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## Changes in soil biological properties in different management and tillage systems in petrocalcic argiudoll



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#### ABSTRACT

We analyzed the effect of different tillage systems under different land-use histories, on biological properties of soil during one year. The experiment was carried out at a Petrocalcic Argiudoll of Tres Arroyos (Buenos Aires, Argentina). The specific aim was to describe and compare the soil organic carbon (SOC), the soil basal respiration (BR) and the activities of the enzyme dehydrogenase, urease and acid-phosphomonoesterase under zero and conventional tillage on soils under pasture and intensive agriculture. The SOC concentration was highest in summer (postharvest) independently of tillage system or land-use history. However, in autumn the plots under conventional tillage showed higher values of SOC than those with zero tillage, independently of land-use history. The BR had a significant benefit in favour of summer pasture soils. The effect of land-use history or the tillage system on the enzymes activity was dependent of sampling season. The soil enzymes were more sensible than SOC and BR. In temporal studies the effect of sampling season is strongest that others factors as tillage systems or land-use history.

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#### 1. Introduction

New technological developments involved in the management of agricultural land has enabled increased the yield production in the grain-producing countries. The effects of agriculture advance modify the soil environment, including physical, chemical and biological properties (Forján and Manso, 2012). These modifications generate changes in the soil biota and therefore the cycling and

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availability of nutrients for crops (Dominguez et al., 2009). Forján and Manso (2012) suggested that when the agricultural cycle is repeated in the same plot decrease the concentration of nutrients, especially N. However, the inclusion of pastures period preserved the physical and chemical soil properties (Studdert et al., 1997) and therefore increased or maintain the soil fertility and productivity.

Conventional tillage practices have been associated with a deterioration in the quality of agricultural soils and loss of integrity, mainly in soils with low stability (Studdert, 2001). However, tillage conservationists such as zero and minimum tillage have been implemented to conserve soil resources in the context of agricultural production. Despite this, conservationist tillage required a source of nutrients and an adequate crop rotation must be taken into account to avoid the perpetuation of pathogens in the soil and / or plant remains.

The soil biological properties can be used as "biological indicators" of soil quality, as they are essential to soil functions and processes. In addition, they are susceptible to alterations by the tillage

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system, the release of agrochemicals or the grazing scheme, and reflect seasonal variations (Gianfreda and Ruggiero, 2006; Laudicina et al., 2011; Paz-Ferreiro et al., 2011). Special attention has been paid to the relationship between the biochemical parameters and the microbial biomass, due to its fundamental role in soil functionality (Nannipieri et al., 2012). Soil enzymes and soil basal respiration have been associated with changes in the microbial activities and with the organic carbon available in the soil (Nannipieri et al., 2012). The activity of dehydrogenase, which is intracellularly localized, is considered an index of total soil microbial activity. Urease, which is localized in microorganisms and animal or plant cells, catalyzes the hydrolysis of urea into ammonia and carbon dioxide (García Izquierdo et al., 2003). Acidphosphomonoesterase is nonspecific and catalyzes the hydrolysis of glycerophosphate and the enzyme activity is closely linked to soil pH (Ekenler and Tabatabai, 2003). In particular, soil enzyme activities have shown to be sensitive indicators of management practices and they are considered of quickly response to climate factors as temperature and moisture (García-Ruíz et al., 2008; Laudicina et al., 2011; Nannipieri et al., 2012; Paz-Ferreiro et al., 2010; Silvestro et al., 2017).

The soil biological properties response to antropogenic pulses and therefore they are considered to evaluate the effect of agricultural management. In the present work, we studied the effect of two different tillage systems on the soil organic carbon and biological properties as respiration and enzymes in soils, with different land-use histories. Our main objectives were: i) to describe and compare the soil organic carbon (SOC), the soil basal respiration (BR) and the activities of the enzyme dehydrogenase, urease and acid-phosphomonoesterase under zero and conventional tillage in soils with contrasting land-use history. This knowledge is relevant to design strategies for management of agricultural system and to maintain an acceptable level of microbial activity and thus achieve a sustainable development.

#### 2. Materials and methods

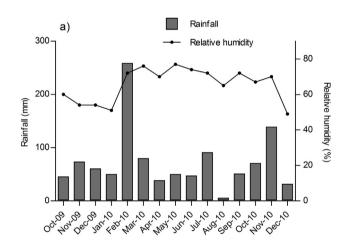
#### 2.1. Study site

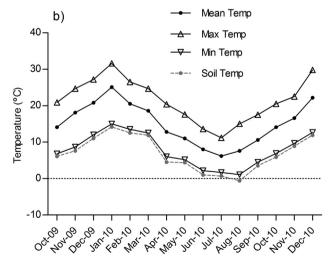
The experiment was carried out at the Barrow Experimental Station of the National Institute of Agricultural Technology (38° 19′ 25″ S; 60° 14′ 33″ W), Tres Arroyos, Buenos Aires province, Argentina. The soil is a Petrocalcic Argiudoll, Series Tres Arroyos, with a depth ranging between 50 and 55 cm, and clay loam texture (SSS, 2014) and has a slightly acid pH (6,4; 1:2,5 soil:water) (USDA, 2006).

The treatment was randomized following a strip-plot design to generate four different treatments with three replicates. The landuse history (LH) as the row factor (two levels) and the tillage system (TS) as the column factor (two levels) (Gómez and Gómez, 1984). The soil samples were collected from plots of 450 m<sup>2</sup> (15 × 30 m) under long and intensive agricultural (IA) history (12 years) and other under agricultural cycles with pasture (PA) Medicago sativa L. and Dactylis glomerata L. (PA; alfalfa/orchardgrass) every 4 years. Each one with two types of tillage [zero tillage (ZT) and conventional tillage (CT)]. The sequence of crops was sunflower (Helianthus annuus L.) as the summer crop, wheat (Triticum aestivum L.) as the winter crop and maize (Zea mays L.) as the summer crop. The practice in this area involves seeding fields with cereal crops with recommended application of herbicides, pesticides and simultaneous application of inorganic fertilizers. Conventional tillage comprised moldboard plowing, disking and field cultivation. The zero tillage comprised the chemical weed control during fallow. For wheat was used Diammonium phosphate (DAP 100 kg ha<sup>-1</sup>) as fertilizer, and the Glyphosate (2 L ha<sup>-1</sup>) was

applied during August 2010 under ZT. In December 2010 for ZT and CT were applied Glyphosate (2 L ha<sup>-1</sup>), 2.4 D 58.4% (1 L ha<sup>-1</sup>), Dicamba (0.1 L ha<sup>-1</sup>) and Metsulfurón-methyl (86.7 g ha<sup>-1</sup>). For sunflower were used Urea (65 kg ha<sup>-1</sup>) as fertilizer during December 2009 and 2010 under ZT, in April 2010 under CT was applied Glyphosate (2 L ha<sup>-1</sup>). In December 2010 under CT and ZT were applied the preemergent flurochloridone (1.5 L ha<sup>-1</sup>) and acetochlor (1.5 L ha<sup>-1</sup>). The agro-climatic conditions of the experimental site are shown in Fig. 1 a,b. provided by the Agrometeorological Station of Barrow Experimental Station (http://siga2.inta.gov.ar/en/datoshistoricos/).

Soil samples were collected randomly through 25 points in each plot from the first 15 cm (roughly 2 kg) of soil with a hydraulic borer and sealed in plastic freezer bags for transfer to laboratory. They were taken in four different seasons related to contrasting crop phenology: summer (postharvest of wheat; December 2009 and 2010), autumn (postharvest of summer crops; April 2010) and winter (tillering of wheat according Zadoks stages; August 2010).





**Fig. 1.** Agroclimatic description of Barrow Experimental Station (38° 19′ 25″ S; 60° 14′ 33″ W), National Institute of Agricultural Technology, Tres Arroyos, Buenos Aires province, Argentina, during the sampling period. a) Rainfall (mm) and relative humidity (%), b) Temperature (°C). Mean annual temperature is 14.9 °C (minimum and maximum temperatures reach 7.4 °C and 20.4 °C respectively) and mean annual precipitation is 750 mm.

#### 2.2. Characterization of soil properties

The SOC was calculated according to Walkley and Black (1934). BR was determined measuring the CO<sub>2</sub> trapped in an alkali solution (NaOH 0.1 N) in a closed system and then 10 days of incubation at 25 °C were titrated with (HCl 0.1 N) (Anderson, 1982).

Soil enzyme activities

Soil enzyme activities were assayed from 20 g air-dried soil (<2mm) with their appropriate substrate and incubated according the specific methodology. Dehydrogenases activity (µg Triphenyl Formazon: TPF. g soil-1h-1) was determined according to Tabatabai (1994). Briefly, 0.5 g soil were incubated in tubes (16  $\times$  150 mm) with 0.01 g CaCO<sub>3</sub> with 0.1 ml of 2,3,5triphenvltetrazolium chloride (TTC) and 0.25 ml of distilled water during 24 h at 37 °C. Then, 10 ml ethanol was added and the tubes were shaken for 1 min. The tubes were washed and filtered (Whatmann N° 5). Red colour absorbance of TPF was measured at 485 nm and contrasted with a calibration curve. Urease activity was determined from 0.5 g soil using urea (10% w/v) as the substrate, and incubating the soil samples for 24 h at 37 °C. Urease activity was determined colorimetrically as the NH<sub>3</sub> released (Nannipieri et al 1980). Acid-phosphomonoesterase activity was determined at pH  $6.5 \,(\mu g \,PNF. \, g \,soil^{-1}h^{-1}) \,(Tabatabai, 1994)$ . A subsample of 0.1 g soil was incubated with 400 µl buffer and 150 µl p-nitrophenol 5 mM (1 h or 1 h 30 min depending on the enzyme activity) in tubes (16  $\times$  150 mm). Then the tubes were frozen (15 min) to stop enzyme activity. A solution of 100 µl CaCl2 0.5 M and 400 µl of NaOH 0.5 M were added to the tubes and they were centrifuged (300 rpm for 10 min). The lecture was done in microplates of 200 ul at 400 nm.

#### 2.3. Statistical analysis

The experimental design corresponds to a strip-plot design, but we cannot account for three factors in this scheme. To compare, SOC and enzyme activities between land-use history (LH) and tillage systems (TS) in different sampling seasons, we first performed a repeated measures analysis to explore the temporal correlation in the plots analyzed in consecutive seasons. Statistical analyses were performed with the lme function from the nlme package (Pinheiro et al., 2013). After that, we performed a strip-plot analysis within each sampling season with LH and TS as fixed factors. We used the strip-plot function to perform the analysis and the Fisher's least significance difference Test (LSD) to evaluate significant differences with the agricolae package (de Mendiburu, 2013).

We also evaluated the correlation between soil variables composition with a principal component analysis (PCA) performed with the Software Infostat v. 2008/P (Di Rienzo et al., 2008).

#### 3. Results

The mean values of SOC varied from 18.13 g .kg $^{-1}$  to 29.90 g . kg $^{-1}$  (Fig. 2 a). We observed that the effect of tillage systems depended on the sampling season (TS  $\times$  SS p = 0.0407). The highest values were observed in summer 2010 (PA-CT 29.77, PA-ZT 26.03, IA-CT 29.90 and IA-ZT 29.60), where the lowest values were observed in IA under CT for autumn and winter 2010. The plots under CT presented higher values of SOC than those under ZT only in autumn 2010 (TS, p = 0.0187). In the remaining sampling seasons, the SOC values were not affected significantly by the landuse history or tillage system.

The mean BR ranged from 209.94  $\mu g$  CO<sub>2</sub>. g soil<sup>-1</sup>h<sup>-1</sup> to 1082.52  $\mu g$  CO<sub>2</sub>. g soil<sup>-1</sup>h<sup>-1</sup>. BR was significantly affected by the land-use history only in summer 2010 (LH, p = 0.0083) (Fig. 2 b). BR was two-fold higher in PA than in IA.

The effect of land-use history and tillage systems on dehydrogenase activity depended on the sampling seasons (LH  $\times$  TS  $\times$  SS, p = 0.0339) (Fig. 2 c). In summer 2009 and autumn 2010, we observed no effect by the land-use history or tillage system. In winter 2010, we observed that the dehydrogenase activity was higher under ZT than under CT (TS, p = 0.0171). In summer 2010, the dehydrogenase activity was affected by the land-use history (LH, p = 0.0034). The dehydrogenase activity was 61.36% higher in PA than in IA.

The activity of urease was affected by the sampling seasons (SS, p < 0.0001). The highest values were observed during summer 2010 (Fig. 2 d). Urease activity was not affected by the land-use history or tillage system.

Acid-phosphomonoesterase activity was affected by the landuse history subject to the sampling seasons (LH  $\times$  SS, p < 0.0001). In autumn 2010, the values of activity were higher in IA than in PA (Fig. 2 e). However, in summer 2010, the response was opposite. This enzyme was not affected by the tillage system.

#### 4. Discussion

SOC is a parameter widely used as a soil quality indicator, but detecting changes in general comprises studies of many years. The SOC values observed in this study were according to those suggested for similar soils (Alvarez et al., 2012; Sainz-Rozas et al., 2011; Silvestro et al., 2017) and were affected by the tillage system depending on the season: SOC was higher under CT than under ZT only in autumn. In agreement with Bonel et al. (2005), we suggest that although the presence of ZT favors the preservation of organic matter, the biological activity was insufficient or poor to incorporate it to the deeper layers of the soil. Under CT, the incorporation of organic matter was deep but increased the mineralization. Therefore, the level of SOC in the soil in both situations would be similar. Similar results were found by Alvarez et al. (2012) for soils of temperate weather. Some researchers have observed that SOC was higher under CT than under ZT for 0-20 cm of depth (Diovisalvi et al., 2008: Dominguez et al., 2009), whereas others that SOC was higher under ZT than under CT (González-Chávez et al., 2010) and others that SOC shows no differences between different agricultural managements (Ferreras et al., 2009). Due to these discrepancies, Bonel et al. (2005) suggested that SOC can be estimated for each soil type in relation with the agroenvironmental conditions. Alvarez and Steinbach (2006) showed that SOC is associated with rainfall and temperature. Because SOC is considered one of the indicators that exerts the most significant influence on soil quality, it should be noted that it varies with the season.

The biological and biochemical parameters are more sensitive and respond quickly to changes introduced in the soil. BR, as a variable to detect total biological activity, was associated with the sampling time and land-use history. The significant effect of land-use history (LH) on BR was observed only in summer 2010. The agroclimatic condition of summer could favor the differences between PA and IA for BR for summer 2010 respect to the other sampling times. The increase in biological activity has been related to the increase in temperature, because warmer temperatures in the soil increase BR and the biological activity, while lower temperatures lead to standing stock of SOC and therefore to low BR (Aon et al., 2001; Franzluebbers, 2002). In our case, the minimum and maximum temperatures in summer 2009 were 2  $^{\circ}$ C - 5  $^{\circ}$ C lower than in summer 2010, and the rainfall was low in December 2009 (Fig. 1). Moreover, the IA management and the lack of pastures in the rotations caused a decrease in SOC (Lavado, 2006) and therefore the BR could be lower due the lack or low level of SOC in the soil. Forján and Manso (2012) suggested that in

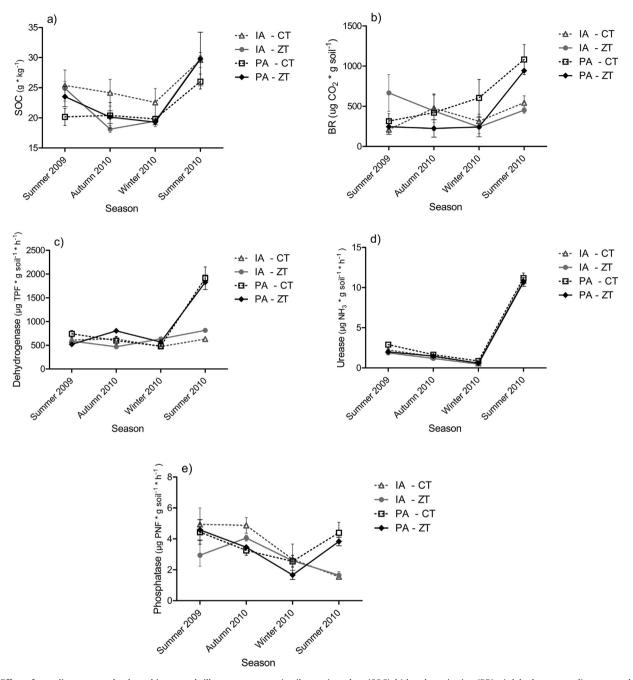


Fig. 2. Effect of sampling seasons, land-use history and tillage systems on a) soil organic carbon (SOC), b) basal respiration (BR), c) dehydrogenase, d) urease and e) acid-phosphomonoesterase. Error bars represent SEM from three independent replicates. IA-CT: Intensive agriculture under conventional tillage; IA-ZT: Intensive agriculture under zero tillage; PA-CT: Soil pasture under conventional tillage; PA-ZT: Soil pasture under zero tillage.

Argiudoll soils, the pastures assure a level of SOC and nutrient pool, due to the plant biomass including the root. However, in summer 2009, we did not find the same pattern, whereby we considered that the combination of land-use history with rainfall and temperature was fundamental. The absence of differences between land-use histories could be due to rainfall differences in both summers (Fig. 1 a, b). We found no effect of the tillage systems on BR. The non-significant differences are due to the long time of the experiment and the effect of the tillage system was absent opposite to the strong effect of land-use history.

The soil enzymes showed a strong relationship with the sampling season. We found effect of either land-use history or tillage systems within each season. Dehydrogenase activity could represent the size and activity of viable soil community. Like BR,

the activity of enzymes indicates biological activity. In this study, this enzyme was far more sensitive than BR. Dehydrogenase activity varied with the sampling time, land-use history and tillage systems. It was higher under ZT than under CT in winter 2010, ZT presented special conditions (moisture and temperature) that increased the biological activity respect to CT (Aon et al., 2001; Ferreras et al., 2009; Madejón et al., 2009). However, in sandy clay loam soil, Melero et al. (2009) found no differences for this parameter between ZT and CT. Mirás Avalos et al. (2007) showed that this enzyme activity was influenced by the sampling season and suggested that this activity was strongly related to the organic matter available in function of time. The increase in dehydrogenase activity has been associated with the stubble crop incorporation to the soil and agroclimatic conditions (Mirás Avalos et al., 2007;

Silvestro et al., 2017). The stronger differences were found for summer 2010 between soil histories. This result could be explained in the same way as for BR.

Urease and acid-phosphomonoesterase activities have been related to the cycles of N and P respectively; therefore, the activity of these enzymes could be regulated indirectly by microorganisms and directly by the soil type (Aon et al., 2001). In our study, urease activity was affected by the seasons only in agree with Aon et al. (2001). They suggested that this enzyme activity varies in function of the sampling time. In their study, urease activity decreased with the time whereas the level of ammonium, nitrates and nitrifying bacteria increased. In our case, the level of urease started decreasing and then increased in summer 2010, probably because the cumulative rainfall was 25% higher in summer 2010 than in summer 2009. This result could be due to the agroclimatic conditions independently of the soil management. Several studies have suggested a strong positive relationship between urease activity and soil moisture (Juan et al., 2009; Nuñez Ramos et al., 2012). However, Paz-Ferreiro et al. (2010, 2011) observed that this enzyme more strongly affected by the altitude, location and management than by the soil moisture and temperature. Palma et al. (2000) observed higher activity under ZT than CT, but this difference was not detected between the sequences (maize-maize versus maize-sovbean).

In this study, acid-phosphomonoesterase activity was affected by the land-use history depending on the sampling time. In autumn 2010, the activity of this enzyme was higher in IA than in PA, whereas in summer 2010, it was the opposite. This result could be explained because in autumn the relative humidity was higher than in the other seasons and this situation may favours the activity of acid-phosphomonoesterase in IA regarding that in PA because in this season the crop presented little growth and did not export it to the yield. However, in summer 2010, the increase of acid-phosphomonoesterase in PA respect to IA could be because the pastures conserve more relative humidity in the soil than the crop. In summer, the crop captures the water available. Dick (1984) suggested that the rotation with continuous crops increases the pH in the soil and therefore this enzyme activity increases as well.

Generally, parameters as BR, dehydrogenase, urease and acid-phosphomonoesterase are related to soil chemical parameters as SOC. Here, we observed a clear correlation between dehydrogenase-BR ( $\rm r^2=0.79$ , p = 0.003), urease-SOC ( $\rm r^2=0.85$ , p = 0.0001) and urease with parameters of microorganism activity as BR ( $\rm r^2=0.67$ , p = 0.0041) and dehydrogenase ( $\rm r^2=0.72$  p = <0.0015). Trasar-Cepeda et al. (2008) found similar results for cropped soils, whereas Mirás Avalos et al. (2007) observed similar results for different soil types. Mirás Avalos et al. (2007) suggested that BR, dehydrogenase activity and soil moisture are related, but influenced by other sources of variation.

Here, we studied the intra-annual variation of the chemical and biochemical properties of soil in a long-term experiment with different land-use histories and tillage systems. The seasonal sampling was the factor that most influenced the different parameters evaluated. The tillage systems caused no differential effect on the soil management according to the land-use history and resulted in no significant effects on the soil environment, except for certain parameters in special space-time points. The continuous use of ZT during this period did not generate a better soil environment than the use of CT. Based on these results, we suggested that these parameters showed the strongest relationship with the seasonal sampling. The positive correlation between SOC and enzymes activity (urease and dehydrogenase) indicated that the availability of carbon substrates favor the activity of the microorganisms and therefore enzyme production. However, these correlations are dependent on seasonality. The soil enzymology is a

relevant tool to detect small changes in the soil, but it also depends on other factors such as the sampling time, the moisture, the temperature and the application of agrochemicals. The enzymatic activities and therefore the microbial activity are favoured by the availability of SOC and by the management of pasture rotation. The inclusion of pastures in the management of agricultural soils guarantees the presence of biomass and therefore carbonate and nitrogenous structures that are fundamental for the pool of soil nutrients.

#### **Declaration of Competing Interest**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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#### **Author Agreement**

All the authors of the manuscript titled Biological properties of soil: answer to different management in petrocalcic argiudoll, are agree to its submission to Journal of the Saudi Society of Agricultural Sciences.

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