0.96 ab	1.15	1.26
	IIa	144.22	0.15 b	1.04 ab	1.14	1.28
	IIb	181.73	0.22 a	1.21 a	1.16	1.59
	IIIa	108.24	0.13 bc	1.20 a	1.22	0.89
	IIIb	168.26	0.08 c	0.48 b	1.15	1.45
	IIIc	171.73	0.18 ab	1.05 ab	1.09	1.57
Conventional Tillage	I	106.77	0.06 d	0.56 b	1.14	0.93
	IIa	118.44	0.12 bc	1.01 ab	1.20	0.99
	IIb	72.22	0.07 cd	0.97 ab	1.32	0.83
	IIIa	103.83	0.08 cd	0.77 ab	1.24	0.84
	IIIb	92.82	0.07 cd	0.75 ab	1.20	0.78
	IIIc	89.24	0.21 a	2.35 a	1.17	0.76
Coefficient of variation (%)		16.6	22	8.37	5.22	27.04

* I- wheat/soybean; IIa- wheat/soybean/vetch/maize, with wheat as the winter cropping; IIb- wheat/soybean/vetch/maize, with vetch as the winter cropping; IIIa- wheat/soybean/vetch/maize/oats/soybean, with oats as the winter cropping; IIIb- wheat/soybean/vetch/maize/oats/soybean, with wheat as the winter cropping; IIIc- wheat/soybean/vetch/maize/oats/soybean, with vetch as the winter cropping. Same or none letters means no significant differences among treatments in the same column within management system (Tukey's $p<0.05$)

explained 79.2% of the information, with a contribution of 43.1% on PC1 and 36.1% on PC2. The most important parameters for the distribution of the treatments along X axis (PC1) were C_{mic} and the C_{mic}/C_{org} ratio and qCO_2, while BSR had a greater influence for the distribution along the Y axis (PC2) (Figure 1), which are key characteristics of the multivariate approach of principal component analysis (PCA) on distinguishing areas as a function of the soil management (SENA et al., 2002).

Data from PCA showed a clear separation between CT and NT, in which NT treatments had clustering closer to the forest area than CT, except for NT-IIIa (Figure 1).

PC1 shows that treatments under NT are closer to the forest area and they are more dispersed than CT treatments (Figure 1). NT is closer to the forest area because under this management system a high quantity of C_{mic} and C_{mic}/C_{org} were accumulated than under CT (Table 1). Treatments NT-IIb and NT-IIIc both had consecutive crop rotations with the same legume plants in the last 2 cropping seasons (soybean/vetch - Table 2), and high values of C_{mic} and C_{mic}/C_{org}. High concentrations of organic C in soil promote greater C_{mic} accumulation (ALVAREZ et al., 1995; VARGAS; SCHOLLES, 2000) which can be used by soil microorganisms for their growth (MOSCATELLI et al., 2005), reflecting the greater concentration of C_{mic} in the soil. This fact could explain the position of both treatments NT-IIb, NT-IIIb and NT-IIIc closest to the forest area (Figure 1).

Treatments distributed along PC2, represented by the Y axis, were most influenced by BSRs. Along this axis, treatments SFA and NT-IIb had showed the greatest

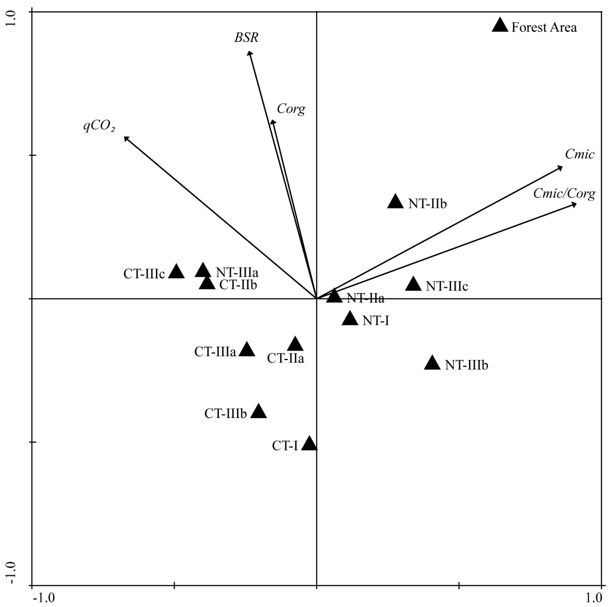

Figure 1 - PCA of the influence of each variety (C_{mic}, BSR, qCO_2, C_{org} and C_{mic}/C_{org}) on the distribution of soil management systems (NT: no-tillage; CT: conventional tillage) and crop rotations I, IIa, IIb, IIIa, IIIb and IIIc (Table 2)

distance from the CT-I and CT-IIIb (Figure 1), in which the first two treatments showed high values of BRS, while low values were found for CT-I and CT-IIIb treatments. Therefore, crop rotation system could have stimulated microbial activity, resulting in greater BSR without an increase in the C_{mic} content.

Within NT treatments, the rotation NT-IIIa was very distinct from the others (Figure 1). Among NT treatments, this rotation presumably shows the greatest C: N ratio because this system had consecutive crop rotations with cereals in the last 2 cropping seasons (maize/oats - Table 2). Possibly such conditions influenced soil organic matter decomposition, reflecting the greater rates of BSR during the studied period.

The ratio of microbial biomass-C to organic carbon (C_{mic}/C_{org}), and the metabolic quotient (qCO_2) associated with soil microbial activity, have been used by many authors as indices to determine the sustainability of agricultural systems. According to Araújo et al. (2008), the ratio C_{mic}/C_{org} was significantly enhanced by organic management, improving soil microbial characteristics and slowly increasing soil organic C. Moscatelli et al. (2007) verified that soil management alters the values of qCO_2. Franchini et al. (2007) reported that the decrease in qCO_2 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, evidence of a smaller relative loss of CO_2 and, consequently a larger accumulation of C.

Conclusions

1. Microbial carbon (C_{mic}), basal soil respiration (BSR), metabolic quotient (qCO_2) and microbial carbon/ organic carbon ratio (C_{mic}/C_{org}) are efficient to determine differences between NT and CT management but only BSR and qCO_2 are suitable to identify significant differences among crop rotations systems.
2. C_{mic}, C_{org} and C_{mic}/C_{org} are key indicators on determining the soil quality, which is clearly observed by the close clustering among crop rotations under NT and forest area as compared to CT.
3. The effects of soil management and crop rotations systems on soil quality indicators are only evident upon an integrated analysis of the data by using mean test and principal component analysis.

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