J. Agr. Sci. Tech. (2017) Vol. 19: 245-257

Mollisol: Biological Characterization under Zero Tillage with Different Crops Sequences

L. B. Silvestro[1], F. Biganzoli[2], H. Forjan[3], A. Albanesi[4], A. M. Arambarri[5], L. Manso[3], and M. V. Moreno[1, 6*]

ABSTRACT

Zero tillage practices have a direct effect on soil microbial communities modifying soil productivity and sustainability. The crop sequences used can change the aforementioned properties, too. In this study, we evaluated the effect of crop sequences under zero tillage management on soil biological and chemical properties including vertical distribution of soil organic carbon, soil basal respiration, and dehydrogenase, acid phosphatase, and urease activity along a seasonal year and at different soil depths. The sequences included in this study were: (I) Single crop per year (sunflower-wheat-sorghum-soybean); (II) Mixed agriculture/livestock with pastures, without using winter or summer forages (wheat sorghum/soybean-canola-pasture); (III) Winter management (wheat-canola-barley-late soybean); (IV) Mixed with annual feed crop (wheat-oat/*Vicia sativa*- soybean or sunflower), and (V) Intensive management (wheat-barley-canola, with alternation of soybean or late soybean). Soil organic carbon decreased with increasing depth, depending on sequences ($P_{seq \times depth}$= 0.0173). Soil basal respiration was higher in the 0-5 cm layer than in the 10-20 cm layer of the topsoil irrespective of the crop sequences (P_{depth}= 0.0062). Dehydrogenase, acid phosphatase and urease activity were affected by crop sequences, sampling season, and depth. Mixed sequences (sequences II and IV), including perennial pastures or annual feed crop could favor dehydrogenase and phosphatase activity. Sequences with cover crops (sequences II and IV) could favor microbial activity and, therefore, improve soil quality.

Keywords: Basal respiration, Enzyme activities, Microbial activity, Soil organic carbon.

INTRODUCTION

Crop management practices can change soil chemical and physical properties affecting the activity of microbial communities, the activity of soil enzymes and, as a consequence, the sustainability of the overall system**.** Zero tillage systems were intended to increase soil Organic Carbon (OC) as compared to conventional tillage (Mishra *et al*., 2010) and the effect on productivity and conservation of soil resources is known (Apezteguía *et al*., 2009). Rotation practices under zero tillage systems have a beneficial effect on the soil and could favor conservation of soil

[1] Laboratory of Functional Biology and Biotechnology (BIOLAB-INBIOTEC-CONICET-CIC), National University of the Center of the Province of Buenos Aires, Argentina.
* Corresponding author; e-mail: vmoreno@faa.unicen.edu.ar
[2] Department of Quantitative Methods and Information Systems, Faculty of Agronomy, University of Buenos Aires, Argentina.
[3] Barrow-INTA Experimental Station, Tres Arroyos, Province of Buenos Aires, Argentina.
[4] Chair in Microbiology and Ecology, Agricultural Faya National University of Santiago del Estero, Argentina.
[5] Spegazzini Institute, FCNyM, National University of La Plata, Argentina.
[6] Agricultural Microbiology, Faculty of Agronomy, National University of the Center of the Province of Buenos Aires, Argentina.

[DOR: 20.1001.1.16807073.2017.19.1.13.8]

moisture, increase soil enzymatic and biological activities and reduce the use of fertilizer (Franzluebbers, 2007). On the other hand, application of continuous cropping without use of pastures for many years decreases both OC and the total soil N concentration (Plaza-Bonilla *et al.*, 2016).

Several works showed the relationship among enzymatic activities, soil basal respiration, and OC with different management of crops and sampling depths, all concluding that tillage systems and crops had effects on soil basal respiration and enzyme activities in the upper portion of soil (Melero *et al.*, 2008; 2009; Paz-Ferreiro *et al.*, 2010; Wang *et al.*, 2008). Also, climatic and seasonal conditions in cultivated areas are a limiting factor for accumulation of OC in the top layer of soil and enzymatic activities (Melero *et al.*, 2009).

Crop management has a direct effect on the microbial community and could be evaluated using soil biological and/or chemical properties (Melero *et al.*, 2011). Soil basal respiration and enzymatic activities allow the evaluation of microbial status and soil physicochemical conditions. Soil basal respiration gives evidence of the soil carbon availability to microorganisms and was used as a measure of the total microbial activity (Ananyeva *et al.*, 2008). Additionally, the activity of several enzymes has been related to the organic matter concentration and provides early indication of change in soil properties associated with cropping practices (Qin *et al.*, 2010). Enzymatic activities have also been used as soil quality indicators in agricultural soils because they show sensitive responses to soil changes and are related to the cycle of nutrients in soil (C, N, P, S) (Geisseler and Horwath, 2009; Qin *et al.*, 2010).

Therefore, different crop sequences produce different changes in soils under zero tillage systems. Soil chemical and biological properties are commonly used as early indicators of such changes. However, these changes can be influenced by sampling procedures.

The aim of this study was to evaluate short-term changes due the crop rotation practices under zero tillage and sampling procedures (season and depth) on OC, BR and enzymatic activities.

MATERIALS AND METHODS

Location and Management of the System

We sampled a field experiment established in 1997 in the Barrow Experimental Station (38° 19’ 25’’ S; 60° 14’ 33’’ W), Tres Arroyos, Buenos Aires Province, Argentina. The experimental site had a slope of 2-3 %, and the top soil had a sandy clay loam texture (259 g kg^{-1} of clay, 269 g kg^{-1} of silt and 472 g kg^{-1} of sand) (USDA, 2006). The soil was classified as a Petrocalcic Argiudoll (depth of the petrocalcic horizon is 50 cm) (SSS, 2014). The agro-climatic conditions of the experimental site are shown in Table 1. Additional detailed soil characterization at the experimental site can be found in Table 2 and Silvestro *et al.* (2013). The plots were arranged in a randomized complete block design and the treatments (crop sequences) randomized, using three replicates of 420 m^2 plot area (14×30 m). The crops used in the sequences were wheat (*Triticum aestivum* L.), sorghum (*Sorghum vulgare Pers.*), soybean (*Glycine max* L.), canola (*Brassica napus* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), sunflower (*Helianthus annuus* L.), and vicia sativa *(Vicia sativa* L.). Sequences consisted of five different crop rotations: (I) Single crop per year (sunflower-wheat-sorghum-soybean); (II) Mixed agriculture/livestock with pastures, without using winter or summer forages (wheat sorghum/soybean-canola-pastures); (III) Winter management (wheat-canola-barley-late soybean); (IV) Mixed with annual feed crop (wheat-oat/*Vicia sativa*-soybean or sunflower), and (V) Intensive management (wheat-barley-canola, with

[DOR: 20.1001.1.16807073.2017.19.1.13.8]

Table 1. Agro-climatic conditions of Barrow, Tres Arroyos, Buenos Aires province, Argentina (38°19'25'' S; 60°14'33'' W).

	Rainfall (mm)		RH (%)		Temperature (ºC)						Frost number		Hours of sun		Soil Temperature at 5-cm depth (ºC)	
					Mean		Maximum		Minimum							
Season	Average [b]	Month	Average	Month	Average	Month	Average	Month	Average	Month	Average	Month	Average	Month	Average	Month
Summer [a]	79.1	61.2	57	54	21	20.8	26.9	27	11.6	12.0	0.1	0	9.6	10	10.3	11
Autumn [b]	67.3	38.8	73	70	14.6	12.8	20.4	20.4	6.0	7.6	3	1.3	6.6	8.6	6.1	4.5
Winter [c]	39.4	5.9	73	65	8.9	7.6	14.3	15.0	2.5	1.1	9.1	13	5.6	6.8	1.1	-0.6

[a] December 2009; [b] April 2010, [c] August 2010, [b] Historical average per month for the 1938-2008 period.

alternation of soybean or late soybean). The cultivars used were Quilmes Ayelen (barley); SW 2836 (canola); A 4613 RG (soybean); A 3726 RG (late soybean); Bonaerense Maja (oat); DK 61 T (sorghum); BIOINTA 2001 (wheat) and DK 3920 (sunflower). Cropping practices involved seeding crops with herbicide application (glyphosate, metsulfuron-methyl, dicamba and 2,4-D) pesticides and simultaneous application of inorganic fertilizers (PDA- diammonium phosphate and urea) (Table 3). Sequence II had a period of three years with pasture and sequence IV had a period with pasture every two years during the 13 years of experiment. The two mixed sequences, i.e. II and IV, had a grazing period while the three remaining sequences I, III, and V were exclusively agricultural. Sequence I was associated with the "traditional" farming in the region with one crop per year. Sequence V was related to management applied by big companies, with the intensification of crop rotation (double annual cropping). Sequence III started with a sequence of one crop per year during six years and then soybean was included as a double crop on the basis of winter crops.

Soil Sampling Procedure

The samples were taken at 3 different times: in summer (December 2009) when the wheat was harvested; during autumn (April 2010) when sequence II had pastures established, whereas sequence IV was covered with oats; at this particular sampling time, sequences I, III, and V were in fallow; and during winter (August 2010), at the fallow period for all the studied sequences. Soil samples (roughly 2 kg) were taken with a hydraulic borer and each cylinder was divided at 0-5, 5-10, and 10-20 cm depth intervals. Soil pH was determined by potentiometric method in a soil:water ratio of 1: 2.5, according to USDA-NRCS (2004).

Table 2. Soil pH values in the 1997, 2004, and 2010 years.

Sequence[b]	Soil pH[a]		
	1997	2004	2010
I	6.6	6.0	5.5
II	6.6	6.0	5.2
III	6.6	5.8	5.7
IV	6.6	6.1	5.9
V	6.6	5.9	6.0

[a] pH: Potentiometric method (1:2,5; Soil: Water) (USDA-NRCS 2004).[b] Crop sequences are defined in the text and Figure 1.

Soil Physical Chemical and Biochemical Analysis

Soil Organic Carbon (OC) was determined on the fine fraction (< 0.5 mm) according to Walkley and Black (1934). Soil Basal Respiration (BR) was determined by measuring the CO_2 trapped in an alkali solution in a closed system. Samples were incubated for 10 days at 25°C (Anderson, 1982).

Dehydrogenase and acid phosphatase activities were determined according to Tabatabai (1994), while urease activity was determined according to Nannipieri *et al.* (1980).

Statistical Analysis

To compare OC, BR, and enzyme activities among sequences at different depths considering the three sampling time, we used a repeated (in space) measures analysis once we discarded temporal autocorrelation. We first explored temporal correlation among plots analyzed in consecutive sampling dates. After that, we performed a mixed effects model to account for spatial autocorrelation between soil samples taken at consecutive depths in the same soil core. We selected the best correlation structure based on Akaike Information Criteria (AIC) and Bayesian Information Criteria **(**BIC) scores of competing models (Zuur *et al.*, 2009).

Table 3. Herbicides (glyphosate, metsulfuron-methyl, dicamba, and 2,4-D) pesticides and simultaneous application of inorganic fertilizers (DPA: Diammonium Phosphate and urea) during experiment.

	Fertilization		Herbicide/Pesticide			
Seq[a]	Winter (June 2009)	Spring (September 2009)	Spring (September 2009)	Summer (February 2010)	Autumn (May 2010)	Winter (June 2010)
I	DPA (100 kg ha^{-1})	Urea (140 kg ha^{-1})	2,4-D (0,400 L ha^{-1}) Dicamba (0,100 L ha^{-1}) Metsulfuron-methyl (6,7 g ha^{-1})	Glyphosate 2 L ha^{-1}	Glyphosate 2 L ha^{-1}	----------
II	DPA (100 kg ha^{-1})	Urea (140 kg ha^{-1})	2,4-D (0,400 L ha^{-1}) Dicamba (0,100 L ha^{-1}) Metsulfuron-methyl (6,7 g ha^{-1})	Glyphosate 2 L ha^{-1}	----------	Glyphosate 2 L ha^{-1}
III	DPA (100 kg ha^{-1})	Urea (140 kg ha^{-1})	2,4-D (0,400 L ha^{-1}) Dicamba (0,100 L ha^{-1}) Metsulfuron-methyl (6,7 g ha^{-1})	Glyphosate 2 L ha^{-1}	Glyphosate 2 L ha^{-1}	----------
IV	DPA (100 kg ha^{-1})	Urea (140 kg ha^{-1})	2,4-D (0,400 L ha^{-1}) Dicamba (0,100 L ha^{-1}) Metsulfuron-methyl (6,7 g ha^{-1})	Glyphosate 2 L ha^{-1}	----------	----------
V	DPA (100 kg ha^{-1})	Urea (140 kg ha^{-1})	2,4-D (0,400 L ha^{-1}) Dicamba (0,100 L ha^{-1}) Metsulfuron-methyl (6,7 g ha^{-1})	Glyphosate 2 L ha^{-1}	Glyphosate 2 L ha^{-1}	----------

[a] Crop sequences are defined in the text and Figure 1.

[DOR: 20.1001.1.16807073.2017.19.1.13.8]

Statistical analyses were performed with the lme function from the nlme package (Pinheiro *et al.*, 2016). We selected the best factor combination for varIdent option (nlme package) to correct for heteroscedasticity when necessary. Significant differences were evaluated with the lsmeans package (Lenth, 2013).

RESULTS

The OC was significantly affected by sequences×depth interaction (Table 4, $P_{seq\times depth}$= 0.0173). Sequences II, III, IV, and V showed the higher values of OC in the first layer (0-5 cm) compared to the other layers (5-10 cm and 10-20 cm). However, sequence I showed more OC in the first layer (0-5 cm) than the last layer of soil (10-20 cm) (Figures 1-a, -b, and c). We found no differences among seasons in OC content (Table 4).

BR showed a similar pattern in all five sequences (Table 4, P_{seq}= 0.1814). We found higher values of BR for summer, followed by winter and autumn (Table 4, P_{season}< 0.0001). Moreover, in all seasons, the 0-5-cm depth showed more BR than the 10-20 cm, but not from 0-5 to 5-10 cm, regardless of the cropping sequences (Figures 2-a, -b, and -c; Table 4; P_{depth}= 0.0062).

All three enzyme activities were affected by the cropping sequences, season, and sampling depth (Table 4). In summer, dehydrogenase activity showed higher activity in the surface soil, except crop sequence II, where it was very high in the three sampled layers of the soil profile (Figure 3-a). In autumn, only sequences I and IV showed higher enzymatic activities in the first layer of soil (0-5 cm) than in the last one (10-20 cm; Figure 3-b). In winter, we observed that, in all sequences, the dehydrogenase activity decreased with increasing depth and sequences II, IV, and V (the mixed agriculture/livestock with pastures, mixed with annual feed crop and intensive management, respectively) showed higher values of enzyme activity than

Table 4. Effect of sampling season, crop sequence, and depth on five soil properties.

	Soil properties				
	Soil Organic Carbon (OC)	Soil Basal Respiration (BR)	Dehydrogenase activity	Phosphatase activity	Urease activity
Season [a]	0.8517 ns	< 0.0001 ***	0.0029 ***	< 0.0001***	< 0.0001 ***
Sequences [b]	0.8286 ns	0.1814 ns	0.0001 ***	< 0.0001 ***	< 0.0001 ***
Depth [c]	< 0.0001***	0. 0062 ***	< 0.0001***	< 0.0001 ***	< 0.0001 ***
Season×Sequences	0.5891 ns	0.9737 ns	< 0.0001***	< 0.0001 ***	< 0.0001 ***
Season×Depth	0.6373 ns	0.5620 ns	0.0026 ***	< 0.0001 ***	<0.0001 ***
Sequences×Depth	0.0173 ***	0.2883 ns	< 0.0001 ***	0.6371 ns	<0.0001 ***
Season×Sequences×Depth	0.8825 ns	0.6783 ns	< 0.0001 ***	< 0.0001 ***	<0.0001 ***

[a] Summer; Autumn, Winter; [b] Sequences are defined in the text and Figure 1; [c] a: 0-5; b: 5-10,c: 10-20 cm. ns: Non significant; ***; *F* value significant at P≤ 0.05.

[DOR: 20.1001.1.16807073.2017.19.1.13.8]

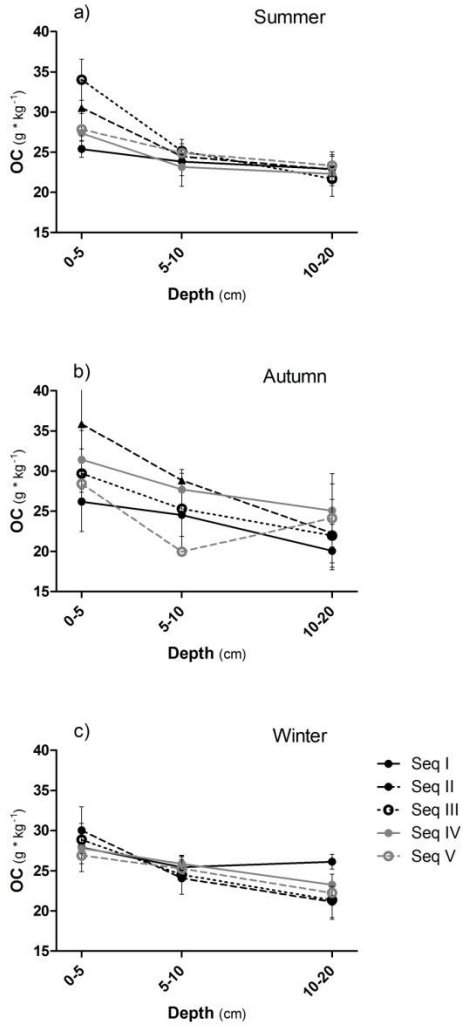

Figure 1. Soil Organic Carbon (OC) under five crop sequences (I, II, III, IV and V), three sampling depths (0-5, 5-10 and 10-20 cm) and three sampling seasons: (a) Summer; (b) Autumn, (c) Winter. (For the sake of clarity we plot each season on a separate panel). Sequences: Seq I: Single crop per year: Sunflower-Wheat-Sorghum-Soybean; Seq II: Mixed agriculture/livestock with pastures: Wheat-Sorghum-Soybean-Canola-Pasture; Seq III: Winter management: Wheat-Canola-Barley-late Soybean; Seq IV: Mixed with annual feed crop: Wheat-Oat/Vicia-Sunflower, Seq V: Intensive management: Wheat-Barley-Canola, Soybean or late Soybean.

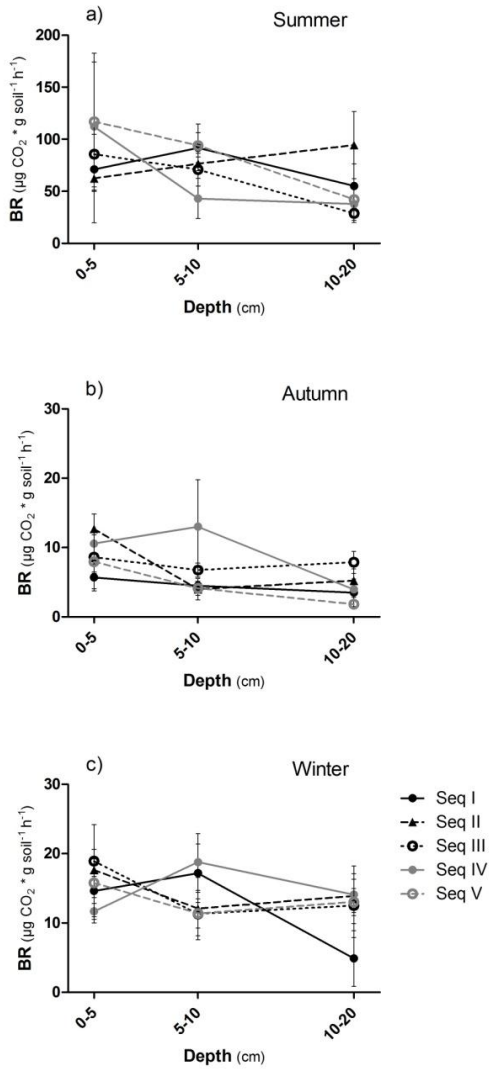

Figure 2. Soil Basal Respiration (BR) of soil under five crop sequences (I, II, III, IV and V), three sampling depths (0-5, 5-10 and 10-20 cm) and three sampling seasons: (a) Summer; (b) Autumn, (c) Winter. Note the difference in scale of the y axes. Sequences are defined in the text and Figure 1.

[DOR: 20.1001.1.16807073.2017.19.1.13.8]

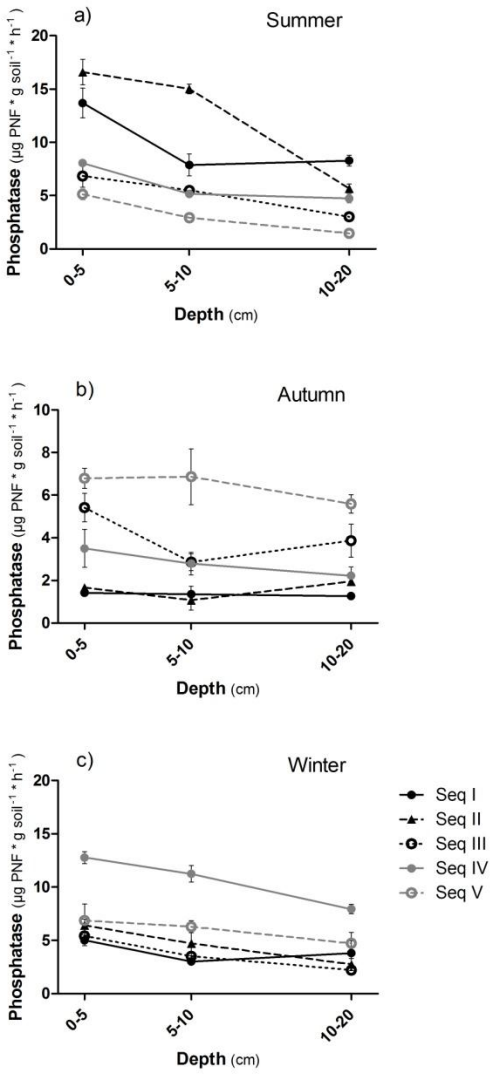

Figure 3. Dehydrogenase activity of soil under five crop sequences (I, II, III, IV and V), three sampling depths (0-5, 5-10 and 10-20 cm) and three sampling seasons: (a) Summer; (b) Autumn, (c) winter. Sequences are defined in the text and Figure 1.

sequences I and III in the first soil layer (Figure 3-c).

In summer, highest phosphatase activity was reached by sequence I and II. The sequences I and IV showed higher enzymatic activities in the first layer of soil (0-5 cm) than in the last one (5-20 cm) and sequences II, III, and V showed differences between layers 0-10 cm and 10-20 cm. (Figure 4-a). In autumn, we did not observe a strong effect of sequences and depth on phosphatase activity, only sequence V showed higher activities than the other sequences (Figure 4-b). In winter, phosphatase activity decreased significantly

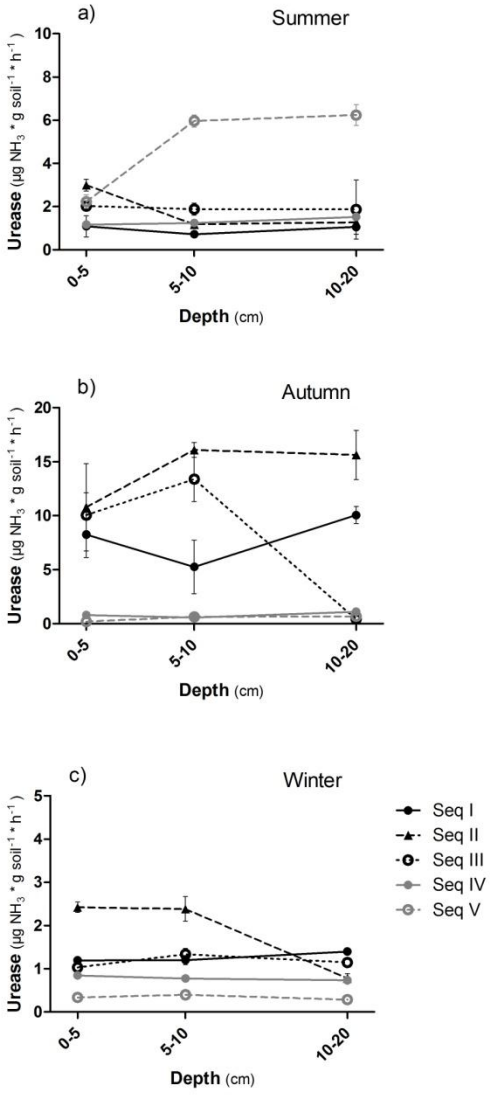

Figure 4. Phosphatase activity of soil under five crop sequences (I, II, III, IV and V), in three sampling depths (0-5, 5-10 and 10-20 cm) and at three sampling seasons: (a) Summer, (b) Autumn, c) Winter. Note the difference in scale of the y axes. Sequences are defined in the text and Figure 1.

[DOR: 20.1001.1.16807073.2017.19.1.13.8]

with increasing depth in sequences II, III, and IV. Sequence IV showed highest values of phosphatase activity (Figure 4-c).

In summer, only in sequence V urease activity increased with increasing depth (Figure 5-a). In autumn, all five sequences showed contrasting pattern of urease activity with depth. Sequences I, II, and III showed higher values of activity than the other sequences, but, in sequence III urease activity decreased to lower values at deepest soil layer, as sequences IV and V (Figure 5-b). In winter, we found lower urease activity with no differences among sequences or depth (Figure 5-c).

DISCUSSION

Soil Organic Carbon (OC) stock was higher in the upper layer of soil (Figure 1). Zero tillage systems leave plant residues on the soil surface resulting in OC stratification (Franzluebbers, 2002). We suggested that the lack of differences among sequences was due to long-term experiment and that the elapsed time was not long enough to show differences. In long-term experiment, the continuing accumulation of OC does not allow differentiation among sequence (Forján *et al.*, 2012). The strong effect of zero tillage also erased a seasonal effect. We suggest that the changes produced by the sampling season were too little to be detected in the OC level, especially in a long-term assay according to other authors (Geisseler and Horwath, 2009).

The results obtained suggested that the highest microbial activity detected through BR was observed in the surface soil. These differences among soil layers suggested that BR showed variation with OC. Moreover, the BR values showed a strong gradient decreasing from summer to winter seasons (Figure 2). High temperature and moisture increase microbial activity, thus increasing BR (Ananyeva *et al.*, 2008). In early summer (postharvest), wheat stubble interacted with agro-climatic conditions to produce differences of an order of magnitude in BR among seasons. On the other hand, the lower BR values in autumn and winter with respect to summer could be in response to the decrease in temperature. This situation could also explain the lack of

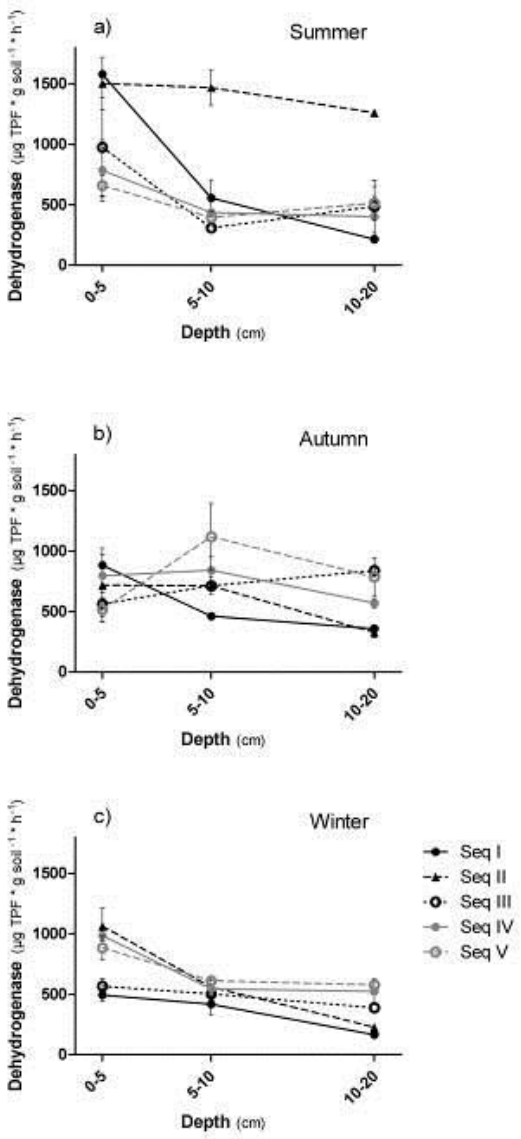

Figure 5. Urease activity under five crop sequences (I, II, III, IV and V), in three sampling depths (0-5, 5-10 and 10-20 cm) and three sampling seasons: (a) Summer; b) Autumn, (c) Winter. Note the difference in scale of the y axes. Sequences are defined in the text and Figure 1.

[DOR: 20.1001.1.16807073.2017.19.1.13.8]

decline in OC (Table 1; Figure 1). This is because the mineralization of organic matter at low temperatures is slower and the stubble remains for more time in the first centimeters of soil surface (Franzluebbers, 2002).

The results showed that changes of enzymatic activity depended on crop sequences and spatio-temporal variables such as sampling season and soil depth. We observed the same gradient decreasing in depth for enzyme activities as for OC stock and BR values. Therefore, we considered that the incorporation of crop residues in soil under zero tillage increased the OC available and, therefore, improved biological activity. Similar results were observed by Aon and Colaneri (2001). They observed a significant decline of enzymatic activity in space as a function of depth in an Aquic Argiudoll. The wide seasonal variation detected in agricultural soil for enzymes has been associated with soil temperature and soil moisture (Alvear *et al.*, 2005; Paz-Ferreiro *et al.*, 2010, 2011). However, several works showed that other sources of variations such as tillage systems and crop sequences had the strongest effect on the enzymes (Alvear *et al.*, 2005; Gianfreda and Ruggeiro, 2006; Paz-Ferreiro *et al.*, 2011). Therefore, the changes observed in the enzyme activities cannot be attributed to a single source of variation.

Dehydrogenase activity was higher in the first centimeters of soil surface, except in sequences II in summer and II, III, and V in autumn. This fact could be explained by the stratification of organic matter as a result of tillage systems (zero tillage). The sequences with pastures (II) and grazing could be explained according to Haynes *et al.* (1991). They suggested that the presence of pasture and grazing animals stimulate microbial activity. Sequences III and V in the crop cycle previous to our sampling had a higher intensity of cropping than the other sequences. The diversity of crops stimulated microbial activity and, therefore, dehydrogenase activity (Gianfreda and Ruggeiro, 2006). In winter, the biological activity estimated through dehydrogenase activity showed a decrease in the effect of depth for all sequences assayed. It was due to the fact that rainfall was lower in winter than in summer and autumn (Table 1); therefore, the moisture was retained in the upper layers associated with plant debris as suggested for these tillage systems. The relationship between OC and dehydrogenase activity revealed the role of the dehydrogenase in the oxidation of organic matter (Aon and Colaneri, 2001; Paz-Ferreiro *et al.*, 2010). Dehydrogenase activity showed a similar pattern to BR, but dehydrogenase activity was more sensitive to changes in the cropping system.

Phosphatase and urease activities are involved in the cycles of P and N, respectively. The activity of these enzymes is regulated indirectly by microorganisms and directly by soil type (Aon and Colaneri, 2001). During the mineralization of organic matter, acid phosphatase activity is associated with soil type, soil management, and soil moisture and temperature (Gómez-Guinan, 2004). In the same way as in other studies (Alvear *et al.*, 2005; Samuel *et al.*, 2011), we observed the highest activity of phosphatase in the first centimeters of soil surface. Therefore, we attributed this fact to the content of OC in the upper portion of soil. The organic matter is responsible for the maintenance of active form of phosphatase enzyme. In relation to this point, Gianfreda and Ruggiero (2006) suggested that phosphatase activity showed a spatial dependence on OC. Ferreras *et al.* (2009) observed for a typical Argiudoll soil that the sequence with cover crop under zero tillage presented higher phosphatase activity than sequences without cover crop.

We observed a stratification of phosphatase activity associated with depth in certain sampling seasons and sequences. In summer, the decline of activity was strongest from 10 cm of depth. Sequence V showed the highest activity of phosphatase in autumn. The presence of cover crops and pulse of soybeans in crops sequences favors phosphatase activity and promotes the

[DOR: 20.1001.1.16807073.2017.19.1.13.8]

mineralization of P in the upper portion of soil. In winter, the sequence with pastures increased phosphatase activity, suggesting that short-term pastures improve the available inorganic P in soil, thus, increasing phosphatase activity (Haynes and Williams, 1999). On the other hand, winter sequence IV showed the highest activity of phosphatase. The presence of cover crops and pulse of soybeans in crops sequences favors phosphatase activity and promotes the mineralization of P in the upper portion of soil (Khan, 1970; Silvestro *et al.*, 2013). Also, we observed a progressive decrease in soil pH from 1997 to 2010 (Table 2). We assume that the decrease in soil pH was due to continuous nitrogen fertilization. Therefore, phosphatase activity was favored, according to Ling *et al.* (2014).

Urease activity and other enzymes have little or no spatial dependence (Gianfreda and Ruggiero, 2006). This study showed that urease activity presented an irregular variation with depth mainly in autumn. This situation indicates that urease activity is not related to the active microbial population, according to Frankenberger and Dick (1983). However, we observed seasonal variation. The increase in urease activity detected in autumn in the sequences I, II, and III could be explained because moisture and temperature were not a limiting factor for this season (Table 1). In summer, high temperature and low rainfall with extended dry periods were registered, while in winter, low temperatures and high number of frost days were registered (Table 1). Therefore, these conditions were determinants for low urease activity in summer and winter in all sequences. The results were similar to that detected by Paz-Ferreiro *et al.* (2011), who indicated that enzyme activities depend on climatic conditions. Also, Nannipieri *et al.* (2012) suggested that enzyme activities decline in summer and winter in relation to the moisture availability and temperature in soil. We suggest that urease activity is influenced by season more than by the sequences of crops and depth in soil. Also, 12 years of continuous nitrogen fertilization could produce the low urease activity. Similar results were observed by Dick *et al*. (1988), who suggested that urease activity decreased with long-term addition of nitrogen fertilizer.

This study represents the intra-annual evaluation of biochemical and chemical properties of soil under zero tillage with different sequences of crop in a long-term experiment. Enzyme activities allow detection of differences in the type of sequences applied to rotation practices in a zero tillage system, except urease activity that depends on the climatic conditions rather than the type of sequence. Mixed sequences, including perennial pastures and annual feed crop could favor dehydrogenase and phosphatase activity and the sequences with sustainable cropping management, including cover crops, could favor microbial activity and, therefore, improve quality of soil.

ACKNOWLEDGEMENTS

This work was supported by grants provided by CONICET (PIP 20130100280 CO- CONICET). L. B. Silvestro is a fellow of CONICET. We thank Prof. M. Oyarzabal for English assistance and Dr. (Soil Science) S. Mestelan for the critical reading of this manuscript.

REFERENCES

1. Alvear, M., Rosas, A., Rouanet, J. L. and Borie, F. 2005. Effects of Three Soil Tillage Systems on Some Biological Activities in an Ultisol from Southern Chile. *Soil Till. Res.*, **82**: 195-202.
2. Ananyeva, N. D., Susyan, E. A., Chernova, O. V. and Wirth, S. 2008. Microbial Respiration Activities of Soils from Different Climatic Regions of European Russia. *Eur. J. Soil Biol.*, **44**: 147-157.
3. Anderson, J. P. E. 1982. Soil Respiration. Part 2. Chemical and Microbiological Properties. In: "*Methods of Soil Analysis",* (Eds.): Page, A. L. and Miller, R. H.

[DOR: 20.1001.1.16807073.2017.19.1.13.8]

American Society of Agronomy, Madison, Wl, PP. 831-871.
4. Aon, M. A. and Colaneri, A. C. 2001. II Temporal and Spatial Evolution of Enzymatic Activities and Physico-chemical Properties in an Agricultural Soil. *Appl. Soil Ecol.*, **18**: 255-270.
5. Forjan, H., Manso and Zamora, M. 2012. Evolución del Contenido de Material Orgánica en Rotaciones Agrícolas. In: "*Actualización Técnica en Cultivos de Cosecha Fina*", (Eds.): Yagüez J. D., Forján H. J. and López Z. Ediciones: INTA, Tres Arroyos, Buenos Aires, 131 PP.
6. Apezteguía, H. P., Izaurralde, R. C. and Sereno, R. 2009. Simulation Study of Soil Organic Matter Dynamics as Affected by Land Use and Agricultural Practices in Semiarid Córdoba, Argentina. *Soil Till. Res.*, **102**: 101-108.
7. Dick, R. P., Rasmussen, P. E. and Kerle, E. A. 1988. Influence of Long-term Residue Management on Soil Enzyme Activities in Relation to Soil Chemical Properties of a Wheat-fallow System. *Biol. Fert. Soil*, **6**:159-164.
8. Ferreras, L., Toresani, S., Bonel, B., Fernández, E., Bacigaluppo, S., Faggioli, V. and Beltrán, C. 2009. Parámetros Químicos y Biológicos como Indicadores de Calidad del Suelo en Diferentes Manejos. *Ciencia del Suelo*, **27**: 103-114.
9. Frankenberger, Jr. W. T. and Dick, W. A. 1983. Relationships between Enzyme Activities and Microbial Growth and Activity Indices in Soil. *Soil Sci. Soc. Am. J.*, **47**: 945-951.
10. Franzluebbers, A. J. 2002. Soil Organic Matter Stratification Ratio as an Indicator of Soil Quality. *Soil Till. Res.*, **66**: 95–106.
11. Franzluebbers, A. J. 2007. Integrated Crop–livestock Systems in the Southeastern USA. *Agron. J.*, **99**: 361–372
12. Geisseler, D. and Horwath, W. R. 2009. Short-term Dynamics of Soil Carbon, Microbiol Biomass, and Soil Enzyme Activities as Compared to Longer-term Effects of Tillage in Irrigated Row Crops. *Biol. Fert. Soil.*, **46**: 65-72.
13. Gianfreda, L. and Ruggiero, P. 2006. Enzyme Activities in Soil. In: "*Nucleic Acids and Proteins in Soil*: *Soil Biology"*, (Eds.): Nannipieri, P. and Smalla, K. Springer- Verlag Berlin Heidelberg, 449 PP.
14. Gómez-Guinan, Y. 2004. Actividad de las Fosfatasas Ácidas y Alcalinas (Extracelulares e Intracelulares) en Hongos de la Rizosfera de *Arachis hypogaea* (Papiloneaceae). *Rev. Biol. Trop.*, **1**: 287-295.
15. Haynes, R. J. and Williams, P. H. 1999. Influence of Stock Camping Behavior on the Soil Microbiological and Biochemical Properties of Grazed Pastoral Soils. *Biol. Fert. Soil.*, **28**: 253-258.
16. Haynes, R. J., Swift, R. S. and Stephen, R. C. 1991. Influence of Mixed Cropping Rotations (Pasture-Arable) on Organic Matter Content, Water Stable Aggregation and Clod Porosity in a Group of Soils. *Soil Till. Res.*, **19**: 77-87.
17. Khan, S. U. 1970. Enzymatic Activity in a Gray Wooded Soil as Influenced by Cropping Systems and Fertilizers. *Soil Biol. Biochem.*, **2**: 137-139.
18. Lenth, R. V. 2013. *Lsmeans: Least-Squares Means. R Package Version 1.10-2.* http://CRAN.R-project.org/package=lsmeans.
19. Ling, N., Sun, Y., Ma, J., Guo, J., Zhu, P., Peng, Ch., Yu, G., Ran W., Guo, S. and Shen, Q. 2014. Response of the Bacterial Diversity and Soil Enzyme Activity in Particle-Size Fractions of Mollisol After Different Fertilization in a Long-term Experiment. *Biol. Fertil. Soil.*, **50**: 901-911.
20. Melero, S., López-Bellido, R.J., López-Bellido, L., Muñoz-Romero, V., Moreno, F. and Murillo, J. M. 2011. Long-term Effect of Tillage, Rotation and Nitrogen Fertiliser on Soil Quality in a Mediterranean Vertisol. *Soil Till. Res.*, **114** : 97-107.
21. Melero, S. López-Garrido, R., Madejón, E., Murillo, J. M., Vanderliden, K., Ordoñez, R. and Moreno, F. 2009. Long-term Effects of Conservation Tillage on Organic Fractions in Two Soils in Southwest of Spain. *Agric. Ecosyst. Environ.*, **133**: 68-74.
22. Melero, S. Vanderlinden, K. Ruiz, J.C. and Madejon, E. 2008. Long-term Effect on Soil Biochemical Status of a Vertisol under Conservation Tillage System in Semi-arid Mediterranean Conditions. *Eur. J. Soil Biol.*, **44** : 437–442.
23. Mishra, U., Ussiri, D. A. N. and Lal, R. 2010. Tillage Effects on Soil Organic Carbon Storage and Dynamics in Corn Belt of Ohio USA. *Soil Till. Res.*, **107** : 88–96.

[DOR: 20.1001.1.16807073.2017.19.1.13.8]

24. Nannipieri, P., Ceccanti, B., Cervelli, S. and Matarese, E. 1980. Extraction of Phosphatase, Urease, Protease, Organic Carbon and Nitrogen from Soil. *Soil Sci. Soc. Am. J.*, **44**: 1011-1016.
25. Nannipieri, P., Giagnoni, L, Renella, G., Puglisi, E., Ceccanti, B., Masciandaro, G., Fornasier, F., Moscatelli, C. and Marinari, S. 2012. Soil Enzymology: Classical and Molecular Approaches. *Biol. Fert. Soil.*, **48**: 743-762.
26. Paz-Ferreiro, J., Trasar Cepeda, C., Leirós, M. C., Seoane, S. and Gil-Sotres, F. 2011. Intra-annual Variation in Biochemical Properties and the Biochemical Equilibrium of Different Grassland Soils under Contrasting Management and Climate. *Biol. Fert. Soil.*, **47**: 633-645.
27. Paz-Ferreiro, J., Trasar Cepeda, C., Leirós, M. C., Seoane, S. and Gil-Sotres, F. 2010. Effect of Management and Climate on Biochemical Properties of Grassland Soils from Galicia (NW Spain). *Eur. J. Soil Biol.*, **46**: 136-143.
28. Plaza-Bonilla, D., Nolota, J., Passota, S., Raffaillaca, D. and Justesa, E. 2016. Grain Legume-Based Rotations Managed Under Conventional Tillage Need Cover Crops to Mitigate Soil Organic Matter Losses. *Soil Till. Res.*, **156**: 33–43.
29. Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. and R Core Team. 2016. nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-128, http://CRAN.R-project.org/package=nlme.
30. Qin, S., He, X., Hu, Ch., Zhang, Y. and Dong, W. 2010. Responses of Soil Chemical and Microbial Indicators to Conservational Tillage *versus* Traditional Tillage in the North China Plain. *Eur. J. Soil Biol.*, **46**: 243-247.
31. Samuel, A. D., Domuţa, C., Şandor, M., Vuşcan, A. and Brejea, R. 2011 Long Term Effects of Agricultural Systems on Soil Phosphatase Activities. *Rom. Agric. Res.*, **28**: 157-163.
32. Silvestro, L. B., Stenglein, S. A., Forjan, H., Dinolfo, M. I., Arambarri, A. M., Manso, L. and Moreno, M. V. 2013. Occurrence and Distribution of Soil *Fusarium* Species under Wheat Crop in Zero Tillage. Span. *J. Agric. Res.*, **11**:72-79
33. Soil Survey Staff (SSS). 2014. *Keys to Soil Taxonomy.* 12th Edition, Natural Resources Conservation Service, United States Department of Agriculture, 372 PP.
34. Tabatabai, M. A. 1994. Soil Enzymes. Part 2. Microbiological and Biochemical Properties. In: "*Methods of Soil Analysis*", (Eds): Weaver, R. W., Angle, J. S. and Bottomley, P.S. SSSA Book Series 5, Madison, Wisconsin, USA, PP. 775-834.
35. USDA. 2006. Keys to Soil Taxonomy. 10th Ed. EEUU, PP. 332.
36. USDA-NRCS. 2004. *Soil Survey Laboratory Methods Manual. Soil Survey Investigations Report Number 42*. Version 4.0, USDA (United States Department of Agriculture)-NRCS (Natural Resources Conservation Service)–National Soil Survey Center.
37. Wang, Q., Bai, Y., Gao, H., He, J., Chen, H., Chesney, R.C., Kuhn, N. J. and Li, H. 2008. Soil Chemical Properties and Microbial Biomass after 16 Years of No-tillage Farming on the Loess Plateau, China. *Geoderma*, **144**: 502-508.
38. Walkley, A. and Black, I. 1934. An Examination of the Degtjareff Method for Determining Soil Organic Matter and a Proposed Modification of the Chromic Acid Titration Method. *Soil Sci.*, **37**: 29-38.
39. Zuur, A. F., Leno, E. N., Walker, N. J., Saveliev, A. A. and Smith, G. 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York, 574 PP.

[DOR: 20.1001.1.16807073.2017.19.1.13.8]

شناسایی ویژگی های زیست شناسی مولی سولها در شرایط بدون خاکورزی در تناوب های زراعی مختلف

ل. ب. سیلوسترو، ف. بیگانزولی، ه. فورجان، ا. البانسی، ا. م. آرامباری، ل. مانسو، و م. و. مورنو

چکیده

کشت وکار بدون خاکورزی تاثیر مستقیمی روی جوامع میکروبی و تغییر باروری و پایداری خاک دارند. تناوب و ترتیب کشت گیاهان هم می تواند این خواص را دگر گون کند. در این پژوهش ، تاثیر تناوب زراعی در شرایط مدیریتی بدون خاکورزی روی خواص بیولوژیکی و شیمیایی خاک شامل توزیع عمودی مواد آلی خاک، تنفس پایه ای (basal respiration) خاک، و فعالیت آنزیم های دی هیدرو ژناز،اسید فسفاتاز، و اوره آز در طی فصل زراعی هر سال و در اعماق مختلف خاک در یک آزمایش دراز مدت ارزیابی شد. تناوب های استفاده شده در این پژوهش عبارت بودند از:I) یک محصول در سال(آفتابگردان-گندم- سورگوم-سویا); II) مخلوطی از گیاهان زراعی و دامی همراه با علوفه چرا بدون استفاده از علوفه زمستانی یا تابستانه; III) مدیریت زمستانه (گندم-کلزا- جو- سویای دیرکشت; IV) مخلوط با گیاهان علوفه سالانه (گندم-چاودار/ماشک- سویا یا آفتابگردان) و V) مدیریت فشرده(گندم- جو-کلزا ، با یک درمیان سویا و سویا ی دیر کشت. نتایج نشان داد که بسته به نوع تناوب ($P_{seq\ x\ depth} = 0.0173$) کربن آلی خاک با افزایش عمق خاک کم می شد و فارغ از نوع تناوب($P_{depth} = 0.0062$) تنفس پایه ای خاک در لایه ۵-۰ سانتی متری بیشتر از لایه ۲۰-۱۰ سانتی متری در خاک سطحی بود. فعالیت آنزیم های دی هیدرو ژناز،اسید فسفاتاز، و اوره آز تحت تاثیر تناوب زراعی، فصل نمونه برداری، و عمق خاک بود. تناوب های مخلوط(تناوب های II وIV) شامل علوفه چرائی چند ساله یا گیاه علوفه ای یکساله می توانند فعالیت آنزیم های دی هیدرو ژناز و اسید فسفاتاز را بهبود بخشند. تناوب های دارای گیاه پوششی (cover crop) شامل تناوب های II و IV به نفع فعالیت میکروبی بوده و در نتیجه کیفیت خاک را بهبود می دهند.

[DOR: 20.1001.1.16807073.2017.19.1.13.8]