Water Air Soil Pollut (2021) 232:295
https://doi.org/10.1007/s11270-021-05211-2

Enzyme Activities as Indicators of Soil Quality: Response to Intensive Soybean and Rice Crops

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Received: 2 February 2021 / Accepted: 4 June 2021

Abstract Soil enzyme activities are often used as indicators of soil contamination. The responses of the activities of specific soil enzymes, dehydrogenase, acid phosphatase, β-glucosidase, carboxylesterase, and urease to different land uses (soybean and rice crops, and a reference site) were analyzed. Changes in activity at the start and end of each crop cycle were quantified. In general, the catalytic activity of all enzymes was lower in both crops than in the reference site. Regarding the soybean crop, all the enzyme activities decreased at the start of the crop cycle (27.5–53%, with respect to reference site values), whereas only acid phosphatase, β-glucosidase and carboxylesterase were lower at the end of the cycle (70.3%, 29.44%, and 45.79%; respectively). In the rice crop, dehydrogenase, acid phosphatase, and β-glucosidase activities were lower at the start of the cycle (27.88%, 50.32%, and 23.21%; respectively), with respect to reference site. However, the enzyme activity was lower at the end of the cycle compared to the reference site (dehydrogenase 20.47%, acid phosphatase 72.72%, β-glucosidase 57.77%, carboxylesterase 27.59%), except for urease activity. Current results suggested that the use of enzyme activities as indicators of soil quality is a viable approach to assess the pesticide impact in agricultural soils of Argentina.

Keywords Soil pollution · Enzyme activity · Soybean crop · Rice crop

1 Introduction

Intensive agriculture largely depends on a high input of agrochemicals such as herbicides, fungicides, and insecticides (Gianfreda & Rao, 2008; Floch et al., 2011; Attademo el al., 2015; EL-Saeid & Alghamdi, 2020), which may import on soil enzyme activities (Raiesi & Salek-Gilani, 2018). Soil enzymes are produced mainly by microorganisms (Lalitha & Santhaguru, 2012) and, to a lesser extent, by plants and

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11270-021-05211-2.

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Published online: 06 July 2021

animals (Sinsabaugh et al., 2002). They play a pivotal role in inorganic matter decomposition and nutrient cycling (Makoi & Ndakidemi, 2008; Maurya et al., 2011). Therefore, soil enzymes are used as indicators of soil quality (Sinsabaugh et al., 2008).

Soil enzymes are mainly oxidoreductases (e.g. dehydrogenase) and hydrolases (e.g. β-glucosidase, urease, phosphatase, and sulphatase). Dehydrogenase is usually used as a indicator of soil microbial activity (Tan et al., 2014; Trasar-Cepeda et al., 1999) and short-term alterations in soil (Nannipieri et al., 2002, 2018). Hidrolases are involved in the nutrient cycles: carbon, phosphorus, nitrogen, and sulphur (Lessard et al., 2014; Yu et al., 2019a). β-glucosidase activity plays an important role in the degradation of low molecular weight carbohydrates, producing monosaccharides as a product of their hydrolysis. Those sugars are an important resource for soil microorganisms (Conn & Dighton, 2000). The use of soil enzymes to assess pesticides impact on soil quality has been a growing interest in the last decade (Paz-Ferreiro & Fu, 2013; Sanchez-Hernandez et al., 2018). Lessard et al. (2014) recommended selecting the most sensitive soil enzymes and including more than one of these molecules in biogeochemical cycle research. Different studies have reported variations in soil enzyme activities induced by land use conversion, with the magnitude of change differing significantly with type of plant community, scale of temporal and spatial analysis, climate, and soil environment conditions (Zhang et al., 2019). Pesticides are expected to affect the activity of soil enzymes (Bollag & Liu, 1990; Gianfreda & Rao, 2008; Riah et al., 2014). In general, soil enzymes were found to respond to pesticides with inhibition of their activity (Riah et al., 2014). For instance, changes in crop management practices (Trivedi et al., 2013) significantly affected microbial community composition and soil attributes after deforestation (Gamboa & Galicia, 2011). Studies on soil health focusing on enzyme activity alteration by pesticides, are scarce in Argentina.

Moreover, soybean production area has considerably increased in the last years (20 million ha of cultivated land, Lupi et al., 2015). Likewise rice production is growing rapidly (206,500 ha 2016/17, Ministry of Agribusiness, Argentina), in the flood plains of Paraná River, in Santa Fe, Entre Ríos and Corrientes provinces, accounting for 90% of the national production (ACPA, 2015). In the present study, we hypothesized that different land uses in central-eastern Argentina could lead to changes — induction or inhibition — in soil enzyme activities in different agricultural management systems with respect to the reference site. The objectives of this study were to (1) explore the responses of dehydrogenase, acid phosphatase, β-glucosidase, carboxylesterase and urease to different land uses, and (2) to compare the variations in soil enzyme activity at the start (sowing) and end (harvest) of a crop cycle. The knowledge of enzymatic activities in these widely distributed agroecosystems in many regions of South American could be used to implement ecological remediation measures, as well as soil monitoring programs.

2 Material and methods

2.1 Study area

The study area is located in San Javier department, Santa Fe province, in the central-eastern region of Argentina (Fig. 1). The area of highest rice and soybean production is located between Romang (San Javier department) and Colonia San Joaquín (Garay department), to the north and south of the locality of San Javier, respectively (López-Lanús & Marino, 2010). Rice and soybean crops are cultivated in low deforested lands. In the area, native grasslands and natural wetlands coexist with land used for intensive farming and cattle breeding (Begenesic, 1998). Unlike dryland crops, rice requires irrigation during some of the growth stages. Water for irrigation of rice fields is obtained from San Javier River, a tributary of Paraná River (Castignani, 2011). The sowing season extends from September to December, and harvest takes place in February and March. The climate is hot and humid, with rainfall exceeding 1000 mm per year and an average annual temperature of 18 °C. The predominant soil type in the region is planesols, but vertisols dominate the soil structure in the south. Luvic phaeozems and humic gleysols with extensive areas of fluvisols, eutric gleysols, and regosols are common (FAO–UNESCO, 1971–1981). Soil in San Javier department is a loam texture (41.2% sand, 38.7% silt, and 19.2% clay), pH of 5.6 ± 0.2, and total organic carbon of 0.64%, nitrogen of 0.069% and organic matter of

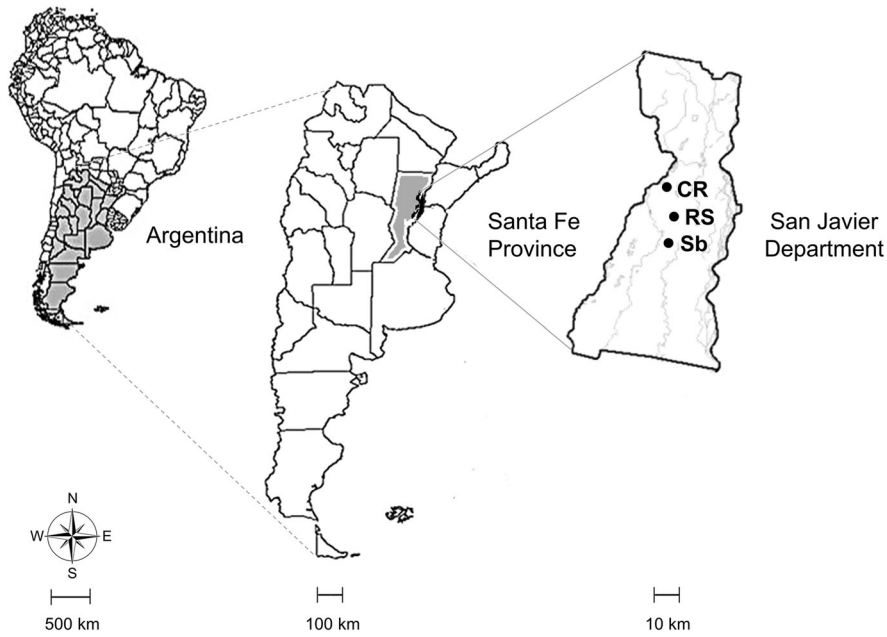

Fig. 1 Location of sampling sites (RS: reference site, Sb: soybean, and CR: rice crop) in Santa Fe province, Argentina

1.1% (INTA, 2019). Moreover, these soils have hydromorphic characteristics, i.e. they have a layer saturated with water over long periods.

Three sites with different management systems (Fig. 1) were selected: a reference site (RS:30° 37′ 42.2″—S 59° 59′ 44″ W); a rice crop (CR: rice monoculture with the use of pesticides in the production process) (30° 36′ 10″—S 60° 04′ 39″ W) and a soybean crop (Sb: soybean monoculture with the use of pesticides in the production process; 30° 40′ 36″—S 59° 58′ 58″ W). TheRS was a natural forest without any agricultural activities within the 3 km surrounding area, where different tree species are dominant, such as *Prosopis nigra, Erythrina crista-galli, Enterolobium contortisiliquum*, and *Vachellia caven*, with the occurrence of some shrubs (*Baccharissa licifolia, B. dracunculifolia*) and herbs (*Paspalum repens, P. prionitis*). The Sb and CR sites were selected because they are the main production activities in the region and South American. Soil forming factors, soil origin and climate conditions are similar in the three sites (INTA, 2019).

In each site, six sampling points were randomly selected at a distance of 50 m among one another. Sampling was conducted at the 0–10 cm surface layer in the morning (8.00–9.00 h). Two samples were taken from each sampling point and mixed to form a composite sample. The samples were transported in plastic tubes placed on ice to the Laboratorio de Ecotoxicologia (Facultad de Bioquímica y Ciencias Biologicas, UNL) and stored at − 30° until analysis of enzymatic activities. Soils were sampled at the start (sowing) and at the end (harvest) of each crop cycle.

2.2 Screening of pesticides in soil samples

A screening of 125 pesticides in soil was carried out in the three sites (CR, Sb, and RS). The compounds were selected taking into account the authorized active ingredients and the current-use pesticides or banned pesticides in Argentina. Pesticides were extracted from soil samples following the QuEChERS approach (Anastassiades et al., 2003) with minor modifications. Briefly, 5 g of soil was soaked in 10 mL of water (0.2% formic acid) for 15 min; then 10 mL of acetonitrile was added, and the sample was shaken for 30 min on a horizontal mechanical shaker. Partition was induced by adding 4 g $MgSO_4$ and 1 g NaCl and completed with 1-min vigorous manual agitation followed by 10-min centrifugation at 15,000 rpm at room temperature. Then, 1 mL supernatant

was cleaned by dispersive solid-phase extraction (d-SPE) with C18 and PSA, before the chromatographic injection. Glyphosate, AMPA (the main metabolite of the microbial degradation of glyphosate) and glufosinate-ammonium were determined separately, as described by Demonte et al. (2018), after a methanol extraction.

For LC-amenable pesticide determination, an ultra-high pressure liquid chromatography was employed (ACQUITY UPLC™, Waters, Milford, MA, USA) coupled to a triple quadrupole mass spectrometer (Micromass TQ Detector from Waters, Manchester, UK) through an orthogonal-Z-spray ionization source (ESI+and ESI−). The separation was performed in a rapid resolution column (C18, 2.1×100 mm, 1.7 µm) using gradient elution, with an acetonitrile and water mix mobile phase, both with 0.1% (v/v) formic acid. For mass detection, two transitions from each compound pseudomolecular ion ([M+H]+or [M−H]−) were used for identification in addition to the retention time, whereas for quantification, the most abundant transition was used. GC-amenable pesticides were determined using an Agilent 7890B gas chromatograph coupled to a triple quadrupole Agilent 7000C mass spectrometer with ionization by Electronic Impact (Agilent Technologies, Santa Clara, CA, USA) and chromatographic separation was done by means of a HP-5MS 5% phenyl methyl siloxane capillary column (30 m×250 µm×0.25 µm, Agilent Technologies). For mass detection, two transitions from each compound ion were used for identification in addition to the retention time, whereas for quantification, the most abundant transition was used. Validation was carried out following the SANTE/11945/2015 document (2015), by determining recovery, selectivity, limits of quantification (LOQ), linearity, precision, and accuracy. The specifications of each pesticide are detailed in Table SD1 and SD2 of the supplementary data.

2.3 Sample preparation for enzyme

Enzyme activities were measured in 1:50 (w/v) soil–water suspensions, following the method by Sanchez-Hernandez et al. (2015). Wet soil (1 g) and distilled water (50 mL) were mixed using Falcon™ tubes at room temperature (20 °C) for 30 min using an orbital shaker (Elmi®Intelli-mixer RM-2L).

2.4 Enzyme activity assays

Dehydrogenase activity was tested using iodonitrotetrazolium chloride as the electron accept (von Mersi & Schinner, 1991). Reduced iodonitrotetrazolium formazan (INTF) formation was determined spectrophotometrically after a 60 min reaction at 40 °C; the results are expressed as µmol INTF h^{-1} g^{-1}dry soil.

Carboxylesterase (EC 3.1.1.1) activity was measured using 4-nitrophenyl butyrate, following Sanchez-Hernandez et al. (2015). Enzyme activities were expressed as µmol of product per hour and gram of dry soil, using calibration curves constructed with 4-nitrophenol.

Acid phosphatase (EC 3.1.3.2) and β-glucosidase (EC 3.2.1.21) activities were tested following Popova and Deng (2010); the reaction medium consisted of 100 µL of soil: water suspension, 100 µL of distilled water and 50 µL of the respective substrates (4-nitrophenyl phosphate or 4-nitrophenyl β-d-glucanopyranoside; 5 mM final concentration) previously dissolved in 20 mM modified universal buffer (pH=6.5). After a 4-h incubation period (continuous shaking at 20 °C), microplates were centrifuged (2500×g, 10 °C and 10 min), and 150 µL aliquots of supernatant were transferred to new microplates. The resulting 4-nitrophenol was immediately (<1 min) read at 405 nm after the addition of 75 µL of 0.5 M NaOH. Standard calibration curves were constructed with 4-nitrophenol.

Urease (EC 3.5.1.5) activity was measured using the method of Schinner et al. (1996). Hydrolytic reactions were conducted by mixing 1 mL 80 mM urea and 1 mL of soil: water suspension in 10-mL tubes, and incubated (orbital shaking) at room temperature (20 °C) for 4 h. Reactions were terminated by adding 5 mL of cold 2 M KCl containing 10 mM HCl. For ammonium extraction, the tubes were agitated for an additional 30-min period and centrifuged (4500×g, 5 min, 10 °C). Supernatants (150 µL) were poured into microplate wells and ammonium was measured after addition of 75 µL of 1:1 (v:v) 0.3 M NaOH: 1.06 M sodium salicylate containing 4.6 mM sodium nitroprusside, followed by the addition of 30 µL of 39.1 mM sodium dichloroisocianide. Microplates were left in the dark for 30 min for color development, and absorbance was read at 690 nm. Urease

activity was expressed as μg NH_4N h^{-1} g^{-1}dry soil using a calibration curve generated with NH_4Cl.

2.5 Data analysis

The effect of land use on soil enzyme activities was tested using a non-parametric Kruskall Wallis test. Data were statistically analyzed using Graph Pad Prism version 6.00 (GraphPad Software, La Jolla, CA, USA). Relationships among enzyme activities were analyzed using a Spearman correlation test. Values were significant at $p < 0.05$.

3 Results

3.1 Pesticide concentrations in soil samples

The results of the screening of pesticide residues in soil in the different soils are shown in Table 1. In Sb, residues of azoxystrobin, carbendazim, glyphosate, and the glyphosate metabolite AMPA were found at the start and end of the crop cycle, whereas azoxystrobin was found at the start of the crop cycle. Conversely, in the CR, three compounds were detected at the start and end of the crop cycle: azoxystrobin, glyphosate, and AMPA, whereas at the end, carbendazim, carboxin, and tebuconazole were detected.

Table 1 Concentration of pesticides in soil under different agricultural management systems: soybean (Sb), rice crop (CR), and reference site (RS). ND: non-detected at the Limit of Quantification in supplementary Table S1. [a] Data are the mean ± SEM of three independent determinations

Sampling sites	Time of sampling	Pesticide	Concentration (μg kg^{-1})[a]
RS	Start		ND
	End		ND
Sb	Start	Azoxystrobin	3 ± 1
		Carbendazim	9 ± 4
		Glyphosate	520 ± 104
		AMPA	265 ± 52
	End	Carbendazim	20 ± 10
		Glyphosate	40 ± 20
		AMPA	147 ± 72
CR	Start	Azoxystrobin	6 ± 3
		Carbendazim	10 ± 5
		Carboxin	6 ± 3
		Tebuconazole	1.0 ± 0.5
		Glyphosate	211 ± 42
		AMPA	188 ± 39
	End	Azoxystrobin	7 ± 3
		Glyphosate	456 ± 93
		AMPA	167 ± 34

3.2 Soil enzyme activities

There was a considerable variation in the activity of the enzymes. Dehydrogenase activity was significantly ($H = 11.3$, $p = 0.0006$) inhibited in CR than in the RS at the start and end of each crop cycle. Likewise, dehydrogenase activity had the lowest values in Sb at the start of the crop cycle ($H = 12.7$, $p = 0.0001$; Fig. 2B). Acid phosphatase and β-glucosidase activities in Sb and CR were significantly lower than in RS, both at the start (Figs. 3 and 4A) and end (Figs. 3 and 4B) of each crop cycle. Carboxylesterase activity was lower ($H = 10.2$, $p = 0.0002$; Fig. 5A) in Sb than in RS at the start of each crop cycle. The esterase activity was also lower ($H = 12.9$, $p = 0.0002$) in Sb and CR (Fig. 5B) than in RS at the end of each crop cycle. Urease activity in Sb was significantly lower ($H = 15.2$, $p = 0.0018$) than that of RS at the start of each crop cycle. There was no significant differences in this hydrolase activity among agroecosystems (Fig. 6A; $H = 3.2$, $p = 0.35$) at the end of each crop cycle. Correlation coefficients among enzymatic activities indicate a significant correlation between urease and dehydrogenase, acid phosphatase, β-glucosidase, and carboxylesterase at start of each cycle crop (Table 2). Carboxylesterase activity significantly correlated with either dehydrogenase, acid phosphatase, or β-glucosidase activity at end of each cycle crop, where there was a significant relationship between β-glucosidase activity and dehydrogenase or acid phosphatase. Similarly, the activities of dehydrogenase and acid phosphatase were significantly correlated at start and end of cycle of crop.

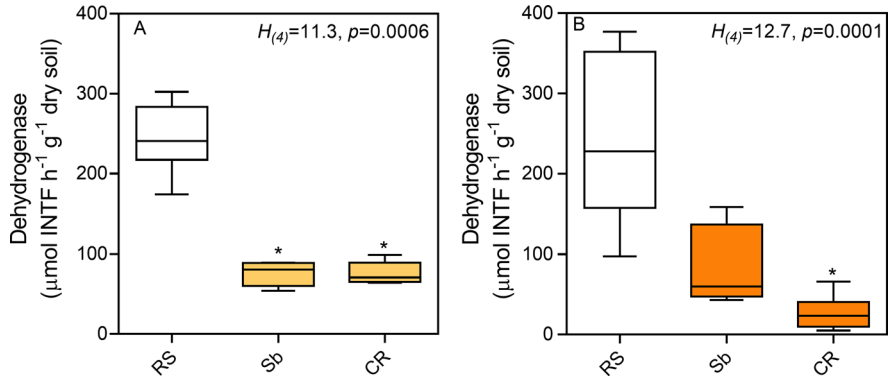

Fig. 2 Dehydrogenase activity in soils of Santa Fe province at the start (**A**) and end (**B**) of the crop cycle. RS: reference site, Sb: soybean, and CR: rice crop. Box-plots indicate the median, the 25th and 75th percentiles (box edges), and the range (whiskers). * $p < 0.05$ compared with the control

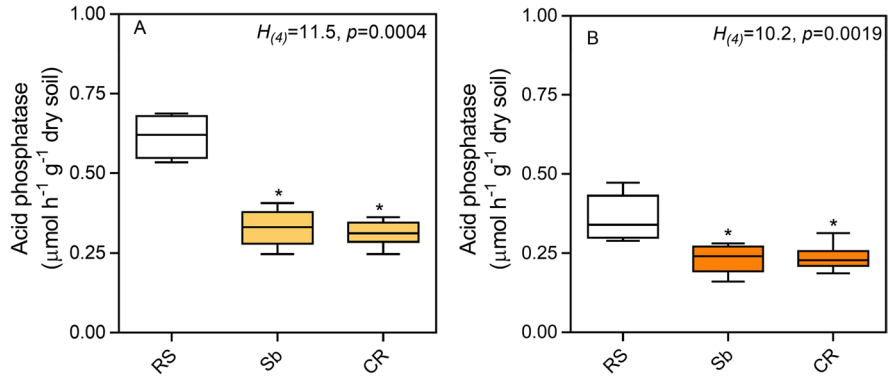

Fig. 3 Acid phosphatase activity in soils of Santa Fe province at the start (**A**) and end (**B**) of the crop cycle. RS: reference site, Sb: Soybean, and CR: c rice crop. Blox-plots indicate the median, the 25th and 75th percentiles (box edges), and the range (whiskers). * $p < 0.05$ compared with the control

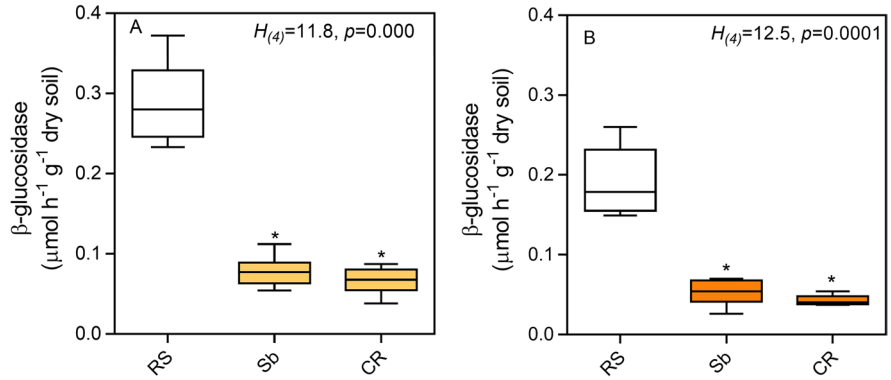

Fig. 4 β-glucosidase activity in soils of Santa Fe province at the start (**A**) and end (**B**) of the crop cycle. RS: reference site, Sb: soybean, and CR: rice crop. Box-plots indicate the median, the 25 th and 75 th percentiles (box edges), and the range (whiskers). *$p < 0.05$ compared with the control

4 Discussion

Agricultural management affects soil enzyme activities. Indeed, the activities were higher in natural or un-disturbed soils than in croplands (Raiesi & Salek-Gilani, 2018). Here, dehydrogenase activity, an indicator of microbial activity (Kuhur et al., 2012), showed significant differences among land uses (soybean, rice and reference site) at the start of each crop cycle; at the end, only the rice field differed with respect to the reference site. However, β-glucosidase, acid phosphatase and carboxylesterase activities exhibited marked differences related to the management type, with both enzymes showing low activities at the start and end of each crop cycle. Enzyme activities are sensitive to agrochemicals (Alvear et al., 2006). Indeed, agrochemicals affect microorganisms and reduce the rate of enzyme production (Borowik et al., 2017; Ochoa et al., 2007). For example, enzyme activity was found to decrease with the application of high doses

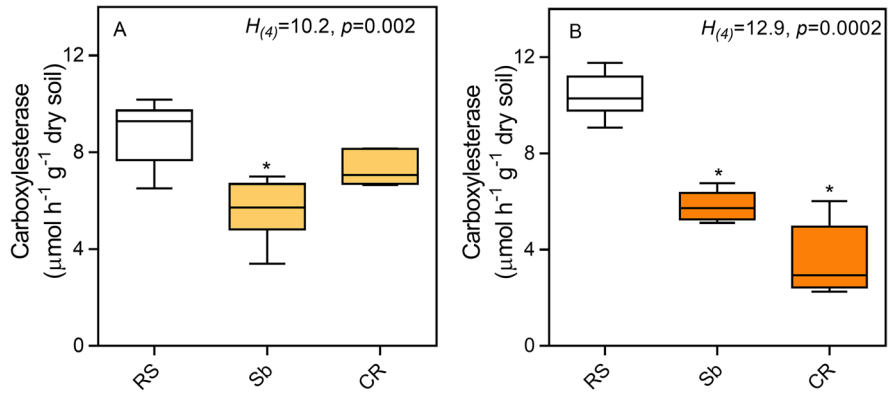

Fig. 5 Carboxylesterase activity in soils of Santa Fe province at the start (**A**) and end (**B**) of the crop cycle. RS: reference site, Sb: soybean, and CR: rice crop. Box-plots indicate the median, the 25th and 75th percentiles (box edges), and the range (whiskers). * $p < 0.05$ compared with the control

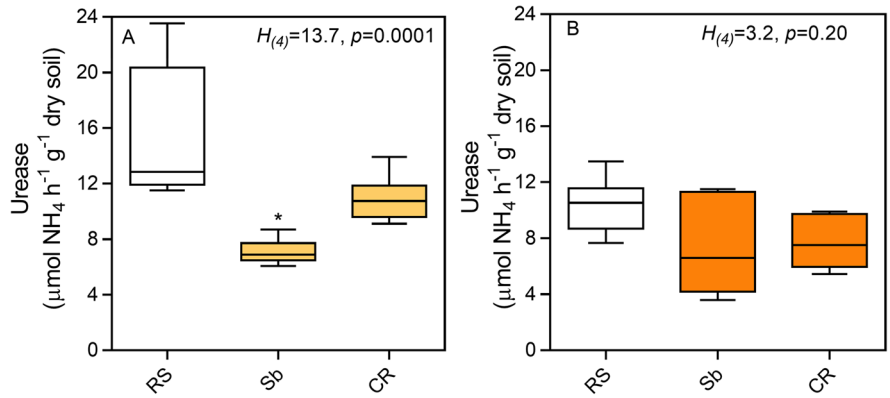

Fig. 6 Urease activity in soils of Santa Fe province at the start (**A**) and end (**B**) of the crop cycle. RS: reference site, Sb: soybean, and CR: rice crop. Box-plots indicate the median, the 25th and 75th percentiles (box edges), and the range (whiskers). * $p < 0.05$ compared with the control

of different agrochemicals (e.g. benomyl, kitazine, mancozeb, mezotrione, and tridimorph; Cycoń et al., 2010; Du et al., 2018). A high number of agrochemicals, such as azoxystrobin, carbendazin, tebuconazole, carboxin, and glyphosate, and metabolite of glyphosate (AMPA) were found in soil samples from soybean and rice crops. In the Argentine Pampas agroecosystems, Alonso et al. (2018) found that most glyphosate in soil samples was below 102–323 µg·kg^{-1}), followed by atrazine (32%; 7–66 µg·kg^{-1}) and then AMPA (22%; 223–732 µg·kg^{-1}). Moreover, fungicides were reported in soil samples from rice fields (azoxystrobin: 8.1 µg·kg^{-1}; Rossi et al., 2020). Although 105 pesticides were searched by different analytical methods, only six were detected in soil. These findings highlight that some contaminants were not detected due to their short half-life, photolysis or the range of detection limits of LC, GC. In addition, the non-observation of the other 99 pesticides not necessarily indicates that they are not present. Therefore, more studies involving different detection limits indifferent seasons considering crop phenology (and, consequently, pesticide applications) should be performed. Moreover, this is the first study that has been carried out in our region including a broawd range of pesticides. Even more, regional comparisons are limited, since there is scarce research in Argentina at multi-residue analysis levels comprising different pesticides in soils (except of glyphosate and AMPA) (Ramirez Haberkon et al., 2021).

Table 2 Correlation coefficients between enzymatic activities. * $p<0.05$ and ** $p<0.01$

		Dehydrogenase	Acid phosphatase	β-glucosidase	Carboxylesterase
Urease	Start	0.65*	0.58*	0.55*	0.79**
	End	0.27	0.28	0.05	0.36
Carboxylesterase	Start	0.38	0.41	0.36	-
	End	0.86**	0.74**	0.84**	-
β-glucosidase	Start	0.59*	0.76**	-	-
	End	0.79**	0.64*	-	-
Acid phosphatase	Start	0.61*	-	-	-
	End	0.63*	-	-	-

Fungicides inhibit the activity of β-glucosidase and dehydrogenase (Baćmaga et al., 2014; Monkiedje et al., 2002). Likewise, Rasool and Reshi (2010), and Sharma et al. (2010) demonstrated that fungicides inhibit phosphatase activities. The destructive effects of the fungicide mancozeb (2000 mg kg^{-1}) on phosphatases, amylase and invertase in soil were reported (Walia et al., 2014). In this context, herbicides like glyphosate found in Sb could have soil inhibited the β-glucosidase activity (Tejada, 2009).

Carboxylesterase activity is an appropriate indicator of pesticide contamination (particularly of organophosphates). Moreover, this esterase is an important detoxification mechanism of oxon metabolites of organophosphate pesticides (Sanchez-Hernandez et al., 2015). Further research is necessary to improve our understanding of the role of these esterases in microbial foraging (Singh, 2014) and to elicit their effectiveness as pesticide exposure biomarkers in the field. Urease activity is a key enzyme in the N-cycle, which derive mainly from plants and microbes (Kong et al., 2008a, b). In this study, urease activity was statistically lower in soybean soil than in the reference soil at the start of each crop cycle. However, herbicides and fungicides have low (Tejada, 2009) or no effect (Yan et al., 2011; Baćmaga et al., 2012) on urease activity. Soil enzymes are sensitive to changes due to disturbances in rice field. In the rice field, soils are flooded during early rice growth stages but not in late growth stages. Reduced enzyme activities under flooded conditions may be a consequence of reduced size of microbial biomass. This phenomenon might occur in those environments where no agrochemicals have been applied (Pulford & Tabatabai, 1988). Soil microbial activity and soil water storage are supposed to be negatively correlated (Zeng et al., 2005), indicating that microbes need oxygen for aerobic respiration (Veverka et al., 2019). By contrast, other studies demonstrated an increase in dehydrogenase activity, which resulted in an increased population of anaerobic microorganisms in rice fields (Srilatha, 2014; Vandana et al., 2012). The effect of season and flooding on enzyme activities in rice fields should be further explored. In addition, crop rotation and mulching of soil surface reduce temperature oscillations, maintaining low soil temperatures and soil moisture during hot and dry seasons, and stimulating microbial activity and crop growth (Dong et al., 2017; Liu et al., 2010; Yu et al., 2019b). Hai-Ming et al. (2014) observed that inclusion of winter cover crops in rotation schemes may improve soil enzyme activities and microbial communities. In our study, the different crops were not under rotation and were under extensive livestock production, which may have affected initial enzyme activity. The highest enzymatic activity observed in forest soils (control) is related to the greater accumulation of soil organic matter (Błońska et al., 2017). Organic matter is a very important nutrient deposit in the nutrient cycle that improves chemical, physical, and biological conditions. Thus, its content influences on both soil fertility and productivity (Steiner et al., 2007).

Finally, our study could be considered a starting point to provide baseline values for dehydrogenase, acid phosphatase, β-glucosidase, carboxylesterase, and urease activities in soybean and rice crops, which can be used for assessing the effects of different soil uses for agricultural activities. In Argentina, the use of the soil and its conservation are a matter of continue debate, and monitoring and evaluating its quality and fertility have begun to gain relevance. Through the Technical Cooperation Mechanism of the Ministry of Foreign Affairs, national experts from the Ministry of Environment and Sustainable Development have trained institutions and farmers from other Latin American countries (Bouza et al., 2016). In this sense, studies on different soil enzyme activities to determine the state of soils are an important contribution not only for agroecosystem assessment but also for management plan establishment.

5 Conclusion

Our results showed a significant decrease of soil enzyme activities in the agricultural soil management schemes, probably because of the intensive use of pesticides. Results include: (1) a lower dehydrogenase activity in soybean and rice than in the forest at the start of the growth cycle of each crop, and at the end of rice crop cycle; (2) β-glucosidase and acid phosphatase activities were low both at the start and the end of soybean and rice cropcycles; (3) carboxylesterase activity was low at the start of the soybean crop cycle, and at the end of soybean and rice crop cycles;

(4) urease activity was low at the start of the soybean crop cycle. The use of enzyme activities as indicators provides useful information to understand the effects of pesticides on the different management practices used in these agroecosystems.

Acknowledgements We are grateful to the farmers (Cooperativa "El Progreso" and Cooperativa "Villa Elisa") for the permission to fieldwork in their farms and to Secretaria de Agricultura Familiar de la Nación Argentina by assistance during the fieldwork.

Author contribution Andres M. Attademo Conception, Design, Execution, Interpretation and Writing.
Juan C. Sanchez-Hernandez: Conception.
Rafael C. Lajmanovich: Design and Interpretation.
Maria Rosa Repetti: Execution and Analyses.
Paola Peltzer: Execution and Interpretation.

Funding This work was carried out under the project "Importancia de los bordes de campo de arroz orgánico en el rol de los vertebrados en el control biológico de plagas" supported by Curso de Acción para la Investigación y Desarrollo (Programa de I+D Orientado a Problemas Sociales y Productivos, UNL), 1 ed by A. M. Attademo and P. M. Peltzer, and programme of academic mobility (PROMAC, UNL).

Data availability Supporting data of the study are available in this published article [supplementary information]. In addition, data sets generated and analyzed during the study are available from the corresponding author upon reasonable request.

Code availability Not applicable.

Declarations

Ethics approval and consent to participate The research has been carried on according to the Ethics Committee of the Facultad de Bioquímica y Ciencias Biológicas (FBCB), Universidad Nacional del Litoral (UNL), Santa Fe, Argentina. http://www.fbcb.unl.edu.ar/pages/investigacion/comite-deetica.php All authors gave their consent to participate.

Consent for publication Not applicable.

Competing interests The authors declare no competing interests.

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