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



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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<sup>-1</sup>), especially when cropped with black oats, than in the conventional tillage system (4.8–6.6 mg kg<sup>-1</sup>). 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.

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## 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

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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<sub>2</sub>) = 5.8; organic carbon = 25.6 g kg<sup>-1</sup>; P = 9.24 mg dm<sup>-3</sup>; Ca = 5.03 cmol<sub>c</sub> dm<sup>-3</sup>; Mg = 2.28 cmol<sub>c</sub> dm<sup>-3</sup>; K = 0.69 cmol<sub>c</sub> dm<sup>-3</sup>; CEC = 11.7 cmol<sub>c</sub> dm<sup>-3</sup>; base saturation = 68.5%; Al = 0 cmol<sub>c</sub> dm<sup>-3</sup>; granulometric fractions: clay = 800 g kg<sup>-1</sup>; sand = 130 g kg<sup>-1</sup> and silt = 70 g kg<sup>-1</sup>. 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<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> 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<sub>2</sub>SO<sub>4</sub>) extracts by the ascorbic acid blue method (Murphy and Riley, 1962). Soil pH was measured in 0.01 M CaCl<sub>2</sub> suspensions (1:2.5 soil:solution ratio).

### 2.4. Microbiological and biochemical analyses

Microbial biomass of C (C<sub>mic</sub>) and N (N<sub>mic</sub>) were estimated by the fumigation–extraction method (Vance et al., 1987) using coefficients K<sub>C</sub> = 0.33 and K<sub>N</sub> = 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<sub>2</sub> 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<sub>mic</sub> was used to calculate the metabolic quotient (qCO<sub>2</sub>) (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 carboxymethyl 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<sup>-1</sup> *p*-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<sub>2</sub>SO<sub>4</sub> 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

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