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Identifying indicators of C and N cycling in a clayey Ultisol under different tillage and uses in winter

Daniel Bini[a,b], Cristiane Alcantara dos Santos[a,b], Laura Priscila Toledo Bernal[a], Galdino Andrade[a], Marco Antonio Nogueira[a,c,*]

[a] Universidade Estadual de Londrina, Centro de Ciências Biológicas, Departamento de Microbiologia, Londrina, PR, Brazil
[b] Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz", Departamento de Ciência do Solo, Laboratório de Microbiologia, Piracicaba, SP, Brazil
[c] Empresa Brasileira de Pesquisa Agropecuária, Embrapa Soja, Caixa Postal 231, CEP 86001-970 Londrina, PR, Brazil

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ABSTRACT

Although tropical and subtropical environments permit two cropping cycles per year, maintaining adequate mulching on the soil surface remains a challenge. In some cases, leaving soils fallow during the winter as an agricultural practice to control pathogens contributes to reduce soil mulching. The aim of this study was to assess attributes associated with C and N cycling in a soil under conventional and no-tillage management, with contrasting uses in winter: black oats (*Avena strigosa* Schreb) as cover crop or fallow. No-tillage increased total C and N, irrespective the winter crop. Cropping black oats under no-tillage resulted in more microbial biomass C and N, and glutaminase activity (15.2%, 65.2%, and 24%, respectively) than no-tillage under fallow. Under conventional tillage, winter cropping did not affect the attributes under study. Available P was higher in the no-tillage system (9.2–12.3 mg kg^{-1}), especially when cropped with black oats, than in the conventional tillage system (4.8–6.6 mg kg^{-1}). A multivariate analysis showed strong relationships between soil microbiological and chemical attributes in the no-tillage system, especially when cropped with black oats. Soil pH, dehydrogenase and acid phosphatase activities were the most effective at separating the soil use in winter. Microbial N, total N, microbial to total N ratio, available P, metabolic quotient (qCO_2), and glutaminase activity were more effective at separating soil management regimes. The no-tillage system in association with winter oat cropping stimulated the soil microbial community, carbon and nutrient cycling, thereby helping to improve the sustainability of the cropping system.

1. Introduction

The intensive soil use typical of conventional tillage leads to soil degradation and declining sustainability in cropping systems. The direct impact of rainfall on the soil surface and exposition to the sun and wind worsen erosion, reduce soil humidity, levels of organic matter and microbial activity and diversity, and consequently impair carbon and nutrient cycling in soils (Babujia et al., 2010; Balota et al., 2003; Franchini et al., 2007). Where winter crops are less profitable and the soil remains exposed and uncultivated until the summer, the soil microbial community undergoes lack of fresh carbon sources (Nogueira et al., 2006).

Conservationist practices aim to improve soil health and the sustainability of cropping systems (Doran et al., 1996; Jackson et al., 2003; Van Bruggen and Semenov, 2000). No-tillage systems, for example, help to improve physical, chemical, and microbiological soil attributes (Babujia et al., 2010; Balota et al., 2004; Mikanová et al., 2009) mainly by increasing and maintaining higher levels of organic C in soil and thereby increasing the production stability (Franchini et al., 2012). Keeping mulch on the soil surface reduces the range of soil temperature, retains soil moisture for longer periods, and establishes a gradient of organic matter transformations in the litter, mimicking what happens in a soil under native vegetation (Hungria et al., 2009).

Mulching is one of the main challenges of no-tillage systems in tropical and subtropical regions, making the use of winter crops for mulch production an important option for crop rotation (Balota et al., 2003, 2004; Franchini et al., 2007; Hungria et al., 2009; Silva et al., 2010). Apart from protecting soils during the winter, plant biomass is an important source of organic carbon for the soil microbial biomass, which helps temporarily immobilize and

* *Corresponding author at*: Embrapa Soybean, Soil Biotechnology, Rod. Carlos João Strass S/N, Distrito de Warta Cx. Postal 231, 86001-970 Londrina, PR, Brazil. Tel.: +55 43 3371 6215; fax: +55 43 3371 6100.
E-mail addresses: marco.nogueira@embrapa.br, marco_nogueira@hotmail.com (M.A. Nogueira).

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reduce losses of nutrients like N and P (Balota et al., 2003; Kaschuk et al., 2010). The soil microbial community plays important roles in the sustainability of cropping systems, because it is involved in the cycling of C and nutrients (Cleveland et al., 2010; Sisti et al., 2004). Soil management systems that stimulate the soil microbial community via organic carbon inputs, rhizodeposition, maintenance of soil moisture, and lower ranges of temperature help to improve sustainability (Balota et al., 2003; Nogueira et al., 2006; Sinha et al., 2009). Measurements of key microbial and biochemical processes, in addition to chemical attributes, have been used as tools to assess C and nutrient cycling in soils and to evaluate soil health (Balota et al., 2003, 2004; Chaer et al., 2009; Melero et al., 2008).

The aim of this study was to assess attributes associated with soil quality in a field trial five years after installed, conducted partly under a no-tillage system and partly under conventional tillage, with contrasting soil uses in winter: cropping with black oats or fallow.

2. Material and methods

2.1. Site description and experimental design

The field trial was installed in 2002 at an experimental site managed by the State University of Londrina, Paraná, Brazil (23°20′31.76′S, 51°12′41.31′W). The soil is classified as Rhodic Kandiudult (Soil Taxonomy, USDA), which chemical characteristics in 2002 at the 0–10 cm layer were: pH (0.01 M $CaCl_2$) = 5.8; organic carbon = 25.6 g kg^{-1}; P = 9.24 mg dm^{-3}; Ca = 5.03 $cmol_c$ dm^{-3}; Mg = 2.28 $cmol_c$ dm^{-3}; K = 0.69 $cmol_c$ dm^{-3}; CEC = 11.7 $cmol_c$ dm^{-3}; base saturation = 68.5%; Al = 0 $cmol_c$ dm^{-3}; granulometric fractions: clay = 800 g kg^{-1}; sand = 130 g kg^{-1} and silt = 70 g kg^{-1}. The local climate is classified as Cfa under the Köppen system (humid subtropical, with hot and humid summers, and mild winters), with not well defined dry season, but most of the 1600 mm of mean annual rainfall falling between September and March.

The experimental design was entirely randomized, consisting of no-tillage (NT) or conventional tillage (CT) systems cropped in rotation with corn or soybean in the summer, in combination with fallow (F) or black oat (O) in winter, resulting in four treatments: NT–O, NT–F, CT–O, and CT–F. The treatments were established in belts against the slope (<12%), along which eight replications were established, forming plots of 8 × 8 m. In winter, black oats were sown mechanically and grown until flowering, when plants were rolled onto the soil surface in the NT, or incorporated into the soil by harrowing in the CT. The summer crop was also mechanically sown. In the NT system the soil was not disturbed and plant residues were kept on the soil surface as mulching, whereas in the CT the soil was plowed once a year and harrowed at least five times a year.

2.2. Soil sampling

Soil sampling was carried out in October 2007 (in the fifth year of the experiment) at a depth of 0–10 cm, and consisted of 15 subsamples for each plot. Subsamples were pooled, sieved (2 mm), and homogenized to form a composite sample. For chemical analyses, an aliquot was air-dried for 72 h. For microbiological and biochemical analyses, samples were stored at 5 °C for three days until analysis. Soil moisture was determined gravimetrically after oven drying at 105 °C for 24 h, so that results could be expressed in dry soil basis.

2.3. Chemical analyses

Total organic carbon was oxidized with $K_2Cr_2O_7$ in the presence of sulfuric acid (Yeomans and Bremner, 1988); total N was digested with sulfuric acid and catalysts, followed by steam distillation and titration (Bremner and Mulvaney, 1982). Available P was assessed in Mehlich I (0.05 M HCl + 0.05 M H_2SO_4) extracts by the ascorbic acid blue method (Murphy and Riley, 1962). Soil pH was measured in 0.01 M $CaCl_2$ suspensions (1:2.5 soil:solution ratio).

2.4. Microbiological and biochemical analyses

Microbial biomass of C (C_{mic}) and N (N_{mic}) were estimated by the fumigation–extraction method (Vance et al., 1987) using coefficients K_C = 0.33 and K_N = 0.68, respectively (Brookes et al., 1985). The microbial quotients for microbial biomasses C and N were calculated as percentages of the soils' total organic C and total N, respectively. Microbial activity was quantified based on the CO_2 released from samples incubated in hermetically sealed vials for 21 days using 0.5 M NaOH as trap (Alef, 1995). The ratio between basal respiration and C_{mic} was used to calculate the metabolic quotient (qCO_2) (Anderson and Domsch, 1993).

For dehydrogenase activity (Casida et al., 1964), triphenyl tetrazolium chloride (TTC) (1:1 soil: 1.5% solution, m:v) was used as substrate and incubated at 37 °C for 24 h. Cellulase activity was assessed in 10-g samples incubated at 50 °C for 24 h, at pH 5.5 using carboxymethy cellulose as substrate, and reducing sugars were determined via the Prussian Blue method (Schinner and von Mersi, 1990). Acid phosphatase activity employed 0.05 mol L^{-1} ρ-nitrophenyl phosphate as substrate, and was incubated at 37 °C for 20 min at pH 6.5 (Tabatabai and Bremner, 1969). Glutaminase activity was assessed in 1-g samples incubated at 37 °C for 2 h, in the presence of 0.5 M L-glutamine as substrate. After incubation, the ammoniacal–N was extracted with a KCl–Ag_2SO_4 solution and quantified by steam distillation and titration (Frankenberger and Tabatabai, 1991).

2.5. Statistical analyses

Before analysis, data were examined for homogeneity of variances and normal distribution. The dataset was subjected to one-way ANOVA and means comparisons by Duncan's test ($p \leq 0.05$), according to an entirely randomized experimental design, and simple Pearson's correlation. A multivariate Principal Component Analysis (PCA) was used to explore how soil attributes and management regimes are related each other by using the software Canoco 4.5 (Ter Braak and Smilauer, 1988). Canonical Discriminant Analysis (CDA) was also applied to identify which attributes were more important to separate the treatments with the software SAS (SAS Institute, 2002). Therefore, Wilk's Lambda, Pillai, Hotelling–Lawley, and Roy's greatest root tests were performed and variables were selected for low colinearity each other, and highly significant effects of treatments ($p \leq 0.0001$). The individual contribution of each variable in separating treatments along the diagram is expressed as the parallel discrimination coefficient [PDC—the product of the standardized canonical coefficient (SCC) and the correlation coefficient (r) between each variable and the canonical discriminant function], where positive values >0.1 are considered highly relevant as discriminating in the CDA (Baretta et al., 2010). The significance of the separation among treatments in the CDA diagram was tested with the LSD test ($p \leq 0.05$) (SAS Institute, 2002).

3. Results

3.1. Chemical attributes

Total organic carbon (TOC) and total nitrogen (total N) were higher in the NT system than in the CT system, independent of

Table 1
Chemical attributes of an Ultisol under no-tillage or conventional tillage cropped with black oat or left as fallow in winter. Means followed by different letters in line are significantly different (Duncan, $p \leq 0.05$). Numbers in brackets represent the standard deviation ($n = 8$).

Chemical attribute	No-tillage		Conventional tillage	
	Oat	Fallow	Oat	Fallow
TOC ($g\ kg^{-1}$)[a]	26.9 (0.8)a	26.7 (1.9)a	24.7 (1.4)b	24.3 (1.6)b
Total N ($g\ kg^{-1}$)	2.4 (0.2)a	2.4 (0.3)a	2.2 (0.1)b	2.1 (0.1)b
Organic matter C/N	11.2 (0.8)a	11.1 (1.3)a	11.2 (0.9)a	11.6 (0.8)a
Available P ($mg\ kg^{-1}$)	12.3 (2.2)a	9.2 (3.2)b	4.8 (0.7)c	6.6 (2.8)c
pH ($CaCl_2$)	5.9 (0.2)a	5.9 (0.2)a	5.5 (0.2)b	5.9 (0.2)a

[a] TOC: total organic C.

winter soil use, with no-effects on the C/N ratio of organic matter (Table 1). Available P was significantly higher in the NT than in the CT, particularly when cropped with oats as mulching in winter. Finally, soil pH was slightly lower in the CT system cropped with black oats (Table 1).

3.2. Microbiological attributes

The NT system cropped with oats in winter had the highest amounts of C_{mic}, similarly to N_{mic}, but N_{mic} also differed between NT and CT (Table 2). The C:N microbial ratio increased from NT–O to CT–F, ranging from 12 to 18. Basal respiration differed only in NT under fallow in winter, for which values were approximately 14% lower than in the other treatments. The metabolic quotient (qCO_2) varied between NT and CT, but was independent of winter soil use. With regard to the microbial quotient (*q*Mic), the NT system associated with oats in winter showed a higher proportion of microbial biomass C in relation to the soil total C than the fallow treatment (Table 2). The *q*Mic for N microbial biomass allowed greater differentiation, in which NT with oats in winter showed higher values.

Peak dehydrogenase activity was found in the CT–F and decreased towards the NT–O. Glutaminase was stimulated under NT, especially with oats, and decreased in the CT system. Conversely, cellulase activity was lower in NT than in CT. The lowest acid phosphatase activity was observed in the CT system under fallow, differing from the other treatments (Fig. 1).

The Pearson's correlation analysis showed a strong relationship between glutaminase activity and TOC and total N, available P, and pH, and most of the microbiological attributes. Notably, glutaminase activity showed a positive correlation with N_{mic} and a negative correlation with cellulase and dehydrogenase activities (Table 3). C_{mic} and N_{mic} showed positive correlations with TOC and total N; N_{mic} was also positively correlated with available P and soil pH. Correlations with N_{mic} were in general stronger than those with C_{mic}. Cellulase was negatively correlated with total N, available P,

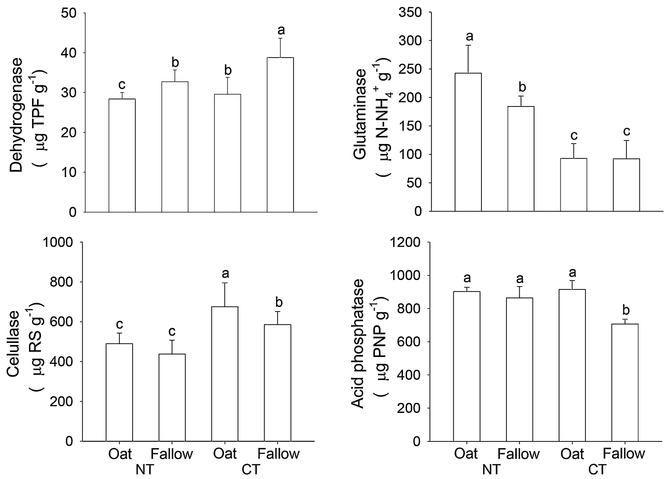

Fig. 1. Enzyme activities in an Ultisol under no-tillage (NT) or conventional tillage (CT) cropped with oat or left as fallow in winter. Means followed by different letters are significantly different (Duncan, $p \leq 0.05$). Vertical bars represent the standard deviation ($n = 8$). TPF—triphenyl formazan; RS—reducing sugar; PNF—*p*-nitrophenol.

soil pH, and N_{mic}, and positively correlated with basal respiration, but did not show a relationship with TOC.

3.3. Principal component analysis

The principal component analysis clearly separated the treatments in the factorial space (Fig. 2). Axis 1 explained 60.5% of the variation and separated NT from CT, while axis 2 explained 19.4% of the variation and separated oat cropping in winter from fallow. Fewer variables were related to CT, including cellulase activity, basal respiration, organic matter C:N, microbial biomass C:N, dehydrogenase activity, and qCO_2, the latter two related to CT under fallow (CT–F). Most variables (C_{mic}, C_{mic}/TOC, Acid P-ase, N_{mic}/tN; G-ase, TOC, N_{mic}, available P, total N and pH) were related to NT, especially when cropped with oats in winter (NT–O).

3.4. Canonical discriminant analysis

The canonical discriminant analysis (CDA) displayed high canonical correlations in the first and second canonical discriminant functions (CDF_1 and CDF_2). The first function accounted for 63% of the discriminating power among the treatments, whereas the second function accounted for 34% (Fig. 3). The CDF diagram showed four statistically distinct groupings, as confirmed by the LSD test ($p \leq 0.05$) for the two canonical functions (Fig. 3). The individual contribution of the selected variables on the variation in this pattern is expressed by the PDC values (Table 4). Thus, pH, dehydrogenase activity, and acid phosphatase activity made important contributions to discriminate among treatments for the CDF_1

Table 2
Microbiological attributes in an Ultisol under no-tillage or conventional tillage cropped with oat or left as fallow in winter. Means followed by different letters in line are significantly different (Duncan, $p \leq 0.05$). Numbers in brackets represent the standard deviation ($n = 8$).

Microbiological attribute	No-tillage		Conventional tillage	
	Oat	Fallow	Oat	Fallow
C_{mic} ($mg\ kg^{-1}$)[a]	870 (123)a	750 (131)b	760 (106)b	755 (47)b
N_{mic} ($mg\ kg^{-1}$)[b]	74 (11)a	60 (13)b	47 (9)c	46 (15)c
C_{mic}/N_{mic}	12 (2)c	13 (3)bc	17 (6)ab	18 (6)a
Respiration ($mg\ CO_2\ kg^{-1}\ d^{-1}$)	48 (4)a	42 (5)b	49 (4)a	50 (3)a
qCO_2 ($mg\ CO_2–C\ g^{-1}\ C_{mic}\ g^{-1}$)	0.6 (0.1)b	0.6 (0.1)b	0.7 (0.1)a	0.7 (0.1)a
*q*Mic–C (C_{mic}/TOC, %)	3.2 (0.4)a	2.8 (0.4)b	3.1 (0.4)ab	3.1 (0.2)ab
*q*Mic–N (N_{mic}/total N, %)	3.1 (0.6)a	2.5 (0.5)b	2.1 (0.4)b	2.1 (0.7)b

[a] C_{mic}: microbial biomass C.
[b] N_{mic}: microbial biomass N.

Table 3
Pearson's correlation among chemical and microbiological attributes in an Ultisol under no-tillage or conventional tillage cropped with oat or left as fallow in winter.

	TOC[a]	Total N	Available P	pH	Basal respiration	C_{mic}[b]	N_{mic}[c]	Dehydrogenase	Celullase	Glutaminase
Total N	0.52^{**}									
Available P	0.61^{***}	0.56^{***}								
pH	ns	0.41^{*}	0.39^{*}							
Basal respiration	ns	ns	ns	ns						
C_{mic}	0.41^{*}	0.36^{*}	ns	ns	ns					
N_{mic}	0.52^{**}	0.55^{***}	0.68^{***}	0.48^{**}	ns	0.48^{**}				
Dehydrogenase	ns	ns	ns	ns	ns	ns	ns			
Celullase	ns	-0.46^{**}	-0.40^{*}	-0.66^{***}	0.45^{**}	ns	-0.40^{*}	ns		
Glutaminase	0.53^{***}	0.44^{***}	0.68^{***}	0.46^{**}	ns	0.41^{*}	0.68^{***}	-0.40^{*}	-0.56^{***}	
Acid phosphatase	ns	ns	ns	ns	ns	ns	ns	-0.63^{***}	ns	0.37^{*}

[*] $p \le 0.05$.
[**] $p \le 0.01$.
[***] $p \le 0.001$.
[a] TOC: total organic carbon.
[b] C_{mic}: microbial biomass C.
[c] N_{mic}: microbial biomass N.

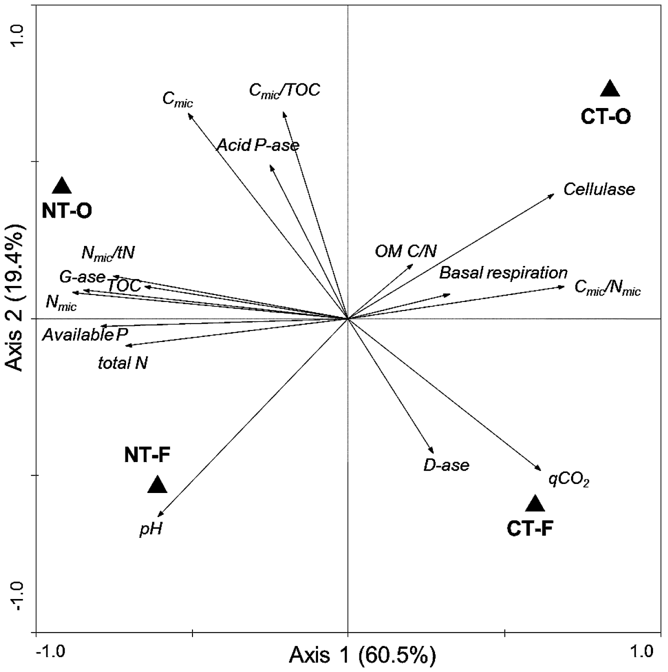

Fig. 2. Principal component analysis (PCA) considering microbial and chemical attributes in an Ultisol under no-tillage (NT) or conventional tillage (CT) cropped with oat (O) or left as fallow (F) in winter. Black triangles are the samples scores' centroids. Acid P-ase: acid phosphatase activity; C_{mic}/N_{mic}: C/N ratio of the microbial biomass; C_{mic}/TOC: percentage of C_{mic} in TOC; C_{mic}: Microbial biomass C; D-ase: dehydrogenase activity; G-ase: glutaminase activity; N_{mic}/tN: percentage of N_{mic} in total N; N_{mic}: Microbial biomass N; OM C/N: C/N ratio of the soil organic matter; qCO_2: metabolic quotient; TOC: total organic carbon.

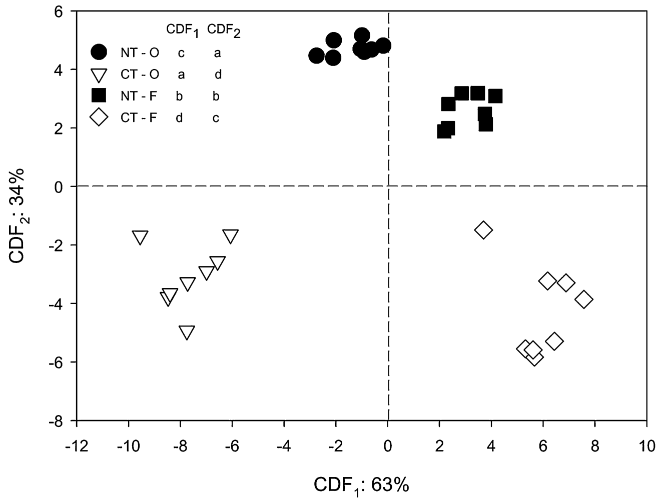

Fig. 3. Discriminating canonical analysis (DCA) based on standardized canonical coeficients from chemical and microbiological attributes obtained in an Ultisol under no-tillage (NT) or conventional tillage (CT) cropped with oat (O) or left as fallow (F) in winter. The groupings were statistically tested (inner), in which different letters mean significant differences by LSD test ($p \le 0.05$).

(oat × fallow), whereas N_{mic}, total N, N_{mic}/tN, available P, qCO_2, and glutaminase activity showed high relevance to discriminate among treatments for the CDF_2 (no-tillage × conventional tillage) (Table 4).

4. Discussion

4.1. Total and microbial C and N in soils

Higher levels of TOC and total N under NT, irrespective of soil use in winter, are attributed to the maintenance of crop residues on the soil surface, which tends to increase TOC in soils. Balota et al. (2003) observed higher C concentrations in soils under NT than in those under CT 22 years after the NT system had been adopted. Similar patterns have been observed under both tropical and temperate conditions (Babujia et al., 2010; Melero et al., 2011; Sun et al., 2011), suggesting that conservationist tillage reduces C mineralization and helps to increase soil C stocks (Murty et al., 2002). Nevertheless, planting oats in winter as a cover crop did

Table 4
Parallel discrimination coefficient (PDC) of canonical discriminating functions 1 and 2 (CDF1 and CDF2) for the different soil management and use in winter, based on microbiological and chemical attributes selected as significant ($p \le 0.0001$) for use in the Canonical Discriminating Analysis. Highlighted PDC values represent high discrimination power (PDC values >0.1).

Attribute	PDC	
	CDF_1	CDF_2
N_{mic}[a]	−0.0878	**0.1472**
Total N	0.0030	**0.4560**
N_{mic}/tN[b]	0.0584	**0.1890**
Available P	0.0408	**0.1073**
qCO_2[c]	−0.0107	**0.1909**
pH	**0.2664**	0.0005
Dehydrogenase	**0.1411**	0.0846
Glutaminase	0.0066	**0.4850**
Acid phosphatase	**0.4185**	0.0572

[a] N_{mic}: microbial biomass N.
[b] N_{mic}/tN: percentage of N_{mic} in total N.
[c] qCO_2: metabolic quotient.

not maximize the increase of TOC and total N, independent of soil management. Increases of TOC and total N in soils are normally observed over the long-term in systems that have a positive balance of C in soils (Dick and Tabatabai, 1993; Nogueira et al., 2006). Mazzoncini et al. (2011) found an increase of TOC in the topsoil in an NT system over 10 yr with winter crops, as a consequence of the more conservative soil management. While no effects of winter crops were observed under CT in the short-term (5 yr), they helped to maintain the soil organic C levels in the long-term (10 yr). Thus, assessments of winter crop effects on agricultural systems should be made over long periods. The likely reason why no effects were observed in TOC and total N in the CT soils cropped with oats in winter was that our study only lasted 5 yr. On the other hand, available P was higher under NT, mainly when cropped with oats in winter, which highlights the potential of oats to improve soil conditions for making P available. One possibility is that the greater input of organic material stimulates microbial activity, including P-solubilizing microorganisms (Khan et al., 2009). Moreover, the oat crop and the microbial biomass immobilize phosphate temporarily, protecting it from fixation in the soil clay and oxides, and later releasing by mineralization. Finally, the organic acids resulting from oat decomposition may coat the P-fixing sites on the clay and oxide surfaces, mainly in oxidic soils (Vance et al., 2003). These results help to support the management strategy based on the application of P to the oat in the winter to be released to the summer crop.

Soil protection is an extra benefit of winter covering crops, since both living plants and their residues minimize the impacts of rainfall on the soil surface and reduce erosion (Balota et al., 2003). In addition, vegetation and mulching maintain a more active and diverse microbial community due to more stable moisture and temperature regimes, and release of available C as rhizodeposition and plant residues (Garcia et al., 2005; Sinha et al., 2009; Geisseler et al., 2011), making a more dynamic C and nutrient cycling driven by microorganisms. In this view, soil microbial biomass (C and N) and activity were sensitive to soil management, mainly with the winter crop, and their positively correlation with TOC and total N suggests limitation of energy sources and nitrogen in the soil (Garcia et al., 2005; Sinha et al., 2009). A more stable and nutrient-rich environment in the NT system cropped with oats in winter benefits the microbial community, increasing the microbial coefficient (*q*Mic) for both C and N microbial biomass. Higher *q*Mic suggest more favorable conditions for the formation of microbial biomass, which works as a labile reservoir of nutrients, especially N. In addition, lower C_{mic}:N_{mic} ratio of microbial biomass in the NT suggests easier mineralization and nutrient cycling. Conversely, the CT system is known to disrupt the soil structure and microsites, promote oxidation of organic matter, and thus impair the soil microbial community (Chodak and Niklińska, 2010; Madejon et al., 2007). By contrast, NT system increases microbial biomass and maintain the fungal hyphae integrity, which also helps improve soil structure and aggregation (Babujia et al., 2010; Balota et al., 2003; Madejon et al., 2007; Mikanová et al., 2009; Silva et al., 2010).

Microbial biomass C and N were more sensitive than TOC and total N in detecting effects of soil management and winter use, and are thus better suited for detecting changes in the short-term.

4.2. Soil microbial and biochemical activities

Soil microbial activity measured as CO_2 release can be stimulated with inputs and the maintenance of organic residues, but tilling can also stimulate CO_2 release as a consequence of aeration and the exposure of organic matter to microbial attack (Anderson and Domsch, 1993; Madejon et al., 2007). This explains the higher respiration rates in the NT system with oats in winter and in both CT treatments. In the NT with oats, the plants were rolled on the soil surface at flowering, depositing a less lignified C source, more prone to microbial attack. However, higher CO_2 release under CT, independent of winter use, is a consequence of tilling that exposes the soil to aeration and accelerates the organic matter to oxidation (Murty et al., 2002). This is undesirable with regards to the sustainability of the system and the maintenance of C stocks in the soil (Murty et al., 2002).

The higher qCO_2 under CT system denotes more stress to the microbial community (Anderson et al., 2004). Despite qCO_2 was effective at distinguishing between tillage systems, it was not effective to distinguish between winter soil uses, whereas dehydrogenase activity was. The highest dehydrogenase activity under CT with winter fallow possibly resulted from the adverse conditions imposed on the microbial community by soil management, like shortage of labile C sources, in addition to other adverse conditions described above. Interestingly, oat cropping in winter resulted in lower dehydrogenase activity in both management systems, suggesting lower levels of microbial stress, which was not revealed by the qCO_2 index. Dehydrogenate activity represents the electron flow in the respiratory chain (Casida et al., 1964) and thus higher activities are in agreement with higher qCO_2 levels. However, Mikanová et al. (2009) observed opposite effects, with less dehydrogenase activity under CT. It must be kept in mind that more factors may affect microbial metabolic activity, such as time elapsed since the establishment of the NT system, the species cropped as winter covering, or soil type (Chodak and Niklińska, 2010; Sinha et al., 2009; Varennes and Torres, 2011).

Cellulase activity was stimulated under CT, especially when cropped with oats in winter. This might be the consequence of greater inputs of organic matter into the soil, in addition to a closer contact with soil due to plowing and harrowing in CT, which made the organic C pools more prone to microbial and soil enzyme attacks. In addition, cellulase activity showed high correlations with soil chemical attributes, especially negative correlations with total N ($p \leq 0.01$) and pH ($p \leq 0.001$). Cellulase thus seems to have greater activity in more acidic and lower N content soils. In fact, lower total N in soils may suggest a higher C to N ratio of the organic matter and plant residues, signifying more recalcitrant C sources, which stimulates cellulase activity (Anderson et al., 2004; Pavel et al., 2004). Since fungi are the main source of cellulase (Anderson et al., 2004), these observations reinforce evidences of fungal favoring under CT, especially when cropped with oat in winter, i.e., lower soil pH, lower organic matter levels, and higher C_{mic}-to-N_{mic} ratio. However, more specific analysis, like phospholipid fatty acid profiling, must be done to check for this possibility.

Glutaminase activity was very promising as indicator of soil management effects on N cycling. Muruganandam et al. (2009) also observed higher enzyme activities under NT than under CT, and were positively correlated with TOC, total N, and microbial biomass, as observed for glutaminase. Moreover, positive correlations with edaphic attributes emphasize its potential as global indicator of changes in soils. Ekenler and Tabatabai (2004) reported strong correlation between glutaminase activity and pH, demonstrating great sensibility to soil pH and soil type. In addition, glutaminase and asparaginase are more catalytically efficient than other soil enzymes, showing significant activity even with low protein concentration in soils. This sensibility in the face of varying environmental conditions, in addition to higher activity, makes glutaminase a reliable and sensitive indicator of changes in soil quality (Ekenler and Tabatabai, 2004).

Acid phosphatase activity decreased in the CT–F, the least conservation-friendly soil management system in this study. In the most conservation-friendly management regime (NT–O) or even under CT with oats cropped in winter (CT–O), acid phosphatase activity remained higher, probably because of more inputs and/or maintenance of organic residues in soil. Despite being generally influenced by P and pH, no significant correlations were observed, as in previous studies (Monokrousos et al., 2006; Olander and Vitousek, 2000). Slight variation in soil pH and P availability may have not been large enough to influence phosphatase activity.

The benefits that conservationist systems offer to soil enzymes are attributed to a greater level of protection in organo-mineral complexes, which prevents them from attack by proteases or other physical, chemical, or biological agents (Melero et al., 2008).

4.3. Global data analysis

Despite the importance of individual attributes in interpreting the effects of soil use and management, a global view encompassing several attributes allows for a more robust analysis. Thus, the DCA clearly separated the effects of soil management and use in winter, where the N cycling-related attributes were more discriminating than the C cycling-related attributes, indicating that soil management led to more evident changes in N cycling. In addition, qCO_2 also showed strong discriminating power concerning soil management, and has been reported as sensitive indicator of disturbances on the microbial community in ecosystems (Baretta et al., 2005; Bastida et al., 2008). The more conservation-friendly soil management regime (NT) improved the microbiological and biochemical attributes, especially those related to N and helped to increase the sustainability of the agricultural ecosystem, irrespectively the use in winter. Conversely, qCO_2 indicated stressful conditions for the microbial community in the CT under fallow, which was also accompanied by higher dehydrogenase activity, indicating a more intense electron flow associated with the microbial metabolism in that condition (Casida et al., 1964).

Given that soil microbial activity is associated with its fertility and quality (Mikanová et al., 2009), conservationist systems like NT are more likely to be sustainable because they favor soil microbial activity and fertility. Thus, soil indicators with good discriminating power are useful tools for monitoring the sustainability of agricultural systems.

5. Conclusions

Five years under NT were sufficient to increase soil chemical attributes such as TOC, total N, and available P, in relation to CT, but no strong effects were found in relation to winter soil use. However, microbiological attributes, especially those associated with N, were more sensitive to soil management and winter use. These included glutaminase activity, microbial biomass N, and qMic–N, which had high discriminating power between soil management regimes and winter use. In particular, glutaminase activity showed to be a very promising indicator of soil health, as an indicator of N-cycling function.

Conservationist soil management regimes are more favorable to C and nutrient cycling. Enzyme activities were sensitive to the different soil management regimes and soil uses in winter, and are promising as indicators for helping interpret the effects of soil use and management on the sustainability of agricultural ecosystems.

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