African Crop Science Journal, Vol. 26, No. 1, pp. 79 - 92
Printed in Uganda.
ISSN 1021-9730/2018 $4.00

DOI: http://dx.doi.org/10.4314/acsj.v26i1.6

EVALUATION OF MICROBIAL AND ENZYMATIC COMMUNITIES IN SOIL AND RIZOSPHERE FROM SOYBEAN PLANTS

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(*Received 2 October, 2017; accepted 13 February, 2018*)

ABSTRACT

Understanding the biological and biochemical soil properties, as well as its enzymatic activity is important in designing an efficient alternative to demonstrate desired modifications in the soil. Such modifications are related to crop systems, cultivation practices or other human activities. The objective of this study was to evaluate the diversity of some microorganisms (*Bacillus, Pseudomonas, Trichoderma* and *Fusarium*) and enzymatic activity in soil from soybean crops with different yields. Soil sampling was determined according to the productivity yields of the property, which were divided into high, medium, and low yield. Microorganisms were evaluated by counting populations of fungi and bacteria through serial dilutions of total bacteria, *Bacillus*, *Pseudomonas*, *Trichoderma* and *Fusarium*. The β-glucosidase, acid phosphatase and arylsulphatase activities were determined by spectrophotometry after one hour of incubation at 37 °C, with the specific substrate p-nitrophenol-β-D-glucopyranoside, p-nitrophenol-phosphate and p-nitrophenyl sulphate, respectively, for each studied enzyme. Urease was determined by the ammonium release method, after incubating the soil with urea for two hours, at 37 °C. Soil samples from the high-yield plot had higher concentrations of *Bacillus* and total bacteria. The low-yield soil showed a higher level of colonies of the genus *Fusarium*. The studied enzyme activities (β-glycosidase, arylsulphatase and urea) were found at lower values in soil samples from the high-yield field and differed statistically from the low-yield field. However, in the rhizosphere samples, these enzymes had a higher activity in the high-yield field. In view of these results, it is possible that the yield of soybean plants influences the number of microorganisms and the enzymatic activity of the soil microbiota.

Key Words: *Bacillus,* p-nitrophenol-β-D-glucopyranoside, *Pseudomonas, Trichoderma* and *Fusarium*

RÉSUMÉ

Comprendre les propriétés biologiques et biochimiques du sol, de même que son activité enzymatique est important dans la mise œuvre d'une alternative efficiente pour démontrer les modifications désirées dans le sol. De telles modifications sont reliées aux systèmes de culture, pratiques culturales ou les autres activités humaines. L'objectif de cette étude était d'évaluer la diversité de quelques microorganismes (*Bacillus, Pseudomonas, Trichoderma* and *Fusarium*) et activité enzymatique dans le sol des cultures du soja avec différents rendements. L'échantillonnage des sols était déterminé selon la productivité de la propriété, qui était divisée en élevée, moyenne et faible rendement. Les microorganismes étaient évalués par comptage des populations des champignons et bactéries à travers des séries de dilutions des bactéries totales, *Bacillus*, *Pseudomonas*, *Trichoderma* and *Fusarium*. Les activités du b-glucosidase, acide phosphatase et arylsulphatase étaient déterminées par la spectrophotométrie après une heure d'incubation à 37ºC, avec le substrat spécifique de p-nitrophenol-b-D-glucopyranoside, p-nitrophenol-phosphate et p-nitrophenyl sulphate, respectivement, pour chaque enzyme étudiée. Uréase était déterminée par la méthode de la libération d'ammonium, après incubation du sol avec l'urée pendant deux heures, à 37ºC. Les échantillons des terres à haut rendement ont les concentrations élevées en *Bacillus* et bactérie totale. Les terres à faible rendement ont montré un niveau élevé de colonies du genre *Fusarium*. Les activités des enzymes étudiées (b-glycosidase, arylsulphatase et urée) étaient trouvées à de faibles valeurs dans les échantillons de terres à haut rendement et diffèrent statistiquement des terres à faible rendement. Néanmoins, dans les échantillons de la rhizosphère, ces enzymes ont une activité élevée sur les terres à rendement élevé. D'après ces résultats, il est possible que le rendement des plants du soja influence le nombre de microorganismes et l'activité des enzymes des sols microbiote.

Mots Clés: *Bacillus,* p-nitrophenol-b-D-glucopyranoside, *Pseudomonas, Trichoderma* et *Fusarium*

INTRODUCTION

Understanding the biological and biochemical soil properties, as well as its enzymatic activity is important in designing an efficient alternative to demonstrate desired modifications in the soil. Such modifications are related to crop systems, cultivation practices or other human activities (Matsuoka *et al.,* 2003). As stated by Gama-Rodrigues *et al.* (2005), soil organic matter has microbial biomass that is very sensitive to environmental and biological changes. According to these authors, responses to variations in the soil cultivation and management system can be detected much faster by analysing microbial biomass and its activity than variations in C and N levels in the soil. Siqueira *et al.* (1994) suggested that soil fertility is not subject only to the physical and chemical properties of the soil, but is also related to the activity and biological interactions taking place in the soil.

The soil consists of various chemical, physical and biological characteristics such as soil organic matter (SOM), water retention capacity, resistance to erosion, nutrient availability, microbial activity, cation exchange capacity, gas emission and organic C, that can be measured to detect its modifications. However, the chosen bioindicators must be measurable, equivalent to these characteristics and enable the analysis and monitoring of changes that occur in this environment (Araújo *et al.,* 2013).

The soil microbial activity indicates its quality and can be measured by the microbial C (Silva *et al.,* 2010) and microbial N (Gama-Rodrigues *et al.,* 2005). This activity may be also be evaluated by enzymatic reactions, e.g., β-glycosidase, urease, acid phosphatase and arylsulphatase; by the respiratory activity and microbial biomass of the soil (Lisboa *et al.,* 2012); or by the most probable number (MPN) of bacteria and fungi in soil (Silveira *et al.,* 2006).

The estimate of some microbial groups can probably indicate how biochemical actions are taking place in the soil. This is because the nutrient cycles in the soil are directly dependent on the microbial action (Silveira *et al.,* 2006). The quantification of enzymes has become an effective soil bioindicator, since enzymatic

activity can be used to evaluate the activity of the microbiota. In this regard, the most studied enzymes are β-glycosidase, arylsulphatase, acid phosphatase and urease (Araújo and Monteiro, 2007). The β-glycosidase enzyme hydrolyses the residues of cellobiose, acting in the final process of the cellulose decomposition (Eivazi and Tabatabai, 1988); therefore, changes in the activity of this enzyme can indicate the soil quality.

Passos *et al.* (2008) observed that the β-glycosidase activity in soil covered for up to 30 days, was higher in non-solarised compared with solarised soil, and that soil with addition of poultry litter showed a 5% increase in β-glycosidase activity.

Arylsulphatase is another enzyme that has been extensively examined in studies on soil quality. This enzyme releases sulphate ions into the soil solution from the hydrolysation of sulphate ester bonds, which are the substrate of the enzyme; thus participating in the sulphur cycle. This enzyme is released by microorganisms and vegetables through exudates of the root system or after death and disruption of root cells (Tabatabai and Bremer, 1970). According to Baligar *et al.* (1988), the arylsulphatase enzyme activity in the soil decreases with a reduction in the amount of organic matter and an increase in soil depth. Pinto and Nahas (2002) asserted that the arylsulphatase enzyme activity in integrated forest soil (integration between agriculture, livestock activities and forest plantation) was significantly higher compared with that of other studied soils (isolated forest, eucalyptus plantation, pasture and corn crop). The same authors observed that soil cultivated with corn had very low enzymatic activity (0.15 µg p-nitrophenol g^{-1} soil dm h^{-1}), which was 152 times lower than the maximum found in integrated forest soil (37.02 µg p-nitrophenol g^{-1} soil dm h^{-1}).

Acid and alkaline phosphatase enzymes are also good indicators of the soil quality, as they are synthesised by microorganisms that mineralise the organic phosphorus in the soil, making it available to plants. Plants and microorganisms can excrete these enzymes, but most of them are produced by the former, because of their rapid metabolism (Dick and Tabatabai, 1992). Gatiboni *et al.* (2008) demonstrated that a lower availability of phosphorus in the soil can cause a decrease in the phosphorus content stored in the soil microbial biomass and an increase in the activity of acid phosphatase enzymes.

Urease, another important enzyme indicator for the soil quality, participates in the mineralisation of nitrogen, one of the main nutrients required by plants. This is an extracellular enzyme synthesised by bacteria, fungi and actinomycetes of the soil. It carries out hydrolysis of urea into carbon dioxide and ammonia, and the latter may have nitrogen immobilised by microorganisms and/or absorbed by plants (Tabatabai and Bremner, 1972). As reported by Vargas *et al.* (2005), urease activity is higher in the no-tillage system, compared with the conventional system, due to the accumulation of organic matter over time. The urease enzyme activity in plantations with crop residues was approximately 30 times higher than that observed in conventionally cultivated soils, and these, when compared with soils under the no-tillage system, showed a four times lower urease enzyme activity (Barreto and Westerman, 1989).

The objective of this study was to evaluate the diversity of some microorganisms and the â-glycosidase, arylsulphatase, phosphatase and urease enzymes in different soybean growing areas in state of Parana, Brazil

MATERIALS AND METHODS

This study was based on soil samples from soybean production areas collected at a property in Candói, Paraná State, Brazil (Table 1).

Soil sampling was determined according to the yield of the stands, as follows: 5.2 Mt ha^{-1}, high yield; 4.2 Mt ha^{-1}, medium yield; and 3.5 Mt ha^{-1}, low yield, represented by yield

TABLE 1. Sampling site (Paraná State (PR)) features in Brazil

Sampling site		Paraná
City		Candói
Coordinate		S-25º 31' 15,6', W-51º 47' 19,8''
Climate features	Type	Cfb - Rainy during winter and summer
	Average annual temperature	16.9 ºC
	Dry season	June to august
	Rainy season	September to February
	Monthly precipitation	150 to 190 mm
Soil features	Type	Cambic aluminum Bruno Latosol
	Texture	Clay
Soil management	Planting system	Crop rotation: soybean, oat, maize, wheat, barley
Sampling	Soil type	Bulk soil and rhizosphere

Adapted from Santos *et al.* (2017)

maps measured by the properties' management system.

Sampling was achieved by collecting 10 subsamples or simple samples in each studied field aiming at the formation of a composite soil sample, harvested in a zigzag pattern. In total, 90 simple samples were collected in all areas. The same procedure was performed three times to compose the replicates (3) of the sampling in each plot with a different productivity history, but varying the initial point of entry of the plot for the collection of subsamples, considering another side of the same plot. The soil samples from the soybean inter-rows were collected with a Dutch soil auger.

Rhizospheric soil samples were composed of fractions of the rhizospheric soil collected from 20 soybean plants in each replicate, three replicates per productivity plot, which was established for the sampling of inter-rows in the studied plots. For this, the soybean plants at the stage of grain maturation were removed from the soil with the roots, and the soil adhered to them was reserved to form the composite sample (sample of soil from the rhizosphere or rhizospheric soil).

Soon after the removal of soil and rhizospheric soil simple samples, the composite samples of each treatment were placed in labelled plastic bags, packaged in a Styrofoam box and immediately sent to Embrapa's Environmental Microbiology Laboratory, located in Jaguariúna (SP, Brazil).

Procedures for soil analysis. In the laboratory, the samples were separated into three parts: (a) one for the evaluation of the microorganisms present in the soil; (b) another for the determination of β-glycosidase, acid phosphatase, urease and arylsulphatase enzymes; (c) and a third part was sent to the Soil Laboratory of Embrapa Maize and Sorghum, Sete Lagoas, MG, Brazil to analyse soil fertility by determination of macro and micronutrients, as well as Al and pH.. Samples for the isolation of the cultivable microorganisms were stored at room temperature. For the enzyme determination, samples were ground through a 2 mm mesh sieve and stored at a temperature of 4 ºC.

Evaluation of microorganisms. The fungal and bacterial populations were counted through

serial dilutions of total bacteria, *Bacillus, Pseudomonas, Trichoderma* and *Fusarium* (Costamilan, 2003; Fontes *et al*., 2003; Gomes *et al*., 2003; Silva *et al.,* 2007; Silva *et al.,* 2011a).

Enzymatic analyses. The β-glycosidase (Tabatabai, 1982; Eivazi and Tabatabai, 1988), acid phosphatase (Tabatabai and Bremner, 1969; Eivazi and Tabatabai, 1977) and arylsulphatase (Tabatabai and Bremmer, 1970) enzyme activities were determined by spectrophotometry after an hour of incubation at 37 °C, with the specific substrate p-nitrophenol-β-D-glucopyranoside, p-nitrophenol-phosphate and p-nitrophenyl sulphate, respectively for each enzyme. After the preparation of a standard curve of p-nitriphenol, we obtained the activities in µg p-nitrophenol g^{-1} soil dm h^{-1}.

Urea was determined by the method of Tabatabai and Bremner (1972), which is based on the determination of the ammonium released after incubation of the soil with urea for two hours at 37 °C. The produced ammonia was measured by distillation and titration (Tedesco, 1985) and expressed in ìg ammonia g^{-1} soil dm h^{-1}.

For the statistical analysis, the values observed for the microorganisms were subjected to an analysis of variance performed in the SISVAR software, whereas the enzymes values were analysed using in the R software. When the treatments differed significantly by the F test, means were compared by the Tukey test at the 5% probability level.

RESULTS

Microorganisms. There were significant differences for the genera *Bacillus*, *Trichoderma* and *Fusarium* and for the total bacteria in the soil (Table 2). The test of means revealed that genus *Bacillus* presented the highest number of colonies in the medium- and low-yield soils (9.94 10^6a and 8.67 10^6a cfu) compared with the soil collected in the high-yield field (1.58 10^6 cfu).

The number of colonies of genus *Trichoderma* was higher in the field with a history of medium yield (28.83 10^6 cfu) compared with the high- (19.33 10^6 cfu) and low-yield (0.08 10^6 cfu) soils. On the other hand, the colonies of genus *Fusarium* were found at higher values in the low-yield soil (9.08 10^6 cfu) compared with the high- and medium-yield soils, which, in turn, showed 2.75 10^6 and 2.42 10^6 cfu, respectively (Table 2).

There were significant differences for the genera *Trichoderma* and *Fusarium* in the soybean plant rhizospheric soil samples (Table 3). The test of means revealed that genus *Trichoderma* showed higher values of colonies in the samples of rhizospheric soil (155.33 10^6 cfu) from the low-yield field, compared with the other rhizosphere samples from the high- and medium-yield plots, with respective values

TABLE 2. Values for colony forming units in different soil samples from a farm in Paraná State in Brazil

Yield	Bacteria total	*Bacillus* spp.	*Pseudomonas* spp.	*Trichoderma* spp.	*Fusarium* spp.
High	10.17 10^{6a}	1.58 10^6b	3.58 10^6a	19.33 10^6b	2.75 10^3b
Medium	3.83 10^6b	9.94 10^6a	3.33 10^6a	28.838 10^6a	2.42 10^3b
Low	2.50 10^6b	8.67 10^6a	4.58 10^6a	0.08 10^6c	9.08 10^3a
LSD (0.05)	11.17	14.48	3.15	8.92	3.43
CV (%)	27.36	39.37	36.40	23.08	29.95

Means followed by same letter do not differ by Tukey's test at 5% probability

TABLE 3. Values for colony-forming units (cfu) in the different rhizospheric soil samples from Paraná State in Brazil

Yield	Bacteria total	*Bacillus* spp.	*Pseudomonas* spp.	*Trichoderma* spp.	*Fusarium* spp.
High	25.92 10^{6a}	110.25 10^{6}a	3.25 10^{6}a	24.17 10^{6}b	6.00 10^{3}b
Medium	22.92 10^{6a}	108.58 10^{6}a	1.94 10^{6}a	14.67 10^{6}c	10.75 10^{3a}
Low	26.00 10^{6a}	109.75 10^{6}a	2.25 10^{6}a	155.33 10^{6}a	11.33 10^{3a}
LSD (0.05)	13.65	45.95	2.95	9.17	1.73
CV (%)	27.55	19.96	26.43	7.18	7.86

Means followed by the same letter do not differ by Tukey's test at 5% probability

of 24.17 10^6 and 14.67 10^6 cfu. The rhizosphere samples also presented higher numbers for *Fusarium* colonies in the low-yield (11.33 10^6 cfu) compared with the high-yield plot (6.00 10^6 cfu). Total bacteria and the genera *Bacillus* and *Pseudomonas* did not show statistical differences between the samples of rhizospheric soil from the different studied plots (Table 3).

Chemical enzymes and soil analysis. There were no significant differences for most of the chemical characteristics of soil and rhizosphere region at the different yields studied (Table 4). However, total enzymatic activity (Table 5) was statistically different across the studied yields. The soil that presented the best enzymatic characteristics for the β-glycosidase and arylsulphatase enzymes was that one collected from medium-yield plots. The high-yield field showed higher values for the acid phosphatase enzyme, whereas the low-yield field had the highest levels of the urease enzyme.

The principal component analysis (PCA) revealed a separation of the soil samples by the yield history of the plots (high, medium and low). Samples from the sites with a history of high and medium yields were distant by approximately 39.0%; while samples from low- and medium-yield fields had a distance of around 18% (Fig. 1-A). The attributes that most influenced the separation of samples (correlation 1) positively were, in descending order, SAT> Al> H + Al> C> CEC> Urease; and negatively, pH> Ca> SB> base saturation. (Fig. 1- C). Where SAT = 0.9975558, Al = 0.9974971, H + Al = 0.9965566, C = 0.9790480, CEC = 0.9780503, Ca = -0.9873851, and SB = -0.9874240. In the separation of samples for correlation 2, the most influential factors were the arylsulphatase enzyme (-0.7904293) and manganese (Mn) (0.7334711) (Fig. 1 - D).

The PCA showed that the samples were separated by the historical yield of the studied plots in the rhizospheric soil collected, with samples distant from each other by approximately 41% horizontally, and vertically by approximately 20% (Fig. 2-A). These differences can be better visualised by the correlation between the studied attributes and the different yields, since it shows which attributes had the most influence on the differentiation of the samples. Overall, in the comparison of samples by yield history, the factors that most contributed positively to the horizontal dissimilarity of the rhizospheric soil were the chemical attributes Mg> CEC> and phosphatase; and negatively, β-glycosidase (Fig. 2-C). Vertically, this differentiation was more influenced positively by urease and negatively by Mn (Fig. 2-D).

DISCUSSION

Microorganisms. Soil from the high-yield plot showed higher densities of *Bacillus* and total bacteria colonies (Table 2), suggesting a greater diversity of microorganisms in the study area.

TABLE 4. Chemical characterisation of dry soil collected after soybean planting in areas with different yields in Brazil

Variable	Rhizosphere				Soil			
	H-R	M-R	L-R	CV (%)	H-S	M-S	L-S	CV (%)
pH H_2O	6.7 a	6.7 a	6.6 a	2.2	6.2 a	6.2 a	6.2 a	2.2
Phosphorus Mehlich (1 mg dm^{-3})	16.9 a	16.1 a	19.1 a	28.0	8.7 a	4.8 a	6.7 a	38.3
Nitrogen (g kg^{-1})	79.6 a	73.8 a	67.5 a	8.6	64.6 a	61.7 a	54.3 a	7.0
Total carbon (g kg^{-1})	46.3 a	42.9 a	41.9 a	7.3	37.6 a	35.9 a	35.0 a	6.3
$H+Al^{2+}$ (cmolc dm^{-3})	4.8 b	5.5 ab	6.1 a	8.6	8.1 a	6.9 b	7.4 ab	5.7
Al^{2+} (cmolc dm^{-3})	0.003 a	0.000 a	0.000 a	300.0	0.010 a	0.010 a	0.013 a	30.0
Ca^{2+} (cmolc dm^{-3})	7.2 a	6.4 a	6.8 a	7.0	4.9 a	4.3 a	4.6 a	7.6
Mg^{2+} (cmolc dm^{-3})	2.5 a	1.8 b	2.0 b	7.9	1.4 a	1.3 a	1.2 a	6.8
K^{+} (mg dm^{-3})	259.6 a	220.1 a	236.9 a	7.1	181.7 a	122.5 a	164.3 a	19.0
SB (cmolc dm^{-3})	9.4 a	9.0 a	9.6 a	7.1	6.7 a	6.4 a	6.8 a	7.1
CEC (cmolc dm^{-3})	15.7 a	13.9 b	14.9 ab	3.2	15.3 a	13.3 b	14.2 ab	4.8
Base saturation (%)	67.7 a	62.5 a	62.6 a	4.6	47.8 a	48.0 a	46.2 a	5.9
Al sat. (%)	0.003 a	0.000 a	0.000 a	300.0	0.1 a	0.2 a	0.2 a	32.0
Cu^{2+} (mg dm^{-3})	2.1 a	1.8 a	1.7 a	31.6	4.2 a	2.8 ab	2.2 b	21.0
Fe^{2+} (mg dm^{-3})	26.4 b	25.0 b	35.9 a	11.8	38.0 a	46.7 a	42.7 a	14.0
Mn^{2+} (mg dm^{-3})	26.4 a	22.5 a	15.3 b	9.3	16.1 a	11.0 a	11.1 a	18.5
Zn^{2+} (mg dm^{-3})	6.4 a	5.0 a	6.0 a	17.4	9.9 a	5.8 a	7.1 a	42.3

Means followed by the same letter in the row do not differ, for each collection, by Tukey's test ($P < 0.05$) performed using the Rstudio statistical software. High rhizosphere yield (H-R), Medium rhizosphere yield (M-R), Low rhizosphere yield (L-R), High soil yield (H-S), Medium soil yield (M-S), Low soil yield (L-S)

TABLE 5. Total enzymatic activity in dry soil collected after soybean planting in areas with different yields in Brazil

Variable	Rhizosphere				Soil			
	H-R	M-R	L-R	CV (%)	H-S	M-S	L-S	CV (%)
β-glycosidase (μg p-nitrophenol g_{soil}^{-1} h^{-1})	19.5 c	25.5 a	22.2 b	2.9	10.3 b	15.3 a	9.3 b	3.8
Phosphatase (μg p-nitrophenol g_{soil}^{-1} h^{-1})	16.8 a	13.7 b	11.6 c	2.4	27.5 a	13.5 b	8.8 c	6.7
Arylsulfatase (μg p-nitrophenol g_{soil}^{-1} h^{-1})	49.9 c	60.9 a	58.4 b	1.5	55.7 a	27.3 b	18.4 c	6.6
Urease (μg ammonia g_{soil}^{-1} h^{-1})	59.0 b	59.0 b	75.6 a	5.6	45.6 b	64.9 a	60.9 a	6.1

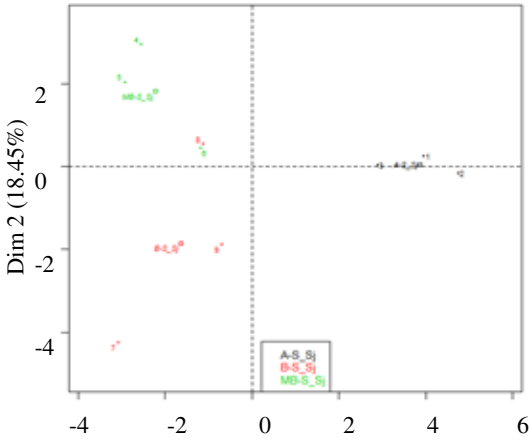
Means followed by the same letter in the row do not differ, for each collection, by Tukey's test ($P < 0.05$) performed using the Rstudio statistical software. High rhizosphere yield (H-R), Medium rhizosphere yield (M-R), Low rhizosphere yield (L-R), High soil yield (H-S), Medium soil yield (M-S), Low soil yield (L-S)

Microorganisms are important in organic matter degradation and soil nutrient dynamics, mediating plant development. Genus *Bacillus* can influence seeds germination and plant growth promotion (Schisler *et al.,* 2004). According to Araújo and Hungria (1999), the *B. subtilis* isolate or its metabolites increase nodulation and yield in soybean. It is widely reported that soils showing greater biodiversity have more classes of microorganisms that operate in the degradation processes of agricultural pesticides and conservation of microbiological processes in situations of environmental stress. This is generally referred to as "biological buffering effect" (Pereira *et al.,* 2007).

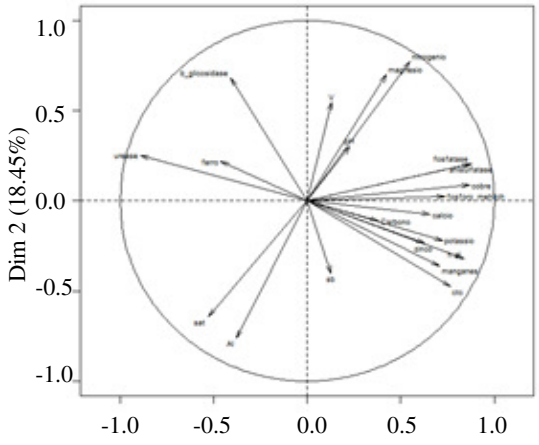
The low-yield soil showed a higher number of colonies of genus *Fusarium* (Table 2). Some species of this genus are pathogenic to soybean, e.g. *Fusarium semiquumum* (Goulart, 2004), *Fusarium graminearum* (Ellis *et al.,* 2011), and *Fusarium solani* (Mart.) Sacc. f. sp. *glycines* (Roy, 1997). Thus, the presence of a higher quantity of this fungus in the soil from the low-yield plots can be considered an important bioindicator for the decrease in the yield of these cultivated areas.

Trichoderma is a genus well-known for promoting plant growth, which may explain the higher number of cfu in the soil collected from the plot with a medium historical yield. According to Silva *et al.* (2011), substrate inoculated with *Trichoderma* species increase growth of cucumber plants by 100%. In addition, many species of *Trichoderma* spp. have been used in the biocontrol of pathogens, such as *Venturia* spp. and *Botrytis* spp. (Mello, 1996). In the rhizosphere samples from the low-yield plot, there was a high number of cfu of genus *Trichoderma*. Although this genus is known as a promoter of plant growth and a pathogen inhibitor, the high number of colonies of this genus may be causing a microbial imbalance near the roots of soybean plants (Vinale *et al.,* 2008). The high number of *Trichoderma* colonies may have caused a microbial imbalance near the roots of the soybean plants. This imbalance may be related

A



B



C

Analysis	Correlation (soil)	P. value	Analysis	Correlation (soil)	P. value
SAT	0.9975558	6.841424e-13	P	-0.6136250	3.381967e-02
Al	0.9974971	7.702451e-13	Zn	-0.6889806	1.320568e-02
H+Al	0.9965566	3.790453e-12	β-glycosidase	-0.7328988	6.696557e-03
Carbon	0.9790480	3.070088e-08	K	-0.9366100	7.242827e-06
CEC	0.9780503	3.867535e-08	Mg	-0.9474775	2.881227e-06
Urease	0.9777082	4.176104e-08	pH	-0.9828953	1.120489e-08
Nitrogen	0.9655222	3.620973e-07	Ca	-0.9873851	2.463312e-09
Phosphatase	0.9209508	2.126387e-05	SB	-0.9874240	2.425718e-09
Fe	0.9121955	3.541559e-05	Base saturation	-0.9943791	4.377131e-11

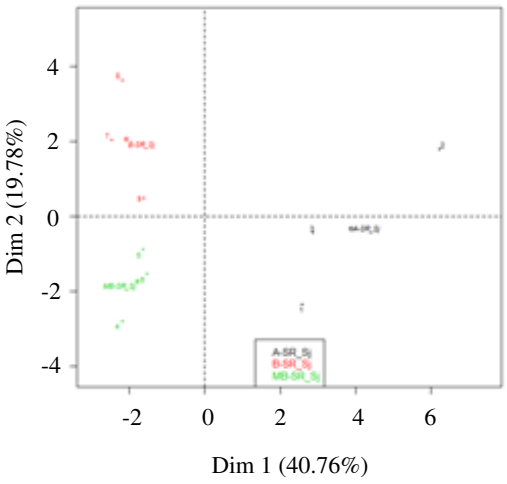
D

Analysis	Correlation (rhizospheric soil)	P. value
Manganese	0.7334711	0.006632152
Copper	0.6403975	0.024873676
β-glycosidase	-0.5005957	0.097391479
Arylsulfatase	-0.7904293	0.002212409

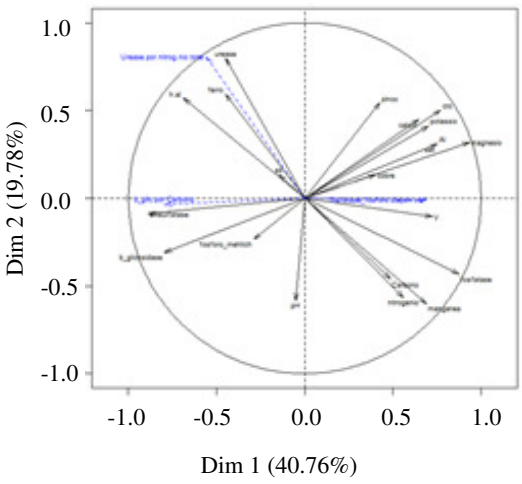
Analysis	Correlation (rhizospheric soil)	P. value
Mn	0.7334711	0.006632152
Cu	0.6403975	0.024873676
β-glycosidase	-0.5005957	0.097391479
Arylsulfatase	-0.7904293	0.002212409

Figure 1. Principal Component Analysis for specific enzymatic activity and chemical attributes of soil collected from a soybean crop in areas with different yields. A - Graph of individual factor PCA. B - Map of several PCA factors. C- Correlation 1 - horizontal distance between samples. D - Correlation 2 - vertical distance between samples.

A



B



C

Analysis	Correlation (Rhizosphere)	P. value	Analysis	Correlation (Rhizosphere)	P. value
Mg	0.93249	0.00025	Phosphatase/$P_{avail.}$	0.68997	0.0397
Phosphatase	0.87329	0.0021	Mn	0.68856	0.04027
CEC	0.77045	0.01512	Ca	0.64873	0.05873
Al	0.75204	0.01942	H+Al	-0.6859	0.04139
Sat	0.75204	0.01942	b-glycosidase	-0.7953	0.0104
Base saturation	0.72387	0.02746	b-glyc/$C_{avail,}$	-0.7953	0.0104
K	0.70086	0.03544	Arylsulfatase	-0.8887	0.00136

D

Analysis	Correlation (Rhizosphere)	P. value	Analysis	Correlation (Rhizosphere)	P. value
Urease/N_{Total}	0.80546	0.00879	pH	-0.5836	0.09898
Urease	0.79729	0.01006	Mn	-0.6036	0.08523
Fe	0.59426	0.09151			

Figure 2. Principal Component Analysis for specific enzymatic activity (dotted blue), chemical doses and biological attributes of soil collected from the soybean rhizosphere area, comparing between areas with different yields. A - Graph of individual factor PCA. B - Map of several PCA factors. C- Correlation 1 - horizontal distance between samples. D - Correlation 2 - vertical distance between samples.

to the production of secondary, volatile and non-volatile metabolites, which have a broad spectrum of antimicrobial action (Vinale *et al*., 2008).

The productivity of plants is linked to the microbiota of the environment, because microbial actions such as biological nitrogen fixation, mycorrhizae and solubilisation of minerals such as phosphate provide nutrients in the largest quantity for plants (Moreira and Siqueira, 2006). Thus, from a microbiological perspective, the occurrence of a more abundant environment can demonstrate its influence in the productivity of soybean plants.

Enzymes and nutrients. In addition to the microorganisms studied, the soil chemistry, b-glucosity, acid phosphatase, arylsulfatase and urease enzymes were also evaluated to verify soil differences in the rhizosphere and in soil between plants, from plots cultivated with soybean with different productivity in the state of Paraná.

The chemical analysis of the soil for most of the attributes did not reveal differences between the studied soils, so we can infer that the yield difference between the plots. This possibly can be due to microbial diversity, its relationships with plants and their activities in the soil (Table 4).

For the specific β-glycosidase, arylsulphatase and urease enzymatic activities, soil from the high-yield soybean plot showed values statistically lower or equal to those obtained from the medium-yield plot (Table 5). However, in the rhizosphere samples, the enzymes had a greater activity in the high-yield field. The values found for these enzymes were lower in the soil collected from the low-yield soybean fields, likely because of the lower microbial activity of these cultivation environments, which, in turn, are able to release these enzymes.

Fernandes *et al.* (1998) reported that the activity of the acid phosphatase enzyme in two types of soil and three conditions of use (plant cultivation, rainforest and pasture) was lower in Oxisols with plant cultivation (385 mol p-nitrophenol g^{-1} h^{-1}) in a conventional system and in native-forest soil (650 mol of p-nitrophenol g^{-1} h^{-1}). In the same soil, the organic phosphorus content of the microbial biomass was also lower under plant-growing conditions (9 mg kg $soil^{-1}$) than in the rainforest (15 mg kg $soil^{-1}$). Thus, according to these authors, the higher acid phosphatase activity observed in soils under forest is possibly due to the higher immobilisation of P in the microbial biomass, reflecting the amount of microorganisms present in that soil. As stated by Conte *et al.* (2002), the microorganisms of soil cultivated in the no-tillage system have their importance in biocycling and P deposition in their cells. In this way, the phosphorus stays available for a longer time in the soil for absorption by the plants.

According to Nogueira and Melo (2003), the arylsulphatase enzyme activity is essential the plantation since this enzyme has the function of transforming organic forms of sulphur into inorganic forms, making them available to plants, and thus modifying their development. The results found for arylsulphatase activity in the fields with high yield history are, therefore, in agreement with the above concept, which determines that soils with a higher activity of this enzyme provide more-developed plants.

Mendes *et al.* (2003) evaluated the biological properties of an Oxisol under the no-tillage and conventional systems in the Brazilian *cerrado* biome that had been systematised for agriculture for 21 years. They reported that the area under conventional planting had significantly lower acid phosphatase and arylsulphatase values than the no-tillage soil. This finding reinforces the idea that soils with more organic residue have higher activities of the acid phosphatase and arylsulphatase enzymes.

In the rhizospheric soil, the β-glycosidase enzyme presented higher values in the samples from the medium-yield plot (Table 5). This enzyme participates in the final phase of

the cellulose degeneration process by the hydrolysis of cellobiose residues, and changes in its activity can alter the soil quality (Passos *et al.,* 2008). Thus, the higher values found in this study for this enzyme may indicate the microbial collapse that may be occurring near the rhizosphere of the plants. The carbon found in the soil was one of the factors that most influenced the yield differences studied, which can be explained by the activity of the β-glycosidase enzyme.

The PCA correlation (Fig. 1 - B) showed that an increase in urease activity is inversely proportional to the contents of Ca, Zn, K, C and Mn; this is because the complex nature of N in soil can lead to a lack of synchrony in availability of nutrients in the soil (Fontoura and Bayer, 2009).

A higher urease activity indicates a decrease in N content (Fig. 2-B), because this enzyme is responsible for the hydrolysis of urea. The volatilisation of ammonia is favoured by the higher urease activity that is usually observed in the topsoil of no-tillage fields (Barreto and Westerman, 1989).

The increase in acid phosphatase activity brought about by the increase in the soil magnesium content (Fig. 2-B) can be attributed to the activating effect of this cation on the activity of this enzyme (Nahas, 2002).

CONCLUSION

The number of microorganisms and the studied enzymatic activities in the soil under soybean crops vary according to the yield level of the plot.

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