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Soil quality assessment based on soil organic matter pools under long-term tillage systems and following tillage conversion in a semi-humid region

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**Running Title:** Soil quality indicators under contrasting tillage systems

### Abstract

A field study was conducted to assess the long-term effects of no-tillage (NT) and conventional tillage (CT), and the short-term effects following tillage conversion -from CT to NT (NT<sub>n</sub>) and from NT to CT (CT<sub>n</sub>) on soil quality (SQ) indicators in a semi-humid climate. First, plots of a long-term tillage experiment on a Luvic Phaeozem initiated in 1986, were split into two subplots in 2012, yielding four treatments: NT, CT, NT<sub>n</sub> and CT<sub>n</sub>. In 2015, composite soil samples were collected from each treatment and from a natural site (Ref) at depths 0-5, 5-10, 10-20 and 0-20 cm. Several indicators were determined: soil organic carbon (SOC) and nitrogen (SON); particulate

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organic C (POM-C) and N (POM-N); potential N mineralization (PMN) and soil respiration ( $R_s$ ). Moreover, bulk density was determined in long-term tillage systems. Different ratios between indicators were calculated, with emphasis on its function in the agroecosystem, *i.e.* functional indicators. Significant differences in SOC, SON and PMN were found between CT and NT at most depths. In contrast, three years after tillage conversion, only a part of the SQ indicators studied were modified mainly at the 0-10 cm depth. The functional indicators showed differences between tillage systems in the long-term and after short-term tillage conversion depending on the depth; however, the PMN/SON ratio demonstrated differences at all depths. Under these conditions, this ratio -related to easily mineralizable N fraction- proved to be a promising indicator for assessing SQ under contrasting tillage systems regardless of the sampling depth.

### **Keywords**

soil organic carbon, no-tillage, long-term experiment, tillage system conversion, functional quality indicator.

### **Introduction**

Over the last few decades, soil quality (SQ) definition and assessment techniques have become a major concern, and scientific information is essential for finding appropriate indicators that can accurately assess SQ (Doran & Parkin, 1994; Karlen, *et al.*, 2001; Andrews *et al.*, 2004). Also, various management systems that preserve or improve SQ have been proposed and tested (Karlen *et al.*, 1997; Karlen *et al.*, 2001). Tillage systems affect many different soil properties and processes (Reeves, 1997; Karlen *et al.*, 2013; Duval *et al.*, 2019). Conventional tillage (CT) favors residue and soil organic matter (SOM) decomposition through disruption of the soil aggregates, enhancing aeration and distributing SOM more uniformly. In contrast, no-tillage (NT) promotes soil aggregation and protects organic compounds against degradation (Martínez *et al.*, 2017). Moreover, NT has been shown to improve, or at least maintain, SQ on the basis of the larger crop yields obtained relative to CT in areas with restricted water availability (Melero *et al.*, 2009 and can preserve or increase SOM in semi-arid conditions (Duval *et al.*, 2019). Other authors (Dimassi *et al.*, 2013) assessed the impact of long-term and short-term (*i.e.* conversion from previous management practices) tillage systems on SOM contents and their depth distribution, showing a SOM stratification in the upper layer under NT in the long-term. In contrast, following tillage conversion SOM was redistributed in depth due to the mixing effect of ploughing. Furthermore,

the effect of changing tillage practices on SQ is still unclear (Wander & Bollero, 1999). More research is needed to understand better the interactions of tillage on the broad spectrum of indicators under different conditions.

Conducting long-term studies is essential to compare the variation of soil properties that serve as indicators in the short- and long-term (Poulton, 1995). Moreover, long-term studies enable an understanding of the time factor in determining the soil properties with ability to express SQ. In addition, they can be valuable in elucidating mechanisms of biologically mediated processes that are important for sustainable agricultural systems (Berti *et al.*, 2016).

Soil organic carbon (SOC) and nitrogen (SON), as well as their labile organic fractions are considered important SQ indicators (Reeves, 1997; De Paul Obade, 2017; Martínez *et al.*, 2018a). In addition, contents of carbon (C) and nitrogen (N) in particulate organic matter (POM) are regarded as the most sensitive SQ indicators of short-term changes (Duval *et al.*, 2013). Biochemical properties, *e.g.* potential N mineralization (PMN) or soil respiration ( $R_s$ ), are generally regarded as potentially useful indicators of SQ, because of their close link to SOM dynamics and nutrient cycling as well as their sensitivity to soil disturbance and changes induced by tillage (Duval *et al.*, 2013; Toledo *et al.*, 2013a; Martínez *et al.*, 2017). Many investigations (Doran & Parkin, 1994; Karlen *et al.*, 1997; Pulido-Moncada *et al.*, 2018) have focused on defining SQ indicators under different conditions; however, a broader approach is demanded for a better understanding of the tillage systems impact on SQ. Toledo *et al.* (2013a) reported that there are functional indicators, that provide information about the agroecosystem performance; however, they are sensitive in relation to many factors, such as soil type, seasons and weather conditions. The ratio between indicators could provide information about the agroecosystem functioning, because it considers the variations in a holistic way and may be more convenient for evaluating changes under different management systems and environmental conditions (Duval *et al.*, 2016). Our hypothesis is that conversion from NT to CT and vice versa would modify the dynamics of SOM organic fractions influencing the SQ indicators and their variations in the short- and long-term; however, its effect will depend on the depth distribution of residue input by crops. The objective of this study was to assess the effects of long-term tillage systems, and after short-term tillage system conversion on indicators related to SOM pools and to identify the most suitable soil attributes for assessing SQ in a semi-humid region of the Argentine Pampas.

## **Material and Methods**

### ***Experimental site***

The study was conducted on a long-term trial established in 1986 at Tornquist (38° 07' 06" S - 62° 02' 17" W) in the southwest of the Pampas, Argentina. The soil was classified as a Luvic Phaeozem (IUSS Working Group WRB, 2015) and was over 2 meters deep, with a loamy texture in the A horizon and clayey-loamy in the B<sub>2</sub> horizon.

According to Thornthwaite (1948), the climate was classified as semi-humid. The rainfall seasonal distribution determined an udic soil moisture regime with irregular distribution (Soil Survey Staff, 2010). The mean annual temperature in this area was 15 °C and the annual precipitation was 735 mm (averaged for the period 1887-2015).

An experiment was initiated to compare two tillage systems: conventional tillage (CT) and no-tillage (NT). The experiment was designed using a randomized complete block with three replicates. The plot size was 660 m<sup>2</sup> (33 m x 20 m). After 26 years (2012), plots were split into two subplots, half of them being managed as previously, *i.e.* CT and NT, whereas the other half being converted to the alternative tillage technique (from NT to CT and from CT to NT), yielding four treatments. In 2015, soil samples were collected from these four treatments (long- and short-term tillage effects) and from a site without any important activity (Ref). Therefore, the treatments studied were:

- No-tillage (NT): long-term direct drilling (1986-2015).
- Conventional tillage (CT): operations performed with a plough and disc harrow during fallow to prepare the soil (1986-2015).
- Recent no-tillage (NT<sub>n</sub>): long-term CT converted to NT and kept under this system for the last 3 years.
- Recent conventional tillage (CT<sub>n</sub>): long-term NT converted to CT and kept under this system for the last 3 years.
- Reference (Ref): natural site adjacent to the tillage system experiment without cultivation, with abundant grass-herbaceous strata and presence of trees (*Acacia sp.*).

The crops grown during the studied rotation were: M, maize (*Zea mays* L.); W, wheat (*Triticum aestivum* L.); S, sunflower (*Helianthus annuus* L.); B, barley (*Hordeum vulgare* L.); and So, sorghum (*Sorghum bicolor* L. Moench). The full crop sequence during the period of study (1986-2015) was: M-W-S-W-S-W-So-B-M-B-M-W-M-W-B-S-W-W-S-B-S-W-M (under pastures)-W (no harvest because of drought)-W-W-So-M-B-W. Details on site managements and crop yields in long-term trials can be found in Martínez *et al.* (2017).

The CT and CT<sub>n</sub> were based on two disk operations to mix the residues with the soil: one in the early summer fallow to the 15-cm depth and another before sowing to 10 cm. Crop residue covered <20% of the soil surface under ploughing. In contrast, NT and NT<sub>n</sub> were characterized by the absence of tillage, with over 30% residues covering the soil surface before crop sowing. Under the NT system, a direct-drill seeder was used to sow directly into the standing residues of the previous crop. A herbicide (2 L ha<sup>-1</sup> of glyphosate) was applied for weed control, at the beginning of the fallow and prior to crop sowing. The plots received 10 kg P ha<sup>-1</sup> year<sup>-1</sup> and 9 kg N ha<sup>-1</sup> year<sup>-1</sup> as diammonium phosphate (18N-20P-0K) at sowing in both tillage systems. The mechanical fallow (CT) was started according to the summer-autumn rainfall occurrence, whereas the chemical fallow (NT) was generally begun in autumn for weed control. The winter wheat was sown in June-July and harvested by late December. The cultivars and management practices used were those recommended for the region (Martínez *et al.*, 2017).

### ***Soil chemical and physical analysis***

A composite soil sample was collected for each of four depths (0-5, 5-10, 10-20 and 0-20 cm) from each replication. The soil was air-dried, sieved and homogenized to 2 mm, and the retained plant residues were discarded. The soil samples were chemically analyzed to determine: SOC by dry combustion using a Leco C automatic analyzer (Leco Corporation, St Joseph, MI); SON by the micro-Kjeldahl method (Bremner, 1996). Particulate organic C (>53 microns) was determined after soil fractionation by particle size using the method described by Duval *et al.* (2013). Carbon and N contents in POM were determined using the same methods as for SOC and SON, respectively.

For biochemical properties, PMN was determined through the anaerobic incubation method developed by Waring & Bremner (1964) and soil respiration (R<sub>s</sub>) by the alkali absorption method (Zibilske, 1994).

Undisturbed soil samples were also taken at the 0-5, 5-10, 10-15 and 15-20 cm depths with a steel cylinders 5 cm in height and 4.7 cm in diameter to calculate bulk density (BD) (Blake & Hartge 1986). Concentrations were then converted to contents using BD data. The equivalent soil mass was not compared between tillage systems because previous studies on the same site had found soil erosion (Galantini *et al.*, 2006; Toledo *et al.*, 2013b).

### ***Functional indicators of soil quality***

Several ratios between indicators –referred to as functional indicators- were evaluated for each depth, following different authors (Toledo *et al.*, 2013a; Martínez & Galantini, 2017; Martínez *et al.*, 2017): i) easily mineralizable N fraction (PMN/SON); ii) proportion of labile N of SON (POM-N/SON); iii) potential supply of N from labile SON (PMN/POM-N), iv) proportion of labile C of SOC (POM-C/SOC); v) susceptibility to biological degradation of SOC ( $R_s$ /SOC), vi) susceptibility to biological degradation of labile SOC ( $R_s$ /POM-C); vii) soil capacity to store and recycle nutrients from labile SOM (POM-C/POM-N); viii) soil capacity to store and recycle nutrients from SOM (SOC/SON).

### ***Statistical Analysis***

Normality and homoscedasticity of data was checked by Bartlett's tests. An analysis of variance (ANOVA) was used to determine differences in SQ indicators between long-term treatments and the non-cultivated site at each depth. For evaluating differences between the long-term tillage systems and the short-term after tillage conversion (NT vs NT<sub>n</sub> and CT vs CT<sub>n</sub>), a simple ANOVA was used. The means for treatments were compared using the least significant difference ( $P < 0.05$ ). To summarize the total variance of the data and to select the most appropriate SQ indicators, a principal component analysis (PCA) was performed including individual and functional indicators for the 0-5 and 0-20 cm data separately, using long- and short-term tillage systems as classification criteria. Indicators with weighted loading values within 10% of the highest weighted loading were selected for each principal component (PC) (Li *et al.*, 2013). The statistical analysis was carried out with Infostat software (Di Rienzo *et al.*, 2018).

## **Results**

### ***Bulk density***

Long-term tillage systems exerted a significant influence on bulk density at all depths, except at the 10-20 cm layer. Highly significant differences were observed at 0-5 cm ( $P = 0.0025$ ) between NT and Ref with CT (Figure 1). Overall, BD values were greater for NT at the 5-10 cm soil depth. Bulk density was larger under NT at 0-20 cm, being significantly different from Ref and CT. To avoid biased interpretations caused by differences in BD between the long-term tillage systems, SOM fractions and biochemical indicators were analyzed in contents.

### ***Soil organic matter fractions***

The SOC showed highly significant differences ( $P < 0.001$ ) between long-term tillage systems and Ref for most depths (0-5, 5-10 and 0-20 cm) (Table 1). At 0-5 cm, significant differences were found between the three long-term treatments, the rank order was: Ref>NT>CT. When comparing short-term effects after tillage conversion, significant differences were found between NT<sub>n</sub> and NT at 0-5 cm and 5-10 cm, with greater SOC values under NT. However, no significant differences were found between CT and CT<sub>n</sub> in SOC values at all depths.

For SON, highly significant differences were found between the long-term treatments at each depth ( $P < 0.001$ ). Distribution of SON varied in the long-term treatment in the 0-5 soil layer, with significant differences between treatments: Ref>NT>CT (Table 1). However, differences between CT and NT disappeared at 5-10 cm depth, and only a difference between both tillage systems and Ref could be detected ( $P = 0.002$ ). Similar results were found at 10-20 and 0-20 cm depth. SON content was significantly greater after tillage system conversion under CT<sub>n</sub> in comparison with CT at 0-5; 10-20 and 0-20 cm. Significant differences were found between NT and NT<sub>n</sub> for all the depths evaluated. However, SON content after system conversion (NT<sub>n</sub>) was greater than in NT only at 10-20 cm depth.

The content of POM-C and POM-N showed significant differences ( $P < 0.05$ ) between long-term treatments at each depth. Similar values between CT and NT were observed at 0-5 cm depth; however, significant differences between treatments were detected with Ref>CT>NT at 5-10, 10-20 and 0-20 cm. In contrast, no significant differences ( $P > 0.05$ ) were found in the POM-C content between NT-NT<sub>n</sub> and CT-CT<sub>n</sub> for most depths (Table 1), except for 10-20 cm depth, where POM-C under CT<sub>n</sub> was higher than under CT. No significant differences in POM-N were detected between CT and NT at 0-5 and 5-10 cm; however, larger values under CT in comparison with NT were found at 10-20 and 0-20 cm, following the same trend as POM-C. With respect to the short-term effect following tillage conversion, larger POM-N values were detected under NT<sub>n</sub> at 10-20 and 0-20 cm, than for NT with no differences ( $P > 0.05$ ) between them at other depths. Considerable POM-N was found in CT<sub>n</sub> at 0-5 and 5-10 cm; however, no significant differences were found between CT and CT<sub>n</sub> at 10-20 and 0-20 cm.

### ***Biochemical indicators***

The long-term effects of tillage on PMN were highly significant ( $P < 0.001$ ) at all depths (Table 1). Significant differences were found between all treatments at 0-5 cm, where Ref>NT>CT. However, this rank order among treatments was modified at the rest of depths, Ref >CT>NT. At

0-20 cm, highly significant differences were found between Ref and both tillage systems ( $P < 0.001$ ), with no differences between CT and NT. When comparing short-term effects of tillage conversion, significant differences were found between  $CT_n$  and CT, with greater PMN values under  $CT_n$  at 0-5 cm, and larger values under CT at 5-10 and 10-20 cm. No significant differences were detected in PMN at 0-20 cm depth. With respect to  $NT_n$  and NT, a greater PMN was detected under  $NT_n$  at 10-20 cm.

Significant differences were found in soil respiration ( $R_s$ ) between treatments at all depths in the long-term plots; the rank order was  $Ref > CT > NT$  at 0-20 cm, and larger values were detected under Ref (Table 1). When comparing long-term tillage systems with tillage system conversion ( $NT-NT_n$  and  $CT-CT_n$ ) there were no significant differences in this indicator at most depths, except for 10-20 cm depth, where  $R_s$  under NT was greater than under  $NT_n$ .

### ***Functional indicators of soil quality***

At all depths, PMN/SON values ( $P < 0.001$ ) were larger in Ref than in CT and NT in long-term treatments (Table 2). Significant differences were found between the tillage systems, with larger values in NT at 0-5 cm, and higher values under CT at all other depths. The short-term effects after tillage conversion showed that PMN/SON values increased at 0-5 cm depth in  $CT_n$  ( $P = 0.003$ ) but they declined significantly at 0-20 cm compared to CT ( $P = 0.021$ ). At 5-10 cm, no differences ( $P > 0.05$ ) were found for this indicator between the long term tillage systems and short-term following tillage conversion. Conversely, opposite results were found at 10-20 cm, with larger values being obtained in  $NT_n$  than in NT ( $P = 0.001$ ), whereas smaller values were found in  $CT_n$  relative to CT ( $P = 0.011$ ).

The PMN/POM-N showed significant differences ( $P < 0.05$ ) between long-term treatments for all depths (Table 2). Some differences occurred between NT and  $NT_n$  at all depths, with larger values under NT at 0-5, 5-10 and 0-20 cm, and higher values under  $NT_n$  at 10-20 cm. When comparing CT with  $CT_n$  there were no significant differences in this indicator at 0-5 and 0-20 cm; however, large values were observed under CT at 5-10 and 10-20 cm.

With respect to POM-C/SOC, significant differences ( $P < 0.01$ ) were found between long-term treatments, with larger values in Ref at all depths other than 0-5 cm (Table 2). At 0-20 cm, significant differences were found among long-term treatments, where  $Ref > CT > NT$ . Under  $NT_n$ , however, larger values ( $P = 0.017$ ) were observed at 0-5 and 5-10 cm in comparison with NT, but no differences were detected at other depths.



The POM-N/SON ratio showed significant differences in the long term at 0-5 and 0-20 cm, with large values in CT and Ref, respectively (Table 2). At 5-10 and 10-20 cm no differences between treatments were detected. With respect to the short-term effects after tillage conversion, values were higher under NT<sub>n</sub>, with significant differences at 0-5 (P=0.031); 5-10 (P=0.006) and 0-20 cm (P=0.023) and under CT<sub>n</sub> at 5-10 cm (P=0.001).

Significant differences (P<0.05) were found for the R<sub>s</sub>/SOC ratio between the long-term treatments for most depths, except for the 0-5 cm soil layer. Similar values were found in Ref and CT at 0-5 and 5-10 cm (Table 2). However, no significant differences were found between NT and CT at 10-20 cm, and long-term treatments presented differences at 0-20 cm, where Ref>CT>NT. The short-term tillage conversion, no significant differences (P>0.05) were found between NT and NT<sub>n</sub> and CT and CT<sub>n</sub> for any depth.

The R<sub>s</sub>/POM-C ratio did not show any significant differences between long-term treatments at all depths (Table 2). Regarding the short-term effects after tillage conversion, no significant differences (P>0.05) were found between tillage systems (NT-NT<sub>n</sub> and CT-CT<sub>n</sub>) for most depths, except for 10-20 cm depth, with higher values of this indicator under NT.

The SOC/SON index did not show any significant differences for most depths (Table 2), except for 0-20 cm, where larger values were observed for CT and NT than for Ref. Short-term effect after tillage conversion, showed that significantly larger values were detected in CT when compared to CT<sub>n</sub> at 0-20 cm.

With respect to POM-C/POM-N, some significant differences were found at 0-5 and 10-20 cm, but not at 5-10 and 0-20 cm (Table 2). At 0-5 cm, Ref and CT differed from NT, whereas NT and CT differed from Ref at 10-20 cm. For short-term effects, no differences were found between NT-NT<sub>n</sub> and CT-CT<sub>n</sub> at 0-5, 5-10 and 0-20 cm; however, at 10-20 cm this ratio was higher under CT<sub>n</sub> and NT when compared, respectively, with CT and NT<sub>n</sub>.

From the PCA, taking account of all indicators, the first two PCs represented more than 63% and 68% of the total variability at 0-5 and 0-20 cm, respectively (Figure 2a, b). At 0-5 cm, the first PC explained 40% of the variance, where the most stable fractions (SOC, SON), PMN and PMN/POM-N contributed positively to this PC. PC1 mainly separates NT from CT, CT<sub>n</sub> and NT<sub>n</sub>. In the case of PC2, POM-C and POM-C/POM-N had the high and positive loadings. This second PC did not permit full separation of treatments. At the 0-20 cm depth, the first PC explained 45% of the variance, where POM-C, R<sub>s</sub>, PMN/SON, POM-N/SON and R<sub>s</sub>/SOC showed positive associations and SON was negatively associated to this component. PC1 mainly separates

treatments under no-till (NT and NT<sub>n</sub>) from treatments with ploughing (CT and CT<sub>n</sub>) irrespective of the time period. Moreover, POM-C/POM-N, PMN/POM-N contributed positively to PC2, whereas SOC was negatively associated to this component. PC2 separates treatments in accordance to the period of time under agricultural system: long-term and short-term after tillage conversion.

## **Discussion**

### ***Soil organic matter fractions***

The results showed that the SOC and SON values increased under NT in the long-term, but only in the upper layer as reported by several authors (Staley *et al.*, 1988; Martínez *et al.*, 2017). Therefore, the redistribution and incorporation of crop residues in subsurface layers under CT did not increase SOC and SON contents at depths before the surface layer. These results in the subsurface were opposite to those found in the literature, maybe because of the smaller contribution of residue input under CT, due to the smaller yields since the beginning of the long-term trial as reported by Martínez *et al.* (2017). Another possible cause of this result could be soil degradation by erosion (Galantini *et al.*, 2006). Despite the large SOC and SON under NT in the surface layer, POM-C and POM-N, did not show significant differences between CT and NT at this depth. This may be related not only to the quantity but also to the quality of residues (Studdert & Echeverría 2006), which are supplied mainly by gramineous crops (wheat, barley and maize) with a high C:N (>40) ratio. For other depths, larger POM-C and POM-N values were detected under CT, possibly due to the contribution of residues in depth and the particular conditions of ploughing. The short-term effects after tillage conversion, were mixed with some decreases and increases being detected in the SOC and SON levels and their particulate fractions. The CT<sub>n</sub> showed an increase in the SON values with respect to CT for most depths, whereas NT<sub>n</sub> did not equal the SOC and SON values under NT at any depth. This may be attributed to a smaller SOM level under long-term CT compared to NT.

Although both biochemical indicators showed significant differences between the long-term tillage systems at all depths, R<sub>s</sub> was a more sensitive indicator because it showed variations in the short-term after tillage conversion. Higher values of PMN values were found at the surface under NT. According to Balesdent *et al.* (2000), this increment may be linked to a higher labile N-pool due to the continuous build-up of crop residues on the topsoil. Although no significant differences were found in C and N in the POM between CT and NT at 0-5 cm, the larger values of PMN under

CT at other depths followed a similar pattern to POM-C and POM-N. This may have occurred because of a more homogeneous distribution of residues at depth, *i.e.* POM, which is highly related to PMN as reported by several authors (Martínez *et al.*, 2017; Martínez *et al.*, 2018b). Moreover, it is important to note that POM-C and POM-N are considered as intermediate C and N levels between slow (SOC and SON) and active fractions such as PMN and  $R_s$  (Six *et al.*, 1999). For this reason, this result may not be only due to an increase in labile fractions in the upper layer, but also to a greater protection of these fractions in the undisturbed soil (Six *et al.*, 1999). In contrast, deeper tillage results in homogeneous POM distribution and thus enhances mineralization potential in deeper layers under CT (Martínez *et al.*, 2017). Also,  $R_s$  showed differences between long-term treatments for all depths, with larger values under CT. Quincke *et al.* (2007) reported that CO<sub>2</sub> emissions increase in intensity and depth of tillage, probably because of greater access to labile SOM and increased aeration after ploughing.

#### ***Functional indicators of soil quality***

Among functional indicators, only the PMN/SON ratio demonstrated differences between long-term tillage systems for all depths. The PMN/SON values - larger in the surface layer for NT but more under CT at other depths- could be related to the place where crop residues are deposited, suggesting that the location of the residues possibly influences the particulate and the active SOM fractions, *i.e.* easy mineralizable N fraction. Moreover, it was observed that the tillage systems after conversion (CT<sub>n</sub> and NT<sub>n</sub>) equaled the effects of long-term NT and CT for most cases. This indicates that easily mineralizable N fraction is an indicator with a great sensitivity for differentiating the contrasting tillage systems in the long- and short-term following tillage conversion. Regardless of the treatment and sampling depth, the PMN/SON values ranged from 0.68 to 3.3%, in coincidence with values reported by other authors (Martínez *et al.*, 2017; Martínez & Galantini, 2017). In general, the results from this study support the hypothesis that conversion from NT to CT and vice versa would modify the dynamics and location of some labile organic fractions, however, its effects on the different functional indicators were not clear, except for PMN/SON ratio. Moreover, for the 0-20 cm layer, POM-C/SOC; POM-N/SON and  $R_s$ /SOC differentiated both the long- and short-term treatments. For this reason, they might be used as SQ indicators to assess the effects of tillage systems on soil quality, when sampling depth is 0-20 cm.

The PCA contributed to a more precise data selection showing which individual and functional indicators were more powerful in differentiating long- and short-term tillage systems from the 0-5

and 0-20 cm data. The PCA showed the effect of tillage system, which mainly explain the variance of SQ indicators. At 0-5 cm, the indicators were affected by the accumulation of SOM (SOC and SON) on soil surface by long-term NT, increasing the labile N and its contribution to PMN (Martinez *et al.*, 2017; Martínez *et al.*, 2018b). The separation between long- and short-term treatments under NT suggested that even after three-years the effect of converting to the NT<sub>n</sub> system was not able to match the accumulated long-term effects -developed over 30 years under no-till- still being similar to CT and CT<sub>n</sub>. Furthermore, time is important to the influence of tillage on SOM pools (Christopher *et al.*, 2009). For that reason, to confirm the magnitude of changes by tillage systems converted under semi-humid conditions, it is recommended to analyze these effects after more than three-years under same tillage system. It can be inferred that SOC, SON, PMN and PMN/POM-N were the most sensitive indicators for differentiating tillage systems at 0-5 cm; however, it is important to note that these indicators differentiate the accumulated long-term NT effects from the other treatments. At 0-20 cm, POM-C, R<sub>s</sub>, PMN/SON, POM-N/SON, R<sub>s</sub>/SOC and SON were more sensitive for differentiating non-tilled and tilled treatments regardless of the number of years under the same system.

## **Conclusions**

In the long term, the tillage system affected labile SOM fractions and their distribution in depth. NT shows a strong stratification of the labile N pool and its mineralizable fraction in the topsoil. In contrast, the labile SOM distribution occurred in deeper layers under CT, although increases with respect to NT were not observed for all indicators. Three years after tillage system conversion, significant changes occurred in the distribution of the SOM fractions due to the effect of the residue location and its contribution between the labile fractions; however, under these particular conditions this short-term effect would not improve the cumulative effect of long-term for most cases.

The PMN/SON ratio proved to be a promising indicator when assessing tillage effects on SQ in the long-term and short-term following tillage conversion, regardless of the sampling depth. However, under the conditions of the current study and considering that functional indicators may be more appropriate for assessing SQ, PMN/SON, POM-N/SON and R<sub>s</sub>/SOC could be used as SQ indicators for differentiating tillage systems when sampling depth is approximately 0-20 cm.

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Figure 1. Bulk density per treatment and depth in 2015. Different letters indicate significant differences ( $P < 0.05$ ) among tillage systems for the same depth. Ref, non-cultivated soil; CT, conventional tillage; NT, no-tillage. Error bars indicate standard deviation.

Figure 2. Biplot of principal component analysis for individual and functional SQ indicators by long-term (NT, CT) and short-term tillage systems after conversion (NT<sub>n</sub>, CT<sub>n</sub>) at 0-5 cm (a) and 0-20 cm (b). SOC, soil organic carbon; SON, soil organic N; POM-C, particulate organic matter C; POM-N, particulate organic matter N; PMN, potential N mineralization; R<sub>s</sub>, soil respiration; PMN/SON, easily mineralizable N fraction, POM-N/SON, proportion of labile N of SON; PMN/POM-N, potential supply of N from labile SON; POM-C/SOC, proportion of labile C of SOC; R<sub>s</sub>/SOC, susceptibility to biological degradation of SOC; R<sub>s</sub>/POM-C, susceptibility to biological degradation of labile SOC; POM-C/POM-N, soil capacity to store and recycle nutrients from labile SOM; SOC/SON, soil capacity to store and recycle nutrients from SOM.

Table 1. Soil quality indicators (mean  $\pm$  standard deviation) at different depths as a function of long-term and short-term tillage conversion.

TS	Depth (cm)	SOC		SON		POM-C			POM-N			PMN		R <sub>s</sub>	
						(Mg ha <sup>-1</sup> )						(kg ha <sup>-1</sup> )		(kg C-CO <sub>2</sub> ha day <sup>-1</sup> )	
Ref	0-5	22.2 $\pm$ 5.9	a	2.1 $\pm$ 0.19	a	5.3 $\pm$ 0.6	a	0.35 $\pm$ 0.03	a	69.8 $\pm$ 4.9	a	23.8 $\pm$ 3.7	a		
NT		14.4 $\pm$ 1.3	b A	1.4 $\pm$ 0.01	b A	2.4 $\pm$ 0.1	b A	0.19 $\pm$ 0.01	b A	33.1 $\pm$ 4.3	b A	9.8 $\pm$ 1.4	b A		
CT		11.4 $\pm$ 1.2	c A	0.9 $\pm$ 0.03	c B	2.5 $\pm$ 0.2	b A	0.17 $\pm$ 0.01	b B	20.7 $\pm$ 0.5	c B	13.8 $\pm$ 6.2	b A		
NT <sub>n</sub>		11.9 $\pm$ 0.2	B	1.0 $\pm$ 0.04	B	2.4 $\pm$ 0.1	A	0.20 $\pm$ 0.01	A	22.3 $\pm$ 2.3	B	10.6 $\pm$ 2.4	A		
CT <sub>n</sub>		11.9 $\pm$ 0.9	A	1.0 $\pm$ 0.01	A	4.5 $\pm$ 0.5	A	0.44 $\pm$ 0.05	A	27.9 $\pm$ 1.4	A	26.5 $\pm$ 3.3	A		
Ref	5-10	16.5 $\pm$ 1.8	a	1.6 $\pm$ 0.07	a	3.5 $\pm$ 0.1	a	0.31 $\pm$ 0.07	a	39.7 $\pm$ 3.3	a	9.4 $\pm$ 0.4	a		
NT		12.4 $\pm$ 0.6	b A	1.2 $\pm$ 0.07	b A	1.4 $\pm$ 0.1	c A	0.15 $\pm$ 0.01	b A	12.4 $\pm$ 1.4	c A	3.3 $\pm$ 0.9	b A		
CT		12.6 $\pm$ 0.9	b A	1.1 $\pm$ 0.04	b A	1.7 $\pm$ 0.1	b A	0.17 $\pm$ 0.00	b B	24.6 $\pm$ 1.9	b A	7.6 $\pm$ 2.0	a A		
NT <sub>n</sub>		9.6 $\pm$ 1.0	B	0.9 $\pm$ 0.03	B	1.5 $\pm$ 0.1	A	0.17 $\pm$ 0.01	A	9.3 $\pm$ 1.5	A	3.8 $\pm$ 0.8	A		
CT <sub>n</sub>		11.7 $\pm$ 0.4	A	1.0 $\pm$ 0.03	B	2.1 $\pm$ 0.6	A	0.21 $\pm$ 0.01	A	21.4 $\pm$ 0.2	B	8.5 $\pm$ 3.0	A		
Ref	10-20	25.4 $\pm$ 3.6	a	2.3 $\pm$ 0.16	a	5.0 $\pm$ 0.1	a	0.45 $\pm$ 0.07	a	49.5 $\pm$ 7.0	a	9.9 $\pm$ 1.9	a		
NT		21.1 $\pm$ 2.2	a A	1.6 $\pm$ 0.07	b B	2.3 $\pm$ 0.1	c A	0.24 $\pm$ 0.01	c B	11.4 $\pm$ 1.2	c B	3.3 $\pm$ 0.6	b A		
CT		21.7 $\pm$ 1.9	a A	1.8 $\pm$ 0.06	b B	2.6 $\pm$ 0.2	b A	0.32 $\pm$ 0.02	b A	22.9 $\pm$ 1.5	b A	4.0 $\pm$ 1.6	b A		
NT <sub>n</sub>		20.2 $\pm$ 1.8	A	1.9 $\pm$ 0.06	A	2.3 $\pm$ 0.2	A	0.29 $\pm$ 0.02	A	22.5 $\pm$ 1.6	A	2.0 $\pm$ 0.2	B		
CT <sub>n</sub>		22.5 $\pm$ 0.6	A	2.0 $\pm$ 0.04	A	3.2 $\pm$ 0.1	A	0.34 $\pm$ 0.03	A	13.6 $\pm$ 0.7	B	2.5 $\pm$ 1.1	A		
Ref	0-20	46.7 $\pm$ 1.3	a	6.0 $\pm$ 0.41	a	16.1 $\pm$ 1.3	a	1.25 $\pm$ 0.06	a	159 $\pm$ 14.9	a	43.6 $\pm$ 3.4	a		
NT		40.6 $\pm$ 0.7	b A	4.2 $\pm$ 0.25	b A	6.0 $\pm$ 0.5	c A	0.59 $\pm$ 0.03	c B	58.4 $\pm$ 2.6	b A	16.8 $\pm$ 0.8	c A		

CT	39.0±0.9	b	<b>B</b>	3.7±0.19	b	<b>B</b>	8.3±1.4	b	<b>A</b>	0.71±0.04	b	<b>A</b>	68.7±3.3	b	<b>A</b>	26.9±5.6	b	<b>A</b>
NT <sub>n</sub>	41.7±2.6		A	3.9±0.05		B	6.6±0.3		A	0.70±0.05		A	55.9±4.7		A	19.5±3.8		A
CT <sub>n</sub>	46.1±1.6		<b>A</b>	4.0±0.03		<b>A</b>	6.8±1.3		<b>A</b>	0.76±0.11		<b>A</b>	62.9±1.5		<b>A</b>	27.5±5.2		<b>A</b>

TS, tillage system. Ref, reference site; CT, conventional tillage; NT, no-tillage; CT<sub>n</sub>, NT converted to CT in 2012; NT<sub>n</sub>, CT converted to NT in 2012. SOC, soil organic carbon (Mg ha<sup>-1</sup>); SON, soil organic N (Mg ha<sup>-1</sup>); POM-C, particulate organic matter C (Mg ha<sup>-1</sup>); POM-N, particulate organic matter N (Mg ha<sup>-1</sup>); PMN, potential N mineralization (kg ha<sup>-1</sup>); R<sub>s</sub>, soil respiration (kg C-CO<sub>2</sub> ha day<sup>-1</sup>). Different lower-case letters indicate significant differences (P<0.05) among the long-term treatments (Ref, NT, CT) for the same depth; different upper-case and bold underlined upper-case letters indicate significant differences (P<0.05) for NT-NT<sub>n</sub> and CT-CT<sub>n</sub> at each depth, respectively.

Table 2. Soil quality indicator ratios (mean  $\pm$  standard deviation) at different depths as a function of long-term and short-term tillage conversion.

TS	Dept h (cm)	PMN/SON PMN/POM-N POM-C/SOC POM-N/SON R <sub>s</sub> /SOC R <sub>s</sub> /POM-C										SOC/SON		POM-C/POM-N			
		(%)												N			
Ref	0-5	3.3 $\pm$ 0.0	a	20.2 $\pm$ 0.	a	25.0 $\pm$ 7.	a	16.1 $\pm$ 0.	b	0.115 $\pm$ 0.0	a	0.46 $\pm$ 0.1	a	10.6 $\pm$ 2.	a	15.1 $\pm$ 1.	a
		5		9		0		9		4		0		9		1	
NT		2.6 $\pm$ 0.1	b A	17.7 $\pm$ 2.	a A	16.7 $\pm$ 1.	a B	13.8 $\pm$ 0.	c B	0.068 $\pm$ 0.0	a A	0.41 $\pm$ 0.0	a A	10.3 $\pm$ 1.	a A	13.7 $\pm$ 0.	b A
		7		7		2		3		1		6		0		5	
CT		2.3 $\pm$ 0.0	c B	12.0 $\pm$ 0.	b A	21.9 $\pm$ 0.	a A	19.5 $\pm$ 0.	a A	0.120 $\pm$ 0.0	a A	0.55 $\pm$ 0.2	a A	12.8 $\pm$ 0.	a A	14.7 $\pm$ 0.	a A
		4		3		8		3		5		5		9		4	
NT <sub>n</sub>		2.2 $\pm$ 0.2	A	11.2 $\pm$ 0.	B	20.3 $\pm$ 0.	A	19.8 $\pm$ 0.	A	0.090 $\pm$ 0.0	A	0.44 $\pm$ 0.0	A	11.9 $\pm$ 0.	A	12.0 $\pm$ 1.	A
		7		9		7		9		2		9		3		0	
CT <sub>n</sub>		2.8 $\pm$ 0.1	A	12.8 $\pm$ 2.	A	25.5 $\pm$ 1	A	22.1 $\pm$ 2.	A	0.112 $\pm$ 0.0	A	0.48 $\pm$ 0.1	A	11.9 $\pm$ 0.	A	13.6 $\pm$ 7.	A
		2		0		1		6		2		3		9		5	
Ref	5-10	2.6 $\pm$ 0.1	a	13 $\pm$ 2.9	a	21.6 $\pm$ 2.	a	20.2 $\pm$ 4.	a	0.057 $\pm$ 0.0	a	0.27 $\pm$ 0.0	a	10.3 $\pm$ 1.	a	11.9 $\pm$ 2.	a
		2				9		5		1		1		5		5	
NT		1.1 $\pm$ 0.0	c A	8.2 $\pm$ 1.0	b A	11.2 $\pm$ 0.	b B	13.2 $\pm$ 1.	a B	0.027 $\pm$ 0.0	b A	0.24 $\pm$ 0.0	a A	10.3 $\pm$ 1.	a A	9.3 $\pm$ 1.5	a A
		7				5		7		1		9		2			
CT		2.2 $\pm$ 0.1	b A	14.2 $\pm$ 1.	a A	13.9 $\pm$ 0.	b A	15.7 $\pm$ 0.	a B	0.061 $\pm$ 0.0	a A	0.44 $\pm$ 0.1	a A	11.5 $\pm$ 1.	a A	10.1 $\pm$ 0.	a A
		1		3		3		7		2		3		0		7	
NT <sub>n</sub>		1.1 $\pm$ 0.1	A	5.6 $\pm$ 0.5	B	16.1 $\pm$ 2.	A	19.6 $\pm$ 1.	A	0.041 $\pm$ 0.0	A	0.25 $\pm$ 0.0	A	10.7 $\pm$ 0.	A	8.9 $\pm$ 0.8	A
		4				1		1		1		5		8			

CT		2.2±0.0	<b>A</b>	10.4±0.	<b>B</b>	18.2±5.	<b>A</b>	21.2±0.	<b>A</b>	0.073±0.0	<b>A</b>	0.43±0.1	<b>A</b>	11.7±0.	<b>A</b>	10.0±3.	<b>A</b>
n		8		6		4		9		3		9		7		0	
Ref	10-20	2.2±0.1	a	11.1±2.	a	20.1±3.	a	20.1±3.	a	0.040±0.0	a	0.20±0.0	a	11.0±2.	a	11.1±1.	a
		5		5		1		9		1		4		1		7	
NT		0.7±0.0	c <b>B</b>	4.8±0.5	b <b>B</b>	10.7±1.	b <b>A</b>	14.6±1.	a <b>A</b>	0.016±0.0	b <b>A</b>	0.15±0.0	a <b>A</b>	13.2±1.	a <b>A</b>	8.8±0.6	b <b>A</b>
		6				1		0		1		3		6			
CT		1.3±0.1	b <b>A</b>	7.1±0.7	b <b>A</b>	12.1±1.	b <b>A</b>	18.2±1.	a <b>A</b>	0.019±0.0	b <b>A</b>	0.15±0.0	a <b>A</b>	12.1±1.	a <b>A</b>	8.0±0.4	b <b>B</b>
		2				8		2		1		5		3			
NT		1.2±0.1	<b>A</b>	7.8±1.0	<b>A</b>	11.6±0.	<b>A</b>	15.0±1.	<b>A</b>	0.010±0.0	<b>A</b>	0.09±0.0	<b>B</b>	10.6±1.	<b>A</b>	8.0±0.4	<b>B</b>
n		0				9		3		1		2		2			
CT		0.7±0.0	<b>B</b>	4.1±0.5	<b>B</b>	14.3±0.	<b>A</b>	16.9±1.	<b>A</b>	0.011±0.0	<b>A</b>	0.08±0.0	<b>A</b>	11.2±0.	<b>A</b>	9.4±0.7	<b>A</b>
n		5				8		3		1		3		2			
Ref	0-20	2.8±0.2	a	14.3±1.	a	21.5±1.	a	18.8±0.	a	0.067±0.0	a	0.31±0.0	a	10.9±0.	b	12.4±1.	a
		2		8		7		8		09		4		1		4	
NT		1.4±0.1	c <b>A</b>	9.6±0.5	b <b>A</b>	12.7±0.	c <b>A</b>	14.1±0.	c <b>B</b>	0.034±0.0	c <b>A</b>	0.28±0.0	a <b>A</b>	11.7±0.	a <b>A</b>	10.3±1.	a <b>A</b>
		0				8		8		01		2		7		1	
CT		1.9±0.0	b <b>A</b>	9.8±0.9	b <b>A</b>	14.9±4.	b <b>A</b>	17.1±0.	b <b>A</b>	0.056±0.0	b <b>A</b>	0.37±0.1	a <b>A</b>	12.0±0.	a <b>B</b>	10.5±1.	a <b>A</b>
		7				0		7		13		0		6		3	
NT		1.4±0.1	<b>A</b>	8.2±0.7	<b>B</b>	15.1±1.	<b>A</b>	17.4±1.	<b>A</b>	0.039±0.0	<b>A</b>	0.26±0.0	<b>A</b>	11.0±0.	<b>A</b>	9.5±0.7	<b>A</b>
n		3				2		0		12		6		8			
CT		1.6±0.0	<b>B</b>	8.4±1.4	<b>A</b>	17.9±3.	<b>A</b>	19.2±2.	<b>A</b>	0.053±0.0	<b>A</b>	0.29±0.0	<b>A</b>	11.5±0.	<b>A</b>	10.9±1.	<b>A</b>
n		4				2		7		14		9		4		1	

TS, tillage systems. Ref, reference site; CT, conventional tillage; NT, no-tillage; CT<sub>n</sub>, NT converted to CT in 2012; NT<sub>n</sub>, CT converted to NT in 2012. PMN/SON, easily mineralizable N fraction; PMN/POM-N, potential supply of N from labile SON; POM-C/SOC, proportion of labile C of SOC; POM-N/SON, proportion of labile N of SON; R<sub>s</sub>/SOC, susceptibility to biological degradation of SOC; R<sub>s</sub>/POM-C, susceptibility to biological degradation of labile SOC; SOC/SON, soil capacity to store and recycle nutrients from SOM; POM-C/POM-N, soil capacity to store and recycle nutrients from labile SOM. Different lower-case letters indicate significant differences (P<0.05) among the long-term treatments (Ref, NT, CT) for the same depth; different upper-case and bold underlined upper-case letters indicate significant differences (P<0.05) for NT-NT<sub>n</sub> and CT-CT<sub>n</sub> at each depth, respectively.

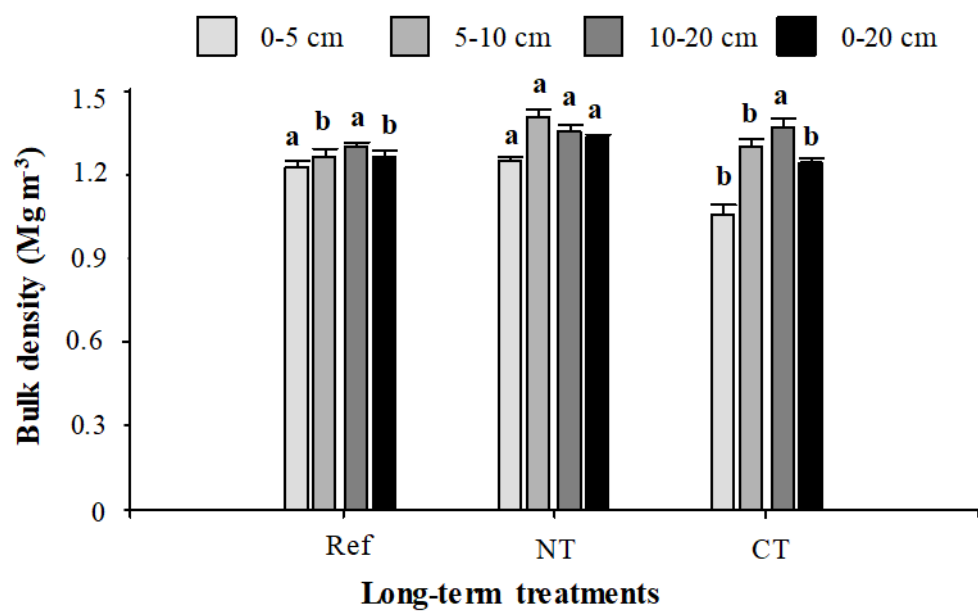


Figure 1.

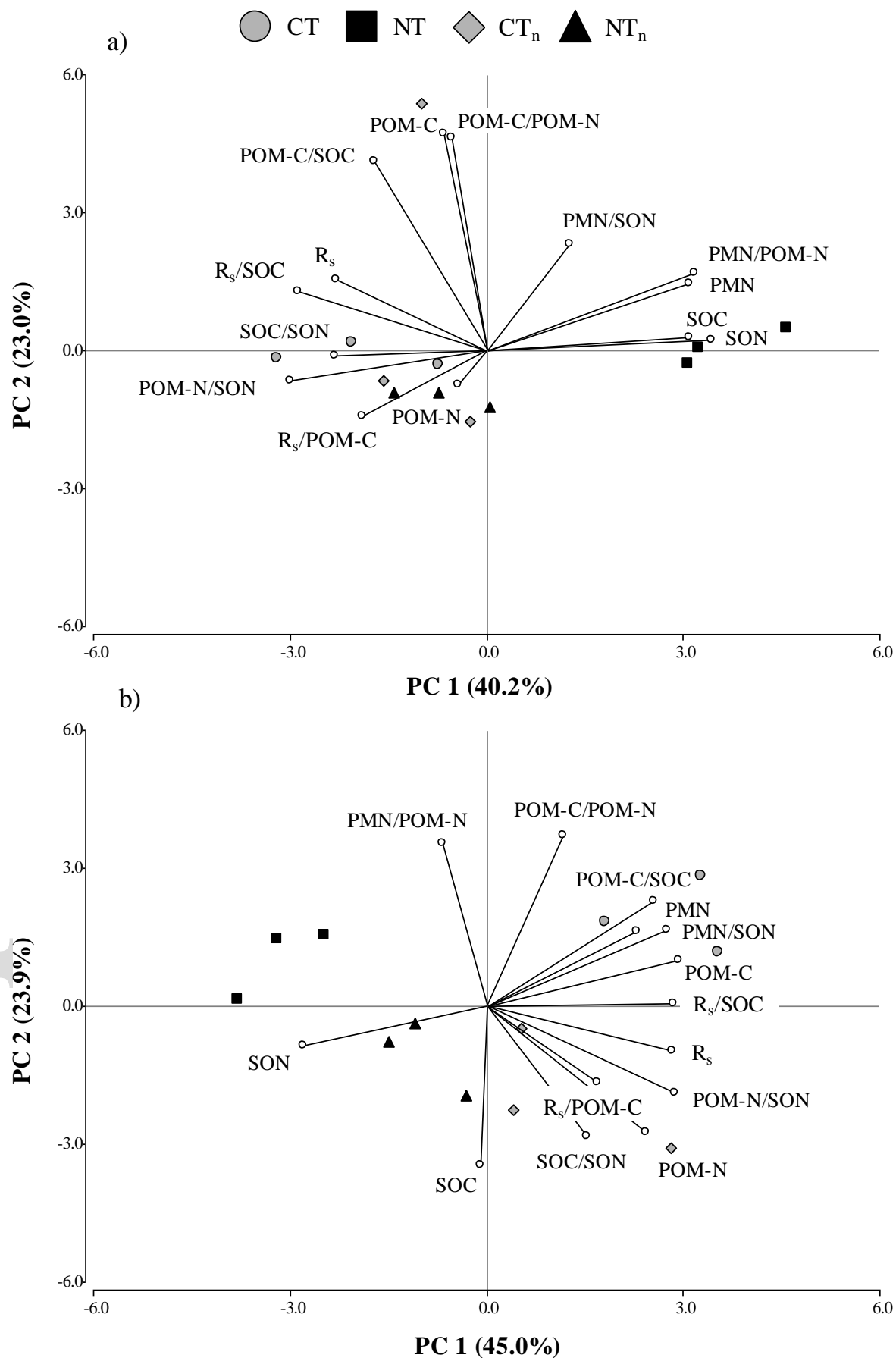


Figure 2.