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Soil enzyme activities under pig slurry addition and different tillage systems

Elcio Liborio Balota*, Oswaldo Machineski and Priscila Viviane Truber

*Laboratório de Microbiologia do Solo, Área de Solos, Instituto Agronômico do Paraná, Cx. Postal 481, 86047-902, Londrina, Paraná, Brazil. *Author for correspondence. E-mail: balota@iapar.br*

ABSTRACT. The objective of this work was to evaluate the changes in soil enzyme activities due to application of pig slurry under different soil tillage systems. The experiment was conducted in a clayey Oxisol in Palotina, PR, using different quantities of pig slurry (0, 30, 60 and 120 m^3 ha^{-1} $year^{-1}$) applied to the soil prior to the summer and winter crop season under conventional tillage (CT) and no tillage (NT), with three replicates. The areas were cultivated with soybean (*Glycine max* L.) and maize (*Zea mays* L.) in the summers of 1998 and 1999, respectively, and with wheat (*Triticum sativum* Lam.) in the winters of both years. The soil samples were collected in March and October of 1998 and 1999 at depths of 0-5 , 5-10 and 10-20 cm. The pig slurry application and the soil tillage systems influenced enzyme activities in the soil. The increase of pig slurry application decreased the ratio of soil enzyme activities to microbial biomass carbon. The phosphatase activity had a negative relationship with soil-available phosphorus. The acid phosphatase activity decreased both under CT and NT systems at all depth studied due to pig slurry application.

Keywords: arylsulfatase, acid phosphatase, swine manure, no-tillage systems, nutrient cycling.

RESUMO. **Atividade enzimática sob solo submetido à adição de dejetos líquidos de suínos em diferentes preparos do solo.** No presente estudo foi avaliado as alterações na atividade de enzimas do solo devido à aplicação de doses crescentes de dejetos líquidos de suínos (DLS) em diferentes sistemas de preparos de solo. O experimento foi conduzido em um Latossolo Vermelho eutrófico textura argilosa em Palotina, PR. Foi estudada a aplicação de diferentes doses de DLS (0, 30, 60 e 120 m^3 ha^{-1} ano^{-1}) em sistema de preparo do solo convencional (PC) e sistema de plantio direto (PD), em três repetições. As dosagens de DLS foram subdivididas, metade aplicada antes do preparo do solo da cultura de verão (soja em 1998 ou milho em 1999) e a outra metade antes do preparo do solo da cultura de inverno (trigo). As amostras de solos foram coletadas em março e outubro de 1998 e 1999 nas profundidades de 0-5, 5-10 e 10-20 cm. O preparo do solo e as diferentes doses de DLS aplicadas influenciaram a atividade das enzimas estudadas. O aumento da dose de DLS aplicada diminuiu a relação da atividade enzimática com a biomassa microbiana de carbono. A atividade da fosfatase ácida relacionou-se negativamente com os teores de P disponível no solo. A atividade da fosfatase decresceu com a aplicação de DLS tanto sob o sistema PC como PD.

Palavras-chave: arilsulfatase, fosfatase ácida, dejetos de suínos, plantio direto, ciclagem de nutrientes.

Introduction

More than 37 million heads of swine are produced in Brazil annually (MIRANDA, 2007). This level of swine production produces a large amount of waste: about 300 million liters of liquid dejections (pig slurry) a day. This volume of pig slurry is so large because of the large volumes of water used for the removal of swine dejections (feces, urine and ration remains) from the production units to a storage tank, where it is stored for at least 120 days to reduce its pollutant potential, before its application to the soil (OLIVEIRA; PARIZOTTO, 1993; SEGANFREDO, 2007).

Almost 50% of Brazilian swine production occurs in the South States (Paraná, Santa Catarina and Rio Grande do Sul), which are characterized by a high density of small farms (MIRANDA, 2007). This leads to the production of a huge amount of pig slurry, which often exceeds the quantity than can be safely applied to the soil. The slurry may also eventually reach the water supply. For these reasons, swine production is considered an activity accompanied by great potential for environmental damage.

The utilization of pig slurry as fertilizer is the most common practice, mainly because it is an easy and inexpensive solution for disposal of swine

dejections. Although it is a valuable fertilizer, the successive application of high amounts of pig slurry to soil can cause soil nutritional disorders and environmental damage (SEGANFREDO, 2007).

Different soil management systems can significantly affect the soil's properties, especially the microbiological properties that play important roles in determining nutrient cycling and soil quality. Microbiological properties can be used as soil quality indicators because soil microorganisms are the second most important (after plants) biological factor in the agricultural ecosystem (YAKOVCHENKO et al., 1996). Therefore, biological and biochemical mediated processes in soils are fundamental to terrestrial ecosystem function (DICK, 1994).

The decomposition of soil organic matter is mediated by microorganisms, which produce enzymes that catalyze the innumerable reactions necessary for their own metabolic processes, decomposition of organic residues, nutrient cycling, and formation of organic matter and soil structure (DICK, 1994). Most soil enzymes are produced by microorganisms and can accumulate in the soil to have a considerable impact on nutrient recycling (DICK, 1997; TABATABAI, 1994). This allows soil enzyme activity to be used to indicate the intensity of certain biochemical processes because the enzyme activity is considered to be a major contributor to the overall microbial activity in soil (DICK, 1992). As enzyme levels increase, the rate of the relevant chemical conversion also increases.

The ecological significance of soil enzyme activities has been discussed in several papers (NANNIPIERI et al., 1990; DICK, 1997). In general, increases in enzyme activity are associated with increased biological activity in the soil, which is considered to be an important indicator of soil quality (DICK, 1992, 1994). Soils managed with organic amendments, such as animal manure, generally have a larger and more active microbial community than those managed with mineral fertilizers (McGILL et al., 1986; FAUCI; DICK, 1994). However, in studies on the effects of soil management, the microbiological properties of the soil have received less emphasis than its chemical and physical properties. For example, there are no studies to determine the effects of pig slurry application on soil enzymes under Brazilian conditions.

The objective of this study was to evaluate the effects that crescent and successive pig slurry application under different soil tillage systems have on soil enzyme activities.

Material and methods

Experimental characteristics and soil sampling

The experiment was established in 1996 at the Experimental Station of IAPAR in Palotina, west of Paraná State (24° 17' S, 53° 50' W). The soil was a clayey Oxisol classified as a Latossolo Vermelho eutrófico (Rhodic Eutrudox), according to Brazilian system of soil classification (BHERING; SANTOS, 2008), having 60% clay, 16% silt and 24% sandy. The soil had a pH of 5.2 ($CaCl_2$), 14.8 mg kg^{-1} of P (Mehlich) and 20.0 g kg^{-1} of organic carbon (Walkley-Black) in the surface layer (0-20 cm).

The experiment was installed in a split-plot block design with tillage as the main plot (100 x 5 m) and pig slurry addition as the subplot (20 x 5 m), which was separated by a 2.0 m buffer with three replicates. Tillage treatments were NT planting into undisturbed soil by opening a narrow trench and CT with one disc plowing at a 20 cm depth and two light harrowings for seedbed preparation. Liquid pig manure was added in four doses (0, 30, 60 and 120 m^3 ha^{-1} $year^{-1}$). Half was applied during the summer crop season, and the other half was applied during the winter crop season. Both applications occurred before the soil preparation. The area was cultivated with soybean (*Glycine max* L.) in summer of 1998 and maize (*Zea mays* L.) in 1999 and wheat (*Triticum sativum* Lam.) in the winter of both years. For both the fall and spring operations each year, crop stubbles were retained on the surface in the NT system and they were tilled conventionally (plowed to 20 cm depth) following harvest.

Five sub-samples of soil were taken randomly within each replicate (0-5, 5-10 and 10-20 cm depths) in March and October of 1998 and 1999, at the end of the summer and winter crops. The fresh soil samples were sieved through a 4 mm mesh to remove large plant material and were stored at 4°C until analysis.

Enzyme activity analyses

Amylase (EC 3.2.1) and cellulase (EC 3.2.1.4) activities were determined according to a modified methodology of Pancholy and Rice (1973) and Deng and Tabatabai (1994a). The activities of arylsulfatase and acid phosphatase were measured according to the methods of Tabatabai (1994). Arylsulfatase activity (arylsulfate sulfohydrolase, EC 3.1.6.1) was determined by using a colorimetric determination of *p*-nitrophenol released when soil samples were incubated with *p*-nitrophenyl sulfate. Acid phosphatase activity (EC 3.1.3) was analyzed with a modified universal buffer (MUB) (pH 6.5) by using a colorimetric determination of *p*-nitrophenol

released when soil samples were incubated with *p*-nitrophenyl phosphate. Activities of arylsulfatase and phosphatase were expressed as μg *p*-nitrophenol (PNP) g^{-1} h^{-1}. All enzyme activities were measured using a one-hour incubation period. All determinations were made in triplicate and were expressed on a dry-weight basis. Within each treatment, data were averaged over the four seasons and two years and were then subjected to ANOVA statistical analysis using the SAS statistical software package. Soil enzyme activities for each tillage system and depth were submitted to regression analysis. It was chosen the significant equation by F test ($p \leq 0.05$) that presented higher correlation coefficient (R^2).

Results and discussion

The average measurements obtained from twelve samples in four seasons' samplings indicate that crescent pig slurry application and soil tillage systems did influence the soil enzyme activities. The different enzymes presented diverse tendencies due to the pig slurry doses, the soil tillage and the depth. In general, enzyme activities in the NT system were higher than those observed in the CT system (Figures 1, 2, 3 and 4).

Amylase activity varied from 176 to 647 μg g^{-1} h^{-1} in the soil under CT and from 529 to 995 μg g^{-1} h^{-1} in the NT system (Figure 1). For CT at the 0-5-cm depth and for both CT and NT at the 5-10-cm depth, the amylase activity increased linearly due to an increase in pig slurry doses. Under NT at the 0-5-cm depth and under CT and NT at the 10-20-cm depth, the amylase activity was significantly increased due to the addition of 30 m^3 ha^{-1} of pig slurry. The activity decreased with the addition of 60 m^3 ha^{-1} of pig slurry, and application of 120 m^3 ha^{-1} of pig slurry led to amylase activity values similar to those observed with the addition of 30 m^3 ha^{-1}. However, regression analysis for these data resulted in a non-significant model.

Cellulase activity varied from 18 to 176 μg g^{-1} h^{-1} in the soil under CT and from 39 to 189 μg g^{-1} h^{-1} in the NT system (Figure 2). At the 0-5-cm depth, the cellulase activity fit a linear model and was inversely proportional to the addition of pig slurry under the NT system. While under CT system the cellulase activity increased with increases of pig slurry addition. At 5-10-cm depth, there was a slight increase in cellulase activity with pig slurry application, and the data fit a quadratic model for both the CT and the NT systems. For the 10-20-cm depth, there was a slight decrease (which fit a quadratic model) for the NT system, and there was a significant linear decrease for the CT system.

The amylase and cellulase activities were within the ranges reported in the literature; additionally, they were similar to those observed in high fertility soils in Brazil (from 350 to 830 μg g^{-1} for amylase and from 67 to 220 μg g^{-1} for cellulose) (BALOTA et al., 2004). These enzymes play important roles in nutrient mineralization because they participate actively in residue decomposition. This decomposition provides readily available carbon for the growth of microorganisms (DENG; TABATABAI, 1994b). Cellulose is the most abundant compound in the biosphere and makes up almost half of the biomass synthesized by photosynthetic fixation of CO_2. Addition of organic material generally increases the enzyme activities in soils, because the added organic compounds represent a supply of readily available substrates for microorganisms. The additional organic compounds lead to increasing glycosidase activity, a chemical activity that plays a major role in the degradation of carbohydrates in soils (DENG; TABATABAI, 1996).

Arylsulfatase activity in the soil varied from 10.1 to 20.1 μg PNP g^{-1} h^{-1} under the CT system and from 14.9 to 33.4 μg PNP g^{-1} h^{-1} under the NT system (Figure 3). At the 0-5-cm depth, the arylsulfatase activity fit a linear model with an inverse relationship to the addition of pig slurry under the NT system. While under CT system, the cellulase activity increased due to an increase in pig slurry addition. The arylsulfatase activity under NT was about 204 and 100% higher than the CT system with application of 0 and 30 m^3 ha^{-1} of pig slurry, respectively. At 120 m^3 ha^{-1} of pig slurry, the values of arylsulfatase are practically the same for both the CT and the NT systems. At the 5-10-cm depth with pig slurry application, there was a slight increase in arylsulfatase activity that fit a quadratic model for the NT system, and there was a significant increase that fit a linear model due to pig slurry addition under the CT system. At the 10-20-cm depth, there was a significant increase in activity that a fit linear model under both the NT and the CT systems.

Arylsulfatase activity levels reported in the literature vary widely (from 4 to 770 μg g^{-1} h^{-1}) in many regions of the world (BALIGAR et al., 1999; BALOTA et al., 2004; GUPTA et al., 1993). In Brazil, the reported levels of arylsulfatase activity vary from 4.0 to 104 μg g^{-1} h^{-1} (BALIGAR et al., 1999; BALOTA et al., 2004). However, the level of arylsulfatase activity obtained in this study was similar to that observed in another area in Paraná

State under clayey soil with high natural fertility and subjected to different soil tillage systems (BALOTA et al., 2004).

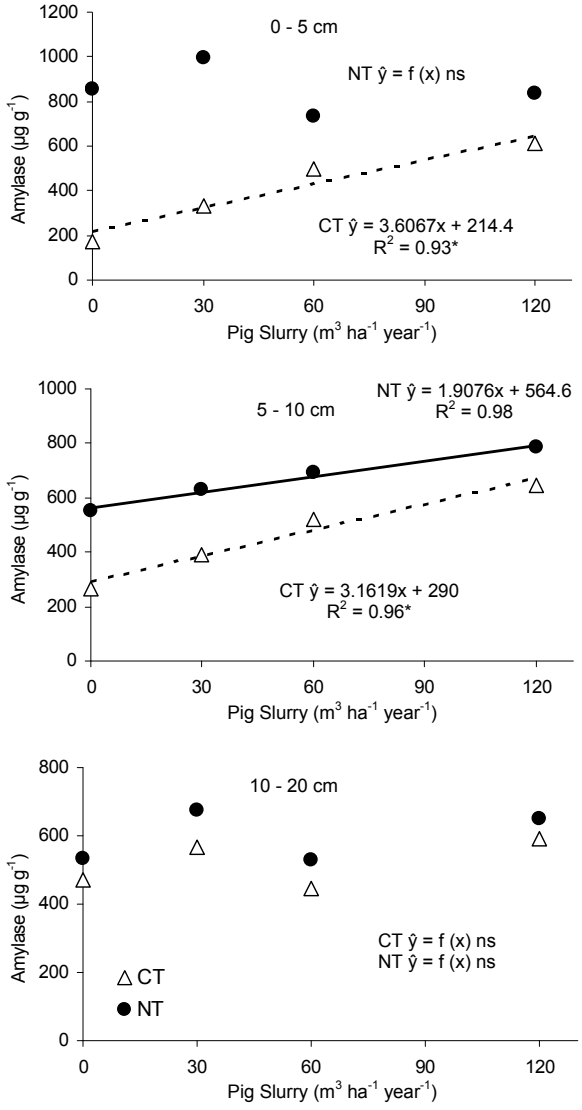

Figure 1. Amylase activity as affected by the application of pig slurry under different soil tillage systems. All values represent the mean of twelve replicates (four sampling seasons and three replicates).
*Significant at 5% of probability; ns: non-significant.

It has been suggested that the addition of organic compounds can significantly increase arylsulfatase activity (DENG; TABATABAI, 1997). The organic carbon from plant residues constitutes a principal reservoir of sulfate esters, which are the substrates for this group of enzymes. Arylsulfatases are one of many types of sulfatases involved in the mineralization of sulfate esters. Most arylsulfatases are not constitutive enzymes, and their synthesis by microorganisms may be controlled by the carbon and sulfur content of the system (DICK, 1997; TABATABAI, 1994).

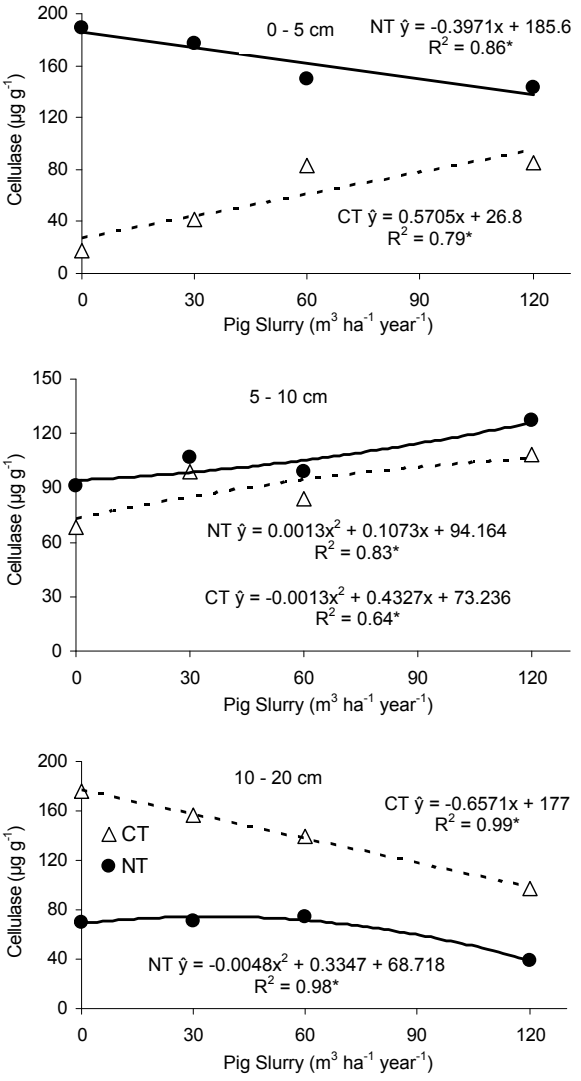

Figure 2. Cellulase activity as affected by the application of pig slurry under different soil tillage systems. All values represent the mean of twelve replicates (four sampling seasons and three replicates).
*Significant at 5% of probability.

Acid phosphatase activity varied from 83 to 300 µg PNP g^{-1} h^{-1} in the soil under CT and from 171 to 387 µg PNP g^{-1} h^{-1} under the NT system (Figure 4). At the 0-5-cm depth, the phosphatase activity decreased linearly due to the addition of pig slurry when using NT system, and the change in activity fit a quadratic model under the CT system. At the 5-10 cm and the 10-20-cm depth, the phosphatase decreased slightly under NT and significantly decreased under CT due to increase of pig slurry addition.

Our results for acid phosphatase activity are consistent with those reported in the literature, which vary widely from 55 to 1165 µg g^{-1} h^{-1} in several studies in Brazil (BALIGAR et al., 1999; CARNEIRO et al., 2004). However, the

phosphatase activity level obtained was about two times lower than that observed in another region of Paraná State under clayey soil with high natural fertility and subjected to different soil tillage (BALOTA et al., 2004).

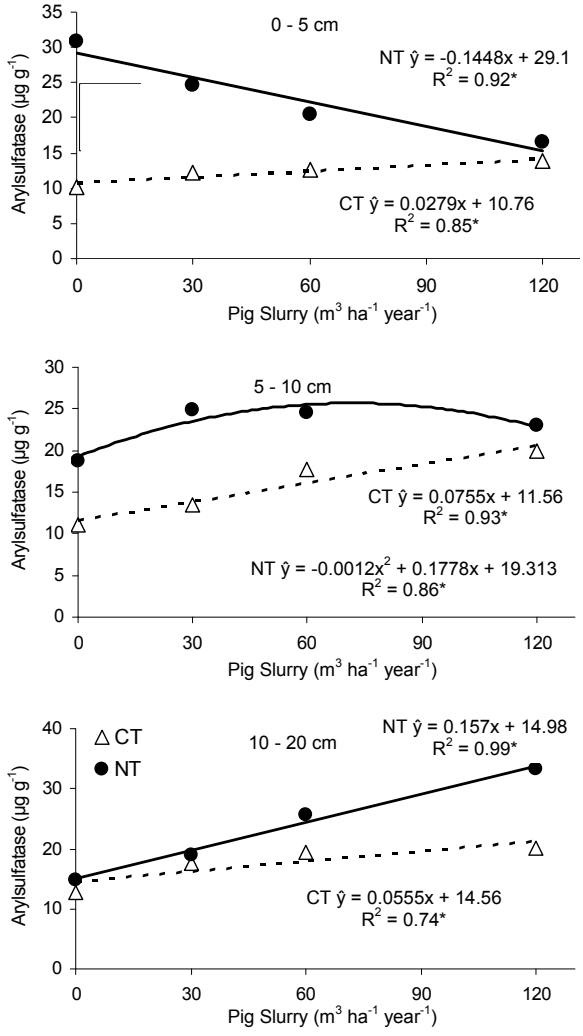

Figure 3. Arylsulfatase activity as affected by the application of pig slurry under different soil tillage systems. All values represent the mean of twelve replicates (four sampling seasons and three replicates).
*Significant at 5% of probability.

Acid phosphatases have been studied extensively because of their importance in soil organic phosphorus mineralization and plant nutrition and because of their optimum activity under acidic conditions (DICK, 1997; TABATABAI, 1994). Phosphatase is the general name of a broad group of enzymes that catalyze the hydrolysis of both esters and anhydrides of H_3PO_4. Because of their high metabolic activity and short lifespan, microorganisms are the most important sources of phosphatases in the soil. It has been suggested that phosphatases are produced when the available phosphorus content reaches critical levels for plant and microorganism growth (SPIERS; McGILL, 1979). In natural systems, such as forests, there is a large amount of microorganism growth without the addition of phosphate fertilizers, even when the level of available phosphorus is low. In these systems, available phosphorus is controlled by the cycling of organic phosphorus, and microbial biomass is an essential component of this process. On the other hand, in agricultural systems, added phosphorus fertilizers may depress phosphatase activity (SPIERS; McGILL, 1979).

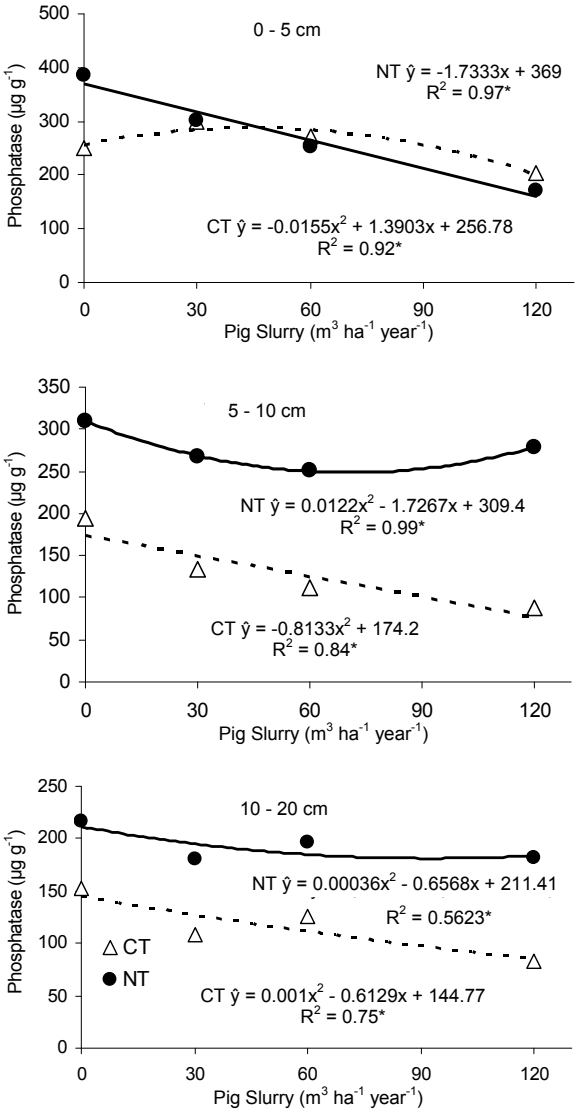

Figure 4. Acid phosphatase activity as affected by the application of pig slurry under different soil tillage systems. All values represent the mean of twelve replicates (four sampling seasons and three replicates).
*Significant at 5% of probability.

Our measurements of the change in soil enzyme activities due to pig slurry application are in agreement of those observed previously by Lalande et al. (2000) and by Rochette et al. (2000).

The application of 60 and 120 Mg ha^{-1} $year^{-1}$ for 19 consecutive years increased the denitrifying enzyme activity by up to 200% for up to three months following the pig slurry application (ROCHETTE et al., 2000). In another long-term field experiment, the application of 90 m^3 ha^{-1} of pig slurry for 18 consecutive years increased the acid phosphatase and arylsulfatase activities at the 0-15 cm depth by 80% when compared to the control 28 days after pig slurry application. At the 15-30 cm depth, there were no observed differences (LALANDE et al., 2000).

The alteration of soil enzyme activities due to pig slurry application may be in response to a higher nutrient immobilization capacity of the microbial community. An increasing supply of organic compounds increases the supply of readily available substrates, such as carbohydrates, for the microorganisms that produce the majority of soil enzymes. Pig slurry is a rich source of both organic and inorganic nutrients. It contains, on a dry matter basis, approximately 3.4% N, 4.2% P and 5.6% K (OLIVEIRA; PARIZOTTO, 1993) as well as several micronutrients needed for crop production. The inorganic nutrients are readily available, while the organic nutrients gradually become available over time. The application of 30, 60 and 120 m^3 ha^{-1} $year^{-1}$ of pig slurry is equal to the addition of 220, 440, and 860 kg ha^{-1} of ammonium sulfate; 570, 1140 and 2280 kg ha^{-1} of simple superphosphate; and 134, 268 and 536 kg ha^{-1} of potassium chloride, respectively. Although pig slurry only provides low levels of available carbon (approximately 1%) for soil microorganisms, it is a source of large amount of nutrients and organic compounds that can stimulate the activity of the microbial biomass.

This great variation in soil enzyme activities due to crescent pig slurry application and soil tillage systems shows that these enzymes are sensitive to soil disturbance. In general, amylase, cellulase and arylsulfatase activity increased with depth under the CT system and decreased with depth under the NT system. The acid phosphatase activity decreased with depth under both the CT and the NT systems. The decrease in soil enzymes with depth may be related to the lower labile C- and O_2 content, which may result in a smaller microbial biomass and lower activity than that in the surface layer. These results indicate that the addition of pig slurry under different soil tillage systems causes diverse effects on the microbial population composition (responsible for producing different enzymes) at different depths.

The decrease in soil enzyme activities due to pig slurry application may be due to a decrease in microorganism growth or due to the stimulation of O_2 demand that consequently creates anaerobic zones (LALANDE et al., 2000). Alternatively, the high rate of pig slurry application may simply create an anaerobic zone in the soil. Also, the application of high amounts of pig slurry was associated with the rainy season and may have caused temporary anaerobic conditions in some soil zones, and effect that could have affected microorganism growth.

The application of high amounts of pig slurry (120 m^3 ha^{-1}) has been associated with the decline of the microbial population and its activity, and it has also been associated with decreased levels of acid phosphatase (LALANDE et al., 2000). However, this decrease in acid phosphatase was also attributed to high soil phosphorus content due to consecutive pig slurry applications. Another important point is that, normally, long-term experiments result in a selection pressure on the microbial community due to the soil management practiced. This selection may promote the development of microorganisms specific for that soil's conditions.

Another effect of manure additions on soil is that the alteration of the organic carbon pool can also protect soil enzymes via association with organic and inorganic colloids that contribute to enzyme stabilization in soils (DICK, 1994; NANNIPIERI et al., 1990). Plant roots also stimulate enzyme activity by creating favorable microhabitats (e.g., increased porosity, water and a diversity of compounds) for microorganisms (DICK, 1997). In addition to the input of carbon contributed to the soil by pig slurry by above- and below-ground biomass, soil carbon is also contributed by root exudates. In general, from 7.0 to 64% of the net carbon fixed by plants is lost through respiration and rhizodeposition in the soil (WHIPPS, 1990). Root exudates can contain more than 200 carbon compounds (KUMAR et al., 2006) including sugars, amino acids, organic acids, hormones, vitamins, polymeric carbohydrates, enzymes and gases (ethylene and CO_2) (WHIPPS, 1990). These compounds, released by roots in the rhizosphere, can be utilized immediately by microorganisms to increasing microbial diversity, number and activity. These increases consequently lead to greater production and release of soil enzymes.

The increase in enzyme activities in the NT system relative to the CT system was 54% for amylase, 16% for cellulase, 53% for arylsulfatase and 48% for acid phosphatase. These results suggest that the decrease in soil disturbance (i.e., no-tillage) can improve microbial activity. Conventional tillage

(CT), which disturbs the soil to prepare the land, reduces soil structure by degrading soil aggregates. On the other hand, the lack of soil disturbance in the NT system increases microbial activity because it favors the formation and stabilization of macro aggregates, which provide habitats for microbiota (BEARE et al., 1994; POWLSON; JENKINSON, 1981). These habitats then allow for the expansion of the microbial community and the production of soil enzymes (DICK, 1992, 1997).

Important ecological information from soil enzymes can be obtained by calculating the ratio of soil enzyme activity to microbial biomass (KANDELER; EDER, 1993). Our results indicate that the increase in pig slurry application decreased the rate of soil enzyme activities to microbial biomass carbon (Figure 5). This is suggests that although the pig slurry application increased the soil enzyme activity, the amount of soil enzyme produced per unit of microbial biomass decreased. According to Kandeler and Eder (1993), this result also suggests that the amount of enzyme immobilized on clay or humic colloids in the soil was also decreased. It has been observed that enzymes produced in excess after the addition of organic compounds are rapidly decomposed (NANNIPIERI et al., 1983). However, it is important to study the mechanisms that increase and maintain a stable biological activity that affects enzyme activities.

Correlations

The enzyme activities were correlated with each other (Table 1), as observed in previous studies (BALOTA et al., 2004; DENG; TABATABAI, 1996, 1997; FRANKENBERGER; DICK, 1983). Amylase and arylsulfatase activity showed a strong relationship with microbial biomass carbon and organic carbon (Table 1), an effect that was not observed for cellulase and phosphatase activity. These close correlations between enzyme activities and organic carbon are likely due to higher carbon levels that support greater microbial biomass and thus have more enzymatic activity (DENG; TABATABAI, 1996, 1997). Furthermore, more organic matter provides a better environmental condition for stabilizing and protecting extracellular enzymes. There was a negative relationship between acid phosphatase activity and extractable phosphorus (Figure 6), confirming the observation that phosphatases are stimulated when soil phosphate levels are low (SPIERS; McGILL, 1979). However, this negative correlation was not found in several other studies (BALIGAR et al., 1999; BALOTA et al., 2004).

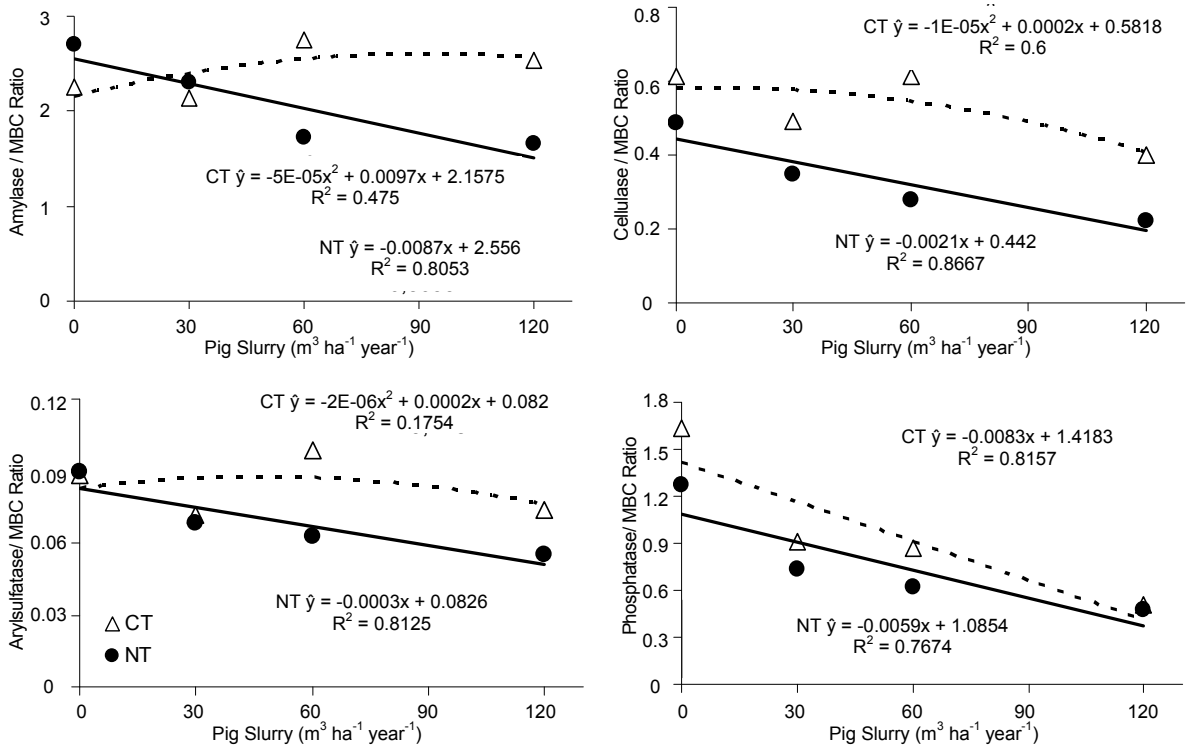

Figure 5. Ratio of soil enzyme activities to microbial biomass carbon (MBC) at the 0-20 depth, as affected by application of pig slurry doses under different soil tillage systems. All values represent the mean of 36 samples (four sampling seasons, three depths and three replicates).

Table 1. Simple correlations (r) between soil enzyme activities and microbial biomass across all treatments, depth and sampling season.

Variables*	Amylase	Cellulase	Arylsulfatase	Acid Phosphatase
Cellulase	0.62*	-	-	-
Arylsulfatase	0.67*	0.27	-	-
Acid Phosphatase	0.25	0.068	0.23	-
MBC	0.78*	0.21	0.56*	0.23
MBN	0.65*	0.35	0.24	0.18
MBP	0.53*	0.40	0.0073	0.033
Organic C	0.78*	0.50	0.40	0.34

*MBC: microbial biomass carbon; MBN: microbial biomass nitrogen; MBP: microbial biomass phosphorus.

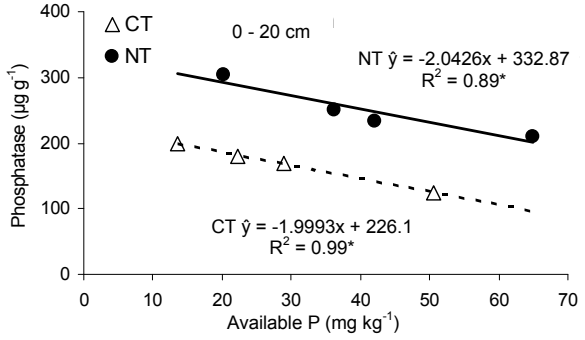

Figure 6. Relationship between acid phosphatase and available phosphorus at the 0-20 depth, as affected by application pig slurry doses under different soil tillage systems. All values represent the mean of 36 samples (four sampling seasons, three depths, and three replicates).
*Significant at 5% of probability.

Our results confirm previous observations that demonstrate the responsiveness of soil enzyme activities to changes in soil management practices. The results demonstrate that the measurement of soil enzyme activities presented a good sensitivity for detecting soil alterations due to the application of pig slurry and changes in soil tillage systems. Although swine dejections have a high potential for causing environmental damage, they can serve as an excellent source of nutrients for crop production and can increase the microbial activity and enzyme activities in the soil. These increases can consequently enhance soil fertility and soil quality.

Conclusion

Pig slurry application and changes in soil tillage systems influenced the soil enzyme activities. The soil enzyme activity was a sensitive measure for detecting changes in the soil due to the application of pig slurry and to changes in soil tillage systems. The increase of pig slurry application decreased the ratio of soil enzyme activities per unit of microbial biomass carbon. The phosphatase activity was inversely related to the available phosphorus in soil. The NT system provides for higher enzyme activities than the CT system.

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