DIVISÃO 3 - USO E MANEJO DO SOLO

Comissão 3.1 - Fertilidade do solo e nutrição de plantas

ORGANIC NITROGEN IN A TYPIC HAPLUDOX FERTILIZED WITH PIG SLURRY

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ABSTRACT

The application of pig slurry may have a different effect on nitrogen dynamics in soil compared to mineral fertilization. Thus, the aim of this study was to determine the different forms of organic N in a Latossolo Vermelho distroférrico (Typic Hapludox) and their relationship to N uptake by crops in response to 10 years of annual application of pig slurry and mineral fertilizer. The treatments were application rates of 0, 25, 50, 100, and 200 m^3 ha^{-1} of pig slurry, in addition to mineral fertilizer, organized in a randomized block design with four replications. The N contents were determined in the plant tissue and in the forms of total N and acid hydrolyzed fractions: ammonium-N, hexosamine-N, α-amino-N, amide-N, and unidentified-N. Annual application of pig slurry or mineral fertilizer increased the total-N content in the 0-10 cm depth layer. The main fractions of organic N in the soil were α-amino-N when pig slurry was applied and unidentified-N in the case of mineral fertilizers. Pig slurry increased the N fractions considered as labile: α-amino-N, ammonium-N, and amide-N. The increase in these labile organic N fractions in the soil through pig slurry application allows greater N uptake by the maize and oat crops in a no-tillage system.

Keywords: manure, N availability, N fractionation.

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RESUMO: *FRAÇÕES DE NITROGÊNIO ORGÂNICO EM LATOSSOLO VERMELHO ADUBADO COM DEJETO SUÍNO*

A aplicação de dejeto suíno pode influenciar na dinâmica do nitrogênio no solo de forma distinta em comparação à adubação mineral. O objetivo deste trabalho foi determinar as diferentes formas de N orgânico em Latossolo Vermelho distroférrico e sua relação com a absorção de N pelas culturas, em resposta a 10 anos de aplicação anual de dejeto suíno e adubo mineral. Os tratamentos foram as doses 0, 25, 50, 100 e 200 m^3 ha^{-1} de dejeto suíno, além da adubação na forma mineral, organizados em blocos casualizados com quatro repetições. Foram determinados os teores de N no tecido vegetal e de N total nas frações hidrolisadas em meio ácido: N-amônio, N-hexosamina, N-α-amino, N-amido e N-não identificado do solo. A aplicação anual de dejeto suíno ou adubo mineral aumentou o teor de N total na camada de 0-10 cm de profundidade. As principais frações do N orgânico no solo foram N-α-amino, quando utilizado dejeto suíno e N-não identificado no caso do adubo mineral. O dejeto suíno aumentou as frações do N consideradas lábeis, N-α-amino, N-amônio e N-amida. Esse aumento das formas lábeis de N orgânico no solo, pela aplicação de dejeto suíno, possibilita maior absorção de N pelas culturas de milho e aveia em plantio direto.

Palavras-chave: esterco, disponibilidade de N, fracionamento de N.

INTRODUCTION

Although swine raising makes use of a great deal of technology, it has paid little concern to the final destination of waste products, principally to rational use of them as a nutrient source for plants. The importance of this organic fertilizer is evident from the expressive volume generated, because each adult pig produces around 8 L d^{-1} of slurry (Oliveira, 1993) and, in 2011, approximately 37 million pigs were slaughtered in Brazil (IBGE, 2013).

The demand for utilization of the waste products generated by swine raising will continue to increase because the increase in pork production has been around 1.77 % a year (Brasil, 2011). National research thus seeks to generate technological resources that establish a balance between production and environmental quality, based on the principle of sustainability. The use of this waste as a raw material in crop fertilization may result in various benefits, leading to improvements in the chemical and biological properties and physical traits of the soil (Cassol et al., 2012), especially in the upper layers, when applied on the soil surface.

Nevertheless, there are still few studies that seek to understand the N dynamic in the soil when organic fertilizers are applied, in comparison to mineral fertilizers with soluble sources. It should be noted that little is known about the chemical nature of organic N in Brazilian soils, especially in areas of application of pig slurry, and it is necessary to carry out studies for characterization of the fractions of the element in the soil, especially in regard to the proportions of labile and non-labile forms.

Although a greater quantity of N is required for most crops, no precise method is yet available to evaluate its availability in the soil because its dynamic in this environment involves diverse processes, such as sorption, adsorption, leaching, volatilization, nitrification, denitrification, immobilization, and mineralization, which are generally mediated by microorganisms and affected by climatic factors (Aita et al., 2006; Lourenzi et al., 2013). Moreover, 95 % of the N in the soil is in the organic form.

Given the great number of N reactions in the soil, fertilization with mineral fertilizers may provide from 11 to 68 % of the total N used by the plant (Lara Cabezas et al., 2000; Giacomini et al., 2009), with the other part coming from the organic N of the soil, although the N compounds of the soil may have high or low lability depending on the type of organic chain they are inserted in (Müller et al., 2011). The synchronism between the release of N from the organic residues and the demand for N by the plants is fundamental, both from the perspective of yield and of reducing the risk of environmental contamination through volatilization of NH_3, leaching of NO_3^-, and emission of N_2O (Giacomini and Aita, 2008).

Considering the demand for knowledge in regard to the effects of application of organic fertilizers, such as pig slurry, on the formation and accumulation in the soil of compounds containing organic N in recalcitrant and labile forms, the aim of this study was to determine the different fractions of organic N in a Latossolo Vermelho distroférrico (Typic Hapludox) and N uptake by plants in a maize-oat succession under no-till subjected to 10 years of application of increasing rates (up to 200 m^3 ha^{-1}) of pig slurry, as compared to mineral fertilizer.

MATERIAL AND METHODS

A field experiment was carried out in the municipality of Campos Novos, SC, Brazil, in an area with a mean altitude of 863 m, located at the geographic coordinates 51° 21’ 47” longitude West and 27° 23’ 34.5” latitude South. The location has a humid mesothermal climate with a mild summer (Cfb), according to the Köppen classification, with annual rainfall of 1,480 mm, relatively well-distributed throughout the year, and mean annual temperature of 16 °C.

Activities began in October 2001 to evaluate the effects of continuous addition of pig slurry on the soil over the years in regard to crop yield, soil chemical, physical, and biological characteristics, and soil quality. Before setting up the experiment, the area was used for commercial crops (maize, soybean, common bean, wheat, and oats) and managed in a no-till system.

Soil in the experiment is classified as a Latossolo Vermelho distroférrico (Embrapa, 2013) [Typic Hapludox], with basalt as the material of origin and with the chemical characteristics shown in table 1. The soil is highly clayey, with a content of 664 and 701 g kg^{-1} in the layers evaluated (Table 2). The soil is characterized as oxidic, with a predominance of crystalline forms of iron oxides (goethite and hematite); in the clay fraction, kaolinite predominates, followed by 2:1 type clay minerals with interlayer hydroxy-Al polymers; in lower proportions, there are gibbsite and quartz (Almeida et al., 2003).

The study evaluated results of the 10th year of carrying out the experiment, composed of the following treatments: pig slurry (PS) at annual rates of 0 (control), 25, 50, 100, and 200 m^3 ha^{-1}, and mineral fertilization (MF). The MF treatment was composed of urea, triple superphosphate, and potassium chloride at annual rates of 130, 44, and 58 kg ha^{-1} of N, P, and K, respectively, which increased to 170, 57, and 67 kg ha^{-1} as of 2007. These values were defined based on recommendations with a view toward maize grain yield of 8 Mg ha^{-1} in the first period, and of 11 Mg ha^{-1} in the second period, according to the manual of the Soil Chemical and Fertility Commission (Tedesco et al., 2004).

The treatments were applied in plots of 12 × 6.3 m, organized in a randomized block experimental design, with four replications. The treatments were always applied in October of each year, from 15 to 20 days after application of glyphosate herbicide to desiccate the winter crop, and from seven to 12 days before planting maize. The pig slurry and the mineral fertilizer were broadcast on the soil surface over the winter crop residue, the first with the aid of a slurry spreader, and the second, manually. The N of the MF treatment was applied in parcels, with 20 % broadcast on the same day as the other treatments and the rest divided into two topdressing applications, the first at the V8 stage and the second at the beginning of maize tasseling.

The slurry used in carrying out the experiment was derived from feeder pigs and was stored with continuous flow in an outside storage tank for around four months prior to application. The pig slurry applied in the experiment was characterized (Table 3), removing a representative sample for duplicate analysis. Dry matter was determined through drying in a forced air laboratory oven at 65 °C. The pH level was determined through reading on a pH meter directly in the PS, and nutrient analyses were made in aliquots from the waste *in natura* (wet base) and carried out as described by Tedesco et al. (1995).

Maize (*Zea mays* L.) and black oats (*Avena strigose* L.) were grown each year in succession under the no-till system (NT), except in the summer

Table 1. Properties of the 0-20 cm layer of the Latossolo Vermelho distroférrico (Typic Hapludox) used to set up the field experiment. Mean values of four samples composed of 10 subsamples

$pH(H_2O)$	pH(SMP)	V	$Al^{3+(1)}$	$Ca^{2+(1)}$	$Mg^{2+(1)}$	P$^{(2)}$	K$^{(2)}$	Clay	TOC
		%	cmol$_c$ kg^{-1}			mg kg^{-1}		g kg^{-1}	
6.1	6.0	87	0.0	8.2	4.6	6.4	97	680	25

Determined according to Tedesco et al. (1995). $pH(H_2O)$: pH in water; pH(SMP): pH in SMP solution; TOC: total organic carbon. (1) 1 mol L^{-1} KCl extractor. (2) P and K: extracted by Mehlich-1.

Table 2. Particle size composition in the 0-5, 0-10, and 10-20 cm layers of a Latossolo Vermelho distroférrico (Typic Hapludox) used for setting up the field experiment

Depth	Clay	Sand	Silt
cm	g kg^{-1}		
0-5	664	53	283
5-10	660	42	298
10-20	701	48	251

Table 3. Chemical properties (dry matter - DM; total nitrogen - TN; and total organic carbon - TOC) of the pig slurry applied annually in the field experiment in the period from 2001 to 2011 in a Latossolo Vermelho distroférrico (Typic Hapludox)

Period (year)	pH	DM	TN	TOC
		kg m^{-3}		
10/2001	6.7	66	3.4	19.2
11/2002	7.1	26	2.6	11.2
10/2003	6.9	32	2.6	12.8
10/2004	7.3	43	3.7	16.6
10/2005	7.8	56	3.2	17.4
10/2006	7.0	114	4.6	35.6
10/2007	7.3	55	2.7	19.7
10/2008	7.1	68	2.4	23.9
10/2009	7.2	69	6.6	25.6
10/2010	7.4	57	3.9	33.4
Total	-	-	-	219
Mean	7.2	59	3.6	22

of 2002/2003, when black beans (*Phaseolus vulgaris* L.) were grown instead of maize, and in the winters of 2005 and 2008, when radishes (*Raphanus sativus* L.) were grown instead of black oats. In the maize crops, a single hybrid cultivar was used at a density of seven plants m^{-2}, with a spacing of 0.60 m between rows. A common cultivar was used for black oats at a density of 60 kg ha^{-1} of seeds, and the cultivar IPR-116 for radish at a density of 10 kg ha^{-1} of seeds, at a row spacing of 0.20 m for both crops. The cultivar Empasc 201 was used for black beans at a density of 20 plants m^{-2}.

Maize was generally planted in the first week of November, whereas the winter crops were always planted in the first half of the month of June each year. All the crops were planted in the no-till system, with a planter composed of a front cutting disk and furrower with a double disk opener. Maize grain yield was determined through manual harvest and mechanical threshing of the ears produced in the useful area (10.2 × 4.3 m) of the plots. For the winter crops, biomass production was evaluated in the useful area of the plots, collecting three subsamples per plot demarcated in a 0.25 m^2.

Soil sampling was carried out in August 2010, with collection from the following layers: 0-2.5, 2.5-5, 5-10, 10-20, 20-30, and 30-40 cm depth. The samples were composed of seven subsamples collected at random points from the diagonal line of the plots, using a soil cylindrical auger. After collection, the soil was placed to dry in a forced air circulation oven at 65 °C and was then ground, sieved in a 2.0 mm screen, and stored in polyethylene containers.

Total nitrogen (TN) contents in the soil and in the plant leaves were determined according to methods described by Tedesco et al. (1995). For leaf sampling, the center third of 30 leaves located below and opposite the ear in the maize tasseling stage, and 50 flag leaves from the oat plants in the initial flowering stage of the crop were collected in each experimental plot (Tedesco et al., 2004). Fractionation of the organic forms of N was based on the extraction and quantification of the N compounds released by acid hydrolysis, based on the protein degradation technique described by Yonebayashi and Hattori (1980) and modified by Camargo et al. (2008).

Two soil samples containing around 10 mg of organic N received three drops of octanol and were subjected to hydrolysis, one with 20 mL of 6.0 mol L^{-1} HCl for a 24 h period, and the other with 20 mL of 1.0 mol L^{-1} HCl for 3 h, both with heating to 110 °C and under reflux in a condenser. The N hydrolyzed fractions, NH_4^+-N, hexosamine-N, and α-amino-N were determined in the neuter hydrolyzed solution of the first sample, while the amide-N fraction was determined in the second. After the hydrolysis procedure, the material was filtered in a slow filter, collecting 60 mL of the hydrolyzed liquid, and this was neutralized to pH 6.5 with NaOH, and the volume was then completed to 100 mL with distilled water.

For hydrolyzed-N, 5 mL from the first sample was transferred to digestion tubes where it remained at 180 °C for 20 min, followed by 330 °C for 2 h or until reaching a greenish-tan color. After cooling, 20 mL of distilled water and 5 mL of 10 mol L^{-1} NaOH were added and distillation began, collecting the ammonia in boric acid indicator solution, and then titration with 0.0025 mol L^{-1} H_2SO_4. For determination of NH_4^+-N, 10 mL from the first sample was transferred to distillation flasks, together with 70 mg of calcined MgO, and then distillation and titration as described above. For determination of amide-N, 10 mL of the second sample was transferred to distillation flasks, together with 70 mg of calcined MgO, and then distillation and titration as described above. The amide-N fraction was calculated by subtracting the value of mineral NH_4^+-N determined in this distillation, composed of mineral NH_4^+-N + amide-N. The value of mineral NH_4^+-N in solution and adhering to the negative charges of the soil was determined through extraction with 1.0 mol L^{-1} KCl according to the method described by Tedesco et al. (1995) for soil analysis.

For the hexosamine-N fraction, 10 mL of the first sample was transferred to distillation flasks, together with 10 mL of a phosphate-borate buffer solution with pH of 11.2, followed by distillation and titration as described above. From this procedure, hexosamine-N + NH_4^+-N was obtained, and hexosamine-N was calculated by subtraction of the NH_4^+-N fraction determined in the second step.

For determination of α-amino-N, 5 mL of the second sample was transferred to distillation flasks, with the addition of 1 mL of 0.5 mol L^{-1} NaOH, followed by heating in a water bath at a temperature of 100 °C until reducing the volume to 2 to 3 mL. After the flask was cooled, 500 mg of citric acid and 100 mg of ninhydrin was added, heating the extract in a water bath once more for 1 more min. After this period, the flask was shaken with a circular movement for a few seconds without removing it from the water bath, where it remained for 10 more min. After cooling, 10 mL of phosphate-borate buffer solution and 1.0 mL of 5 mol L^{-1} NaOH was added, and then the distillation and titration process described above was carried out. The unidentified-N is estimated by subtraction of the sum of the hydrolyzed forms of organic N from the value of hydrolyzed-N determined in the first step, in which unidentified-N = hydrolyzed-N - (NH_4^+-N + amide-N + hexosamine-N + α-amino-N).

The results were subjected to analysis of variance by the F test, considering a randomized block design, with evaluation of the effect of the treatments and comparison of mean values by the Duncan test ($p < 0.05$) and regression analysis for the effect of the application rate of pig slurry on the content of the organic N fractions of the soil and uptake of these forms of N by plants.

RESULTS AND DISCUSSION

Pig slurry and mineral fertilization influenced the total content and the forms of N in the soil only up to the 10 cm depth soil layer, without changes in the deeper layers up to 40 cm. The TN content accumulated in the soil increased with the application rates of pig slurry (PS); however, the application rate of 100 m^3 ha^{-1} was similar to those of 50 and 200 m^3 ha^{-1}, but, in this case, only in the 5-10 cm layer (Table 4). These results indicate that the PS at rates greater than 100 m^3 ha^{-1} promotes N accumulation in the soil, especially in the surface layer, which may also result in greater losses through volatilization of ammonia, N_2O, and N_2, leaching by N-NO_3^- in the soil profile, and surface runoff of the ammonia, nitrate, amide, and organic forms.

At the rates of 25 and 50 m^3 ha^{-1} of PS, TN contents similar to mineral fertilizer (MF) may be seen (Table 4). The greater TN contents in the soil exhibited at the rates of 100 and 200 m^3 ha^{-1} of PS are explainable by the large addition of the nutrient in these treatments in carrying out the experiment, with a total of 660 and 1,320 kg ha^{-1} of TN in 2009, and of 390 and 780 kg ha^{-1} in 2010, respectively, in contrast with the application of 170 kg ha^{-1} of mineral N in the form of urea each year. It is important to note that mineralization of organic N of the soil occurs at a variable rate as a result of the edaphic and climatic characteristics, usage and management practices, type of soil, and quality of the crop or organic residue (Aita et al., 2006; Lourenzi et al., 2013; Schirmann et al., 2013). New approaches in regard to organic N have characterized different fractions of this nutrient in the soil, such as recalcitrant organic N and labile organic N, which have an effect on the rate of mineralization (Müller et al., 2011; Paungfoo-Lonhienne et al., 2012).

The proportion of total hydrolyzed-N in relation to the accumulated TN of the soil in the different treatments ranged from 69 to 76 %

Table 4. Total nitrogen content (TN) in layers up to 40 cm in a Latossolo Vermelho distroférrico (Typic Hapludox), subjected to 10 years of annual fertilization with mineral fertilizer (MF) and pig slurry (PS) at the rates of 0 (control), 25, 50, 100, and 200 m^3 ha^{-1} in a no-till system. Mean values of four replications

Depth	MF	Control	PS 25	PS 50	PS 100	PS 200
cm			g kg^{-1}			
0-2.5	3.9 Ab	3.2 Ac	3.8 Ab	4.1 Ab	4.6 Aa	4.5 Aa
2.5-5	3.2 Bbc	2.8 Bd	3.0 Bcd	3.5 Bb	3.9 Ba	3.9 Ba
5-10	2.5 Cbc	2.2 Cd	2.3 Ccd	2.4 Cbc	2.6 Cab	2.7 Ca
10-20	1.8 Db	1.9 Dab	1.8 Db	1.9 Dab	2.0 Dab	2.1 Da
20-30	1.6 D	1.7 D	1.5 DE	1.5 DE	1.7 DE	1.6 E
30-40	1.3 E	1.1 E	1.2 E	1.3 E	1.3 E	1.4 E

Mean values followed by different letters (lowercase letters in the rows and uppercase letters in the columns) differ by the Duncan test ($p \leq 0.05$).

Table 5. Contents of organic nitrogen hydrolyzed in acid medium (hydrolyzed-N) and in its diverse fractions in soil layers up to a depth of 10 cm in a Latossolo Vermelho distroférrico (Typic Hapludox) subjected to 10 years of annual fertilization with mineral fertilizer (MF) and pig slurry (PS) at the rates of 0 (control), 25, 50, 100, and 200 m^{-3} ha^{-1} in a no-till system. Mean values of four replications

Treatment	Hydrolyzed-N	NH_4^+-N	Hexosamine-N	α-amino-N	Amide-N	Unidentified-N
	mg kg^{-1}					
			0-2.5 cm			
MF	2,978 Da	741 Cc	27.1 Cf	528 Ed	217 Ce	1,465 Ab
Control	2,291 Fa	469 Ed	131.6 Ae	548 Ec	141 De	1,001 Bb
PS 25	2,830 Ea	705 Dd	29.5 Cf	839 Dc	233 BCe	1,024 Bb
PS 50	3,030 Ca	792 Bd	39.9 BCf	947 Cb	271 ABe	979 Bb
PS 100	3,130 Ba	851 Ac	52.0 BCe	1,110 Ab	294 Ad	824 Cc
PS 200	3,308 Aa	884 Ac	84.1 Be	1,155 Ab	304 Ad	881 Cc
Mean	2,928 a	740 d	60.7 f	854 c	243 e	1,029 b
			2.5-5 cm			
MF	1,944 Ba	635 Dc	19.1 f	324 Dd	199 Be	766 Ab
Control	1,606 Da	455 Dc	32.1 e	598 Cb	206 Bd	389 CDc
PS 25	1,949 Ca	622 Cb	54.6 e	621 Cb	191 Bd	461 Bc
PS 50	2,207 Ba	717 Bc	31.2 f	869 Bb	214 Be	376 Dd
PS 100	2,518 Aa	783 ABc	51.2 f	948 Ab	259 Be	476 Bd
PS 200	2,552 Aa	790 Ac	76.3 f	983 Ab	331 Ae	447 BCd
Mean	2,129 a	667 c	44.1 f	724 b	234 e	486 d
			5-10 cm			
MF	1,689 Ca	540 Ab	33.0 e	377 Cc	164 d	574 ABb
Control	1,611 Ca	469 Bc	8.7 f	389 Cd	137 e	607 Ab
PS 25	1,635 Ca	543 Ab	10.4 e	341 Cc	161 d	582 ABb
PS 50	1,769 Ba	553 Ab	25.1 e	488 Bc	168 d	534 Bb
PS 100	1,869 Aa	574 Ab	32.1 d	524 Bb	186 c	553 ABb
PS 200	1,899 Aa	595 Ab	65.9 e	612 Ab	180 d	446 Cc
Mean	1,745 a	546 b	29.2 e	455 c	166 d	549 b
			0-10 cm			
MF	2,075 Da	614 BCc	28.0 Bf	402 Fd	186 CDe	845 Ab
Control	1,780 Ea	468 Dc	45.2 ABe	481 Ec	155 Dd	651 Bb
PS 25	2,012 Da	605 Cc	26.2 Bf	536 Dd	187 CDe	662 Bb
PS 50	2,194 Ca	653 Bc	30.4 Bf	698 Cb	206 BCe	606 Cd
PS 100	2,346 Ba	685 Ac	41.8 ABf	777 Bb	231 ABe	601 Cd
PS 200	2,414 Aa	716 Ac	73.0 Af	840 Ab	248 Ae	555 Dd
Mean	2,137 a	624 b	40.8 d	622 b	202 c	653 b

Connected mean values followed by different letters (lowercase in the row and uppercase in the column) differ by the Duncan test ($p \leq 0.05$). Hydrolyzed-N = $\sum$ (NH_4^+-N + amide-N + hexosamine-N + α-amino-N).

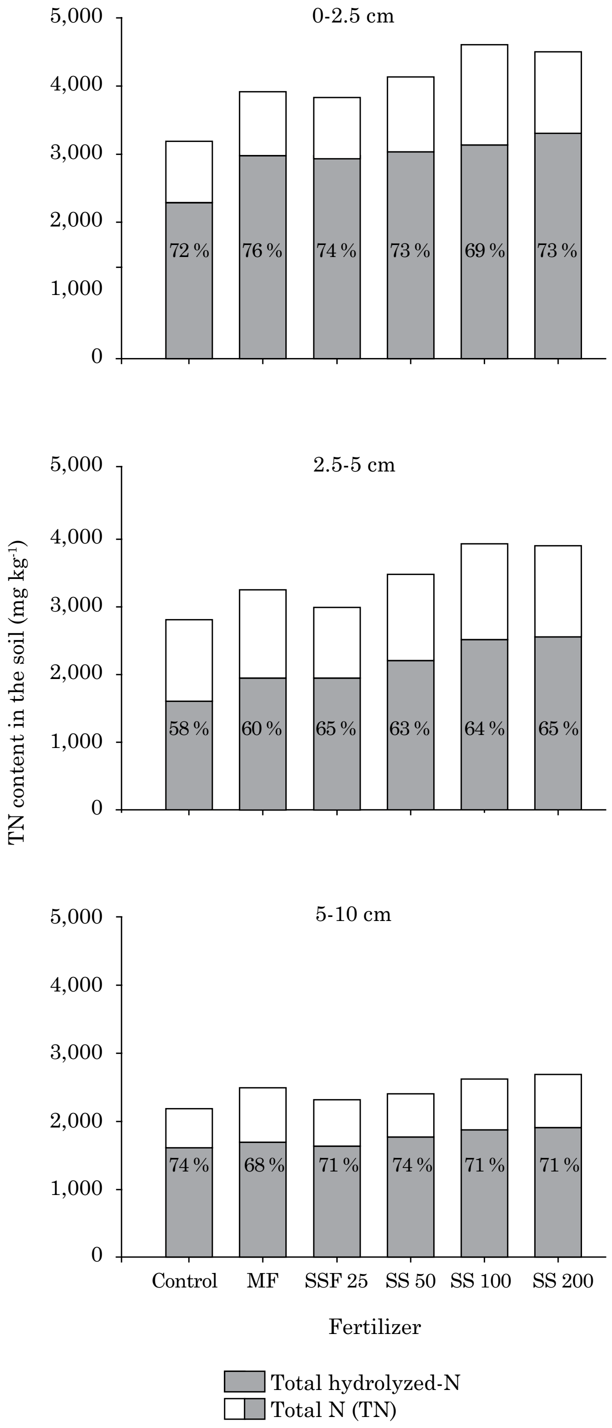

Figure 1. Total nitrogen content (TN) and total hydrolyzed-N content in 6 mol L^{-1} HCl, and respective percentage of the latter in relation to the former in the 0-2.5, 2.5-5, and 5-10 cm depth layers in a Latossolo Vermelho distroférrico (Typic Hapludox) subjected to 10 years of annual fertilization with mineral fertilizer (MF) and pig slurry (SS) at the rates of 0 (control), 25, 50, 100, and 200 m^{-3} ha^{-1} in a no-till system. Mean values of four replications.

in the 0.0-2.5 cm layer, from 58 to 65 % in the 2.5-5 cm layer, and from 68 to 74 % in the 5-10 cm layer, a behavior similar to the TN of the soil (Figure 1). Using this same analytical procedure, Camargo et al. (1997) found the proportion of 79.2 % in the 0-20 cm layer of an Ultisol, and Yonebayashi and Hattori (1980) found 78.5 % in alluvial soils with a clayey texture planted to rice. It is important to note that within the organic N fraction hydrolyzed by the method, the N fractions are determined in the forms of NH_4^+-N, hexosamine-N, α-amino-N, amide-N, and unidentified-N (Table 5).

The fact that the method did not extract all the organic N present in the soil in hydrolysis may be explained by the existence of non-hydrolysable forms, which form clay-metal-humus complexes in the soil. The interaction of organic molecules occurs with polyvalent cations and mineral particles of the clay fraction and oxides, especially by iron oxides in the form of hematite (Fe_2O_3) and goethite (FeOOH), predominant in the soil under study. These forms, in general, characterize occlusion of soil organic matter by physical-chemical mechanisms of protection, which, once established in the soil microaggregates, confer their trait of recalcitrance and limit the accessibility of decomposer microorganisms, enzymes, and the action of acid hydrolysis of the method used in this study (Passos et al., 2007; Plaza et al., 2013).

The greatest contents of hydrolyzed-N occurred in the 0-2.5 cm layer, decreasing with depth, with the rate of 200 m^3 ha^{-1} of PS having the greatest expression in all the layers, although it was similar to the rate of 100 m^3 ha^{-1} in the 2.5-5 and 5-10 cm layers (Table 5). It is noteworthy that during acid hydrolysis of organic N in the soil by the method used, there is degradation of the proteins (Stevenson, 1994), with deamination of monomers and oligomers of sugars from soluble amides, polymers, and amino acids (González-Prieto et al., 1997).

The application rates of 100 and 200 m^3 ha^{-1} of pig slurry applied on the soil also resulted in a greater content of NH_4^+-N in the soil up to 5 cm depth, and in the combination of the layers from 0-10 cm. In the 5-10 cm layer, there was no difference among the fertilizers, which differed only from the control (Table 5). It should be noted that within the forms of greatest lability of organic N in the soil, this fraction is the one that exhibits the greatest contents.

In the hexosamine-N fraction, there was a difference between treatments in the 0-2.5 cm layer and in the combination of the 0-10 cm layers. In the uppermost layer, the greatest content was found in the control, without application of N. Nevertheless, considering the profile of greatest thickness represented by the 0-10 cm layer, the greatest contents are found with 100 and 200 m^3 ha^{-1} of PS (Table 5). The greater hexosamine-N content in the control treatment in the surface layer may be related to the lower microbial activity due to greater limitation in nutrients, a condition that does not allow as much utilization of this form of N by the mineralization process.

It should be noted that the hexosamine-N fraction is considered labile and represents the lowest contents in the soil in relation to the other fractions evaluated. According to Camargo et al. (1999), under conditions that favor the proliferation of microbial biomass, the hexosamine-N content in the soil increases, but over time it may decrease with the limitation of the substrate. González-Prieto et al. (1997) observed decrease in the contents of this fraction after application of mineral fertilization in maize and pasture, suggesting that this form is part of an initial active pool of mineralizable N, which is related to the transitory increase in the soil microbial population.

The application of PS led to an increase in the amide-N content in the soil in the 0-2.5 cm layer, however, only at PS application rates greater than or equal to 50 m^3 ha^{-1}, which allowed results superior to the MF (Table 5). This increase was also observed in the 2.5-5 cm layers, however, only at the application rate of 200 m^3 ha^{-1}. Considering the combined soil layers from 0-10 cm, the application rates greater than or equal to 100 m^3 ha^{-1} also led to greater contents of this N fraction in the soil. The amide-N fraction is recovered in the form of NH_4^+, which is released during the mildest hydrolysis of fractionation in 1.0 mol L^{-1} HCl for 3 h, which indicates that this may be one of the forms of organic N that contributes to availability of the nutrient to the plants.

Similar to the previous fraction, there was an increase in the α-amino-N contents as a result of increasing application rates of PS. The application rate of 50 m^3 ha^{-1} exceeds the MF in all the soil layers evaluated, and application rates greater than or equal to 100 m^3 ha^{-1} up to the 5 cm depth and 200 m^3 ha^{-1} in the 5-10 and 0-10 cm layers lead to the greatest contents of this N fraction in the soil (Table 5). This form of N is present in amino acids which make up proteins that underwent a peptide bond during protein synthesis (Camargo et al., 1999) where N is bound to the alpha group of the carbon, with high bonding energy by hydrogen bridges, Van der Waals forces, and covalent complexes to humic colloids (Stevenson, 1994). González-Prieto and Carballas (1991) observed a decrease in the α-amino-N fraction in cropped soils when compared to natural soils, suggesting that this form may be easily mineralizable and potentially available to plants.

The unidentified-N fraction exhibited the greatest contents in the uppermost soil layer from 0.0-2.5 cm, decreasing with depth. The MF treatment exhibited the greatest contents in the first two soil layers, as well as in the combined layers from 0-10 cm. The application rates greater than or equal to 100 m^3 ha^{-1} of PS decreased this fraction in the soil in the 0-2.5 cm layer (Table 5). It should be remembered that unidentified-N is considered to be the fraction of greatest recalcitrance in relation to the other fractions, with the organic N thus bound to the molecules of greatest molecular weight (Camargo et al., 1997).

The application of increasing rates of pig slurry (PS) allowed increasing quadratic behavior for the NH_4^+-N, hexosamine-N, amide-N, and α-amino-N fractions in all the soil layers up to a 10 cm depth, except for hexosamine-N in the 0-2.5 cm layer, which exhibited decreasing quadratic behavior up to the application rate of 100 m^3 ha^{-1} (Figure 2). As for the unidentified-N fraction, there was decreasing quadratic behavior due to the application of increasing rates of PS, except for the 2.5-5 cm layer, which did not show significant regression.

For the organic N fractions in relation to the total hydrolyzed-N of the soil, it may be observed that MF leads to the existence of more recalcitrant forms in the soil, which in this case make up the unidentified-N fraction, mainly in the surface layers up to a depth of 5 cm, whereas in the application of increasing rates of PS, greater concentrations of α-amino-N and NH_4^+-N prevail. Thus, there is the following sequence in decreasing order for MF: unidentified-N>NH_4^+-N>α-amino-N>amide-N>hexosamine-N. For PS, the following sequence is observed as of the application rate of 50 m^3 ha^{-1}: α-amino-N> NH_4^+-N>unidentified-N>amide-N>hexosamine-N (Figure 3). It should be noted that the greater evidence of the α-amino-N in the soil as a result of the application of PS, indicates that we are dealing with more complex compounds with greater stability, associated with humic compounds (Nguyen and Shindo, 2011).

Thus, the organic N fractions with greater representation were those with a greater degree of recalcitrance, i.e., unidentified-N for MF and α-amino-N for PS, which lead to the formation of organic colloids of greater molecular weight and lower availability in the soil, results that corroborate with those observed by Zhang et al. (2011). Probably, the lower presence of organic N in its more labile forms is related to the greater speed of mineralization by soil microorganisms, and these forms of organic N are indicators of availability of N to the plants in a short period of time.

The annual application of rates greater than or equal to 100 m^3 ha^{-1}, and greater than or equal to 50 m^3 ha^{-1} allowed a greater N content in the plant tissue in the maize and oat crops,

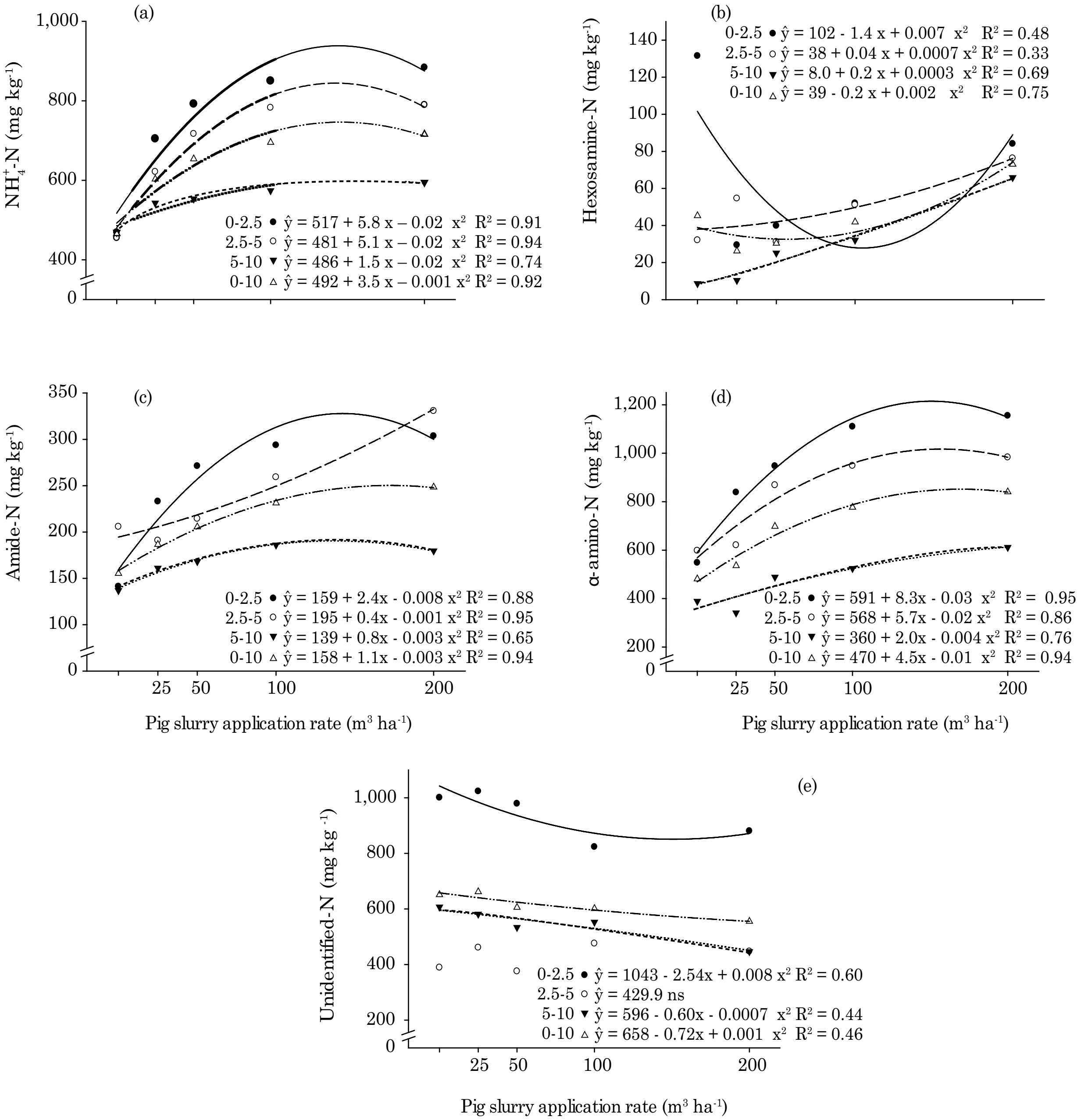

Figure 2. Contents of (a) NH_4^+-N, (b) hexosamine-N, (c) amide-N, (d) α-amino-N, and (e) unidentified-N in the 0-10 cm layer in a Latossolo Vermelho distroférrico (Typic Hapludox) subjected to 10 years of annual fertilization of pig slurry at the rates of 0, 25, 50, 100, and 200 m^{-3} ha^{-1}.

Table 6. Nitrogen content in the leaf tissue of maize and oat subjected to 10 years of annual fertilization with mineral fertilizer (MF) and pig slurry (PS) at the application rates of 0 (control), 25, 50, 100, and 200 m^{-3} ha^{-1} in a no-till system. Mean values of four replications

Plant	MF	Control	PS 25	PS 50	PS 100	PS 200	Mean
				g kg^{-1}			
Maize	26.5 Ab	10.6 e	20.6 Ad	24.2 Ac	29.5 Aa	28.4 Aa	23.3 A
Oat	13.8 Bb	11.6 c	13.4 Bb	15.8 Ba	17.3 Ba	16.7 Ba	14.8 B
Mean	20.2 b	11.1 d	17.0 c	20.0 b	23.4 a	22.6 a	

Mean values followed by different letters (lowercase letters in the row and uppercase letters in the column) differ by the Duncan test ($p \leq 0.05$).

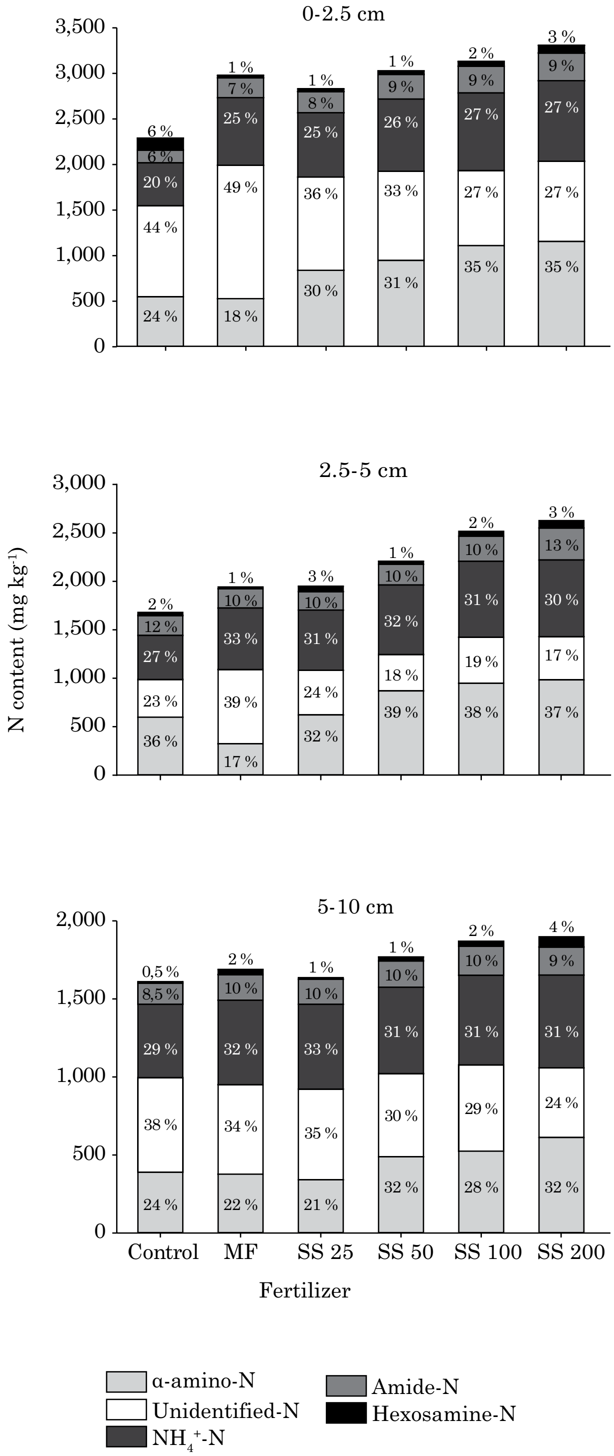

Figure 3. Proportion of forms of organic N in the soil in relation to hydrolyzed-N in the soil in the layers up to 10 cm depth in a Latossolo Vermelho distroférrico (Typic Hapludox) subjected to 10 years of annual fertilization with mineral fertilizer (MF) and pig slurry (SS) at the rates of 0 (control), 25, 50, 100, and 200 m^{-3} ha^{-1} in a no-till system. Mean value of four replications.

respectively (Table 6). These results indicate that the greater efficiency of N uptake by crops at the greater rates of PS are explainable due to the greater quantity of N in the production system, as well as through the fact that greater contents of organic N with a lower degree of recalcitrance in the soil are present in these treatments.

The relation between N content in the maize and black oat plant tissue and the forms of organic N in the soil allow the inference that the increase in the content of the hydrolyzable NH_4^+-N fraction in the 0-10 cm layer leads to increasing linear behavior in the nutrient content in the leaves of both crops (Figure 4), most notably in maize. In regard to the hexosamine-N fraction in the same soil layer, there was no significant fit between the hydrolyzed content and the N leaf content, a result that is explained by the low proportion of N recovered in this form when compared to the others (Figure 3).

The increase in the contents of amide-N and α-amino-N up to 240 and 740 mg kg^{-1} (Figure 4), respectively, in the 0-10 cm soil layer also led to a greater N content in the plant tissue, both in maize and in black oats, indicating that they are included in the labile forms of soil organic N.

The explanation for the concentration of N in the leaf tissue of maize and oats occurring in association with the contents of the element recovered in the NH_4^+-N, amide-N, and α-amino-N hydrolyzed fractions also finds support in studies that indicate that the plants are capable of taking up soil organic N in the form of simpler organic compounds (Komarova et al., 2008; Soper et al., 2011). Thus, the uptake of forms of organic N by plants may contribute as a source of N, as well as simpler inorganic forms (Näsholm et al., 2009; Tegeder and Rentsch, 2010). This was observed in studies that showed that the plants grow in a similar way, or an even better way, with amino acids as a source of N, when compared to inorganic sources (Cambui et al., 2011; Soper et al., 2011). Nevertheless, others report that the amino acids may inhibit growth (Näsholm et al., 2009; Paungfoo-Lonhienne et al., 2012). However, studies on more complex forms of N as a source of N to the plants, and their mechanisms of assimilation and transformation in the soil and in the plant are still in initial stages.

Comparing the increase in the organic N fraction in the form of unidentified-N, to N uptake by the maize and black oat plants, there was decreasing linear behavior in the 0-10 cm layer (Figure 4). These results suggest that this form of N does not contribute to providing this nutrient to these crops and, therefore, may not be used for evaluation of availability in a short period of time.

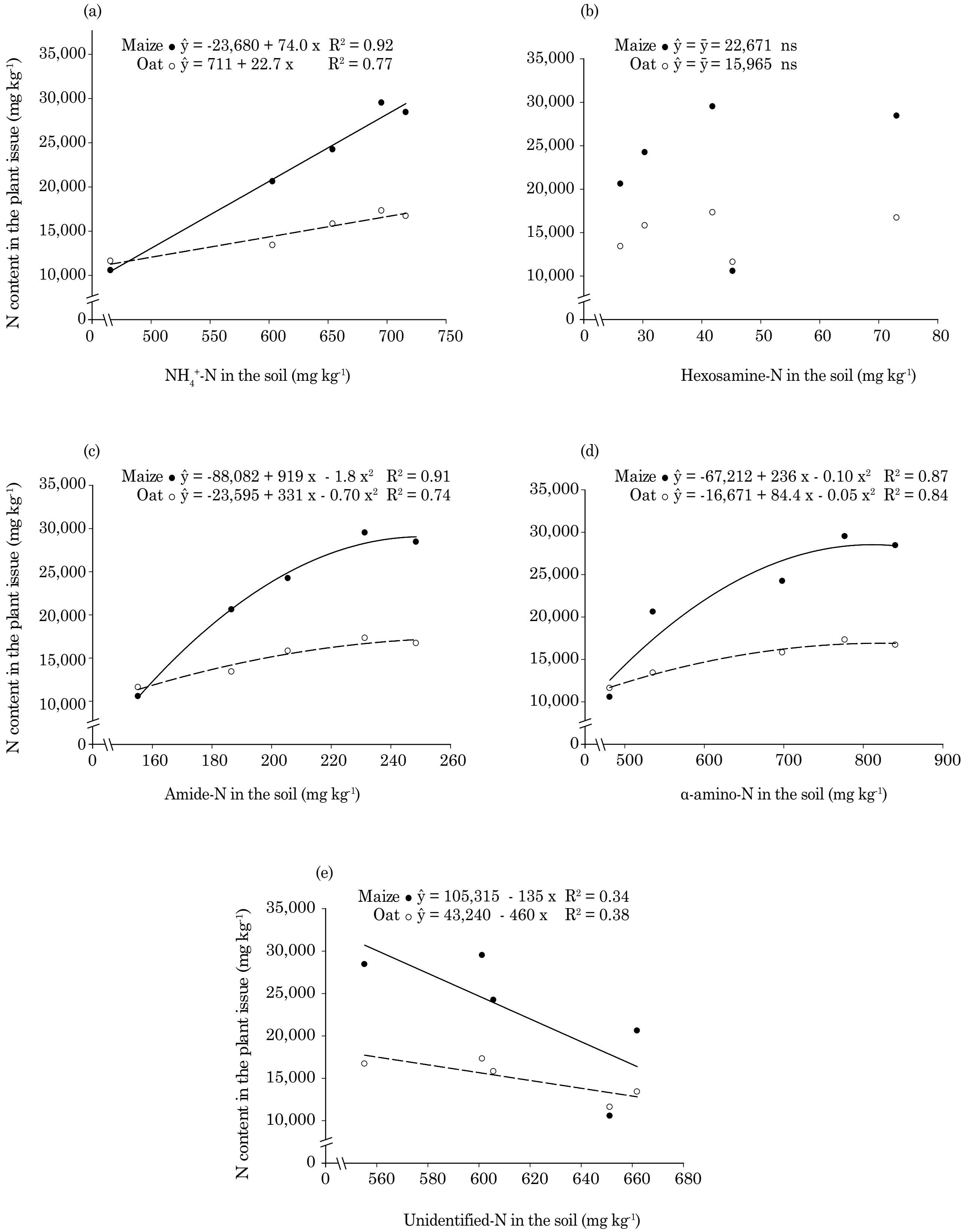

Figure 4. Relation between the N contents in the form of (a) NH_4^+-N, (b) hexosamine-N, (c) amide-N, (d) α-amino-N, and (e) unidentified-N in the soil in the 0-10 cm layer, and N content in the leaves of maize and oat subjected to 10 years of annual fertilization with pig slurry at the application rates of 0, 25, 50, 100, and 200 m^{-3} ha^{-1} in a no-till system.

CONCLUSIONS

Annual fertilization with mineral fertilizer and pig slurry at application rates greater than 25 m^3 ha^{-1} over 10 years increases the total nitrogen contents in the layer up to a depth of 10 cm in a Typic Hapludox cropped in a no-till system with a maize-black oat succession.

The main fractions of organic N in the soil, hydrolyzable in acid medium, are α-amino-N when pig slurry is used, and unidentified-N in mineral fertilization, although pig slurry also contributes to an increase in the less recalcitrant forms, such as NH_4^+-N and amide-N.

The increase in labile forms of N in the soil, such as α-amino-N, amide-N, and NH_4^+-N, allows greater uptake of N by the maize and black oat crops.

REFERENCES

Aita C, Port O, Giacomini SJ. Dinâmica do nitrogênio no solo e produção de fitomassa por plantas de cobertura no outono/inverno com o uso de dejetos de suínos. R Bras Ci Solo. 2006;30:901-10.

Almeida JÁ, Torrent J, Barrón V. Cor de solo, formas do fósforo e adsorção de fosfato em Latossolos desenvolvidos de basalto do extremo-sul do Brasil. R Bras Ci Solo. 2003;27:985-1002.

Brasil. Ministério da Agricultura, Pecuária e Abastecimento. Assessoria de Gestão Estratégica. Projeção do agronegócio 2009/2010 a 2019/2020. Brasília, DF: 2011 [citado 2012 Nov 10]. Disponível em: http://www.agricultura.gov.br/arq_editor/file/MAIS%20DESTAQUES/Proje%C3%A7%C3%B5es%20Agroneg%C3%B3cio%202009-2010%20a%202019-2020.pdf.

Camargo FAO, Gianello C, Vidor C. Nitrogen fractions in the microbial biomass in soils of southern Brazil. R Bras Ci Solo. 1999;23:181-85.

Camargo FAO, Gianello C, Vidor C. Tempo de hidrólise e concentração de ácido para fracionamento do nitrogênio orgânico do solo. Pesq Agropec Bras. 199;32:221-27.

Camargo FAO, Gianello C, Rheinheimer S, Kaminski J, Bissani CA. Fracionamento do nitrogênio, fósforo e enxofre orgânicos. In: Santos GA, Silva LS, Canellas LP, Camargo FO, editores. Fundamentos da matéria orgânica do solo: Ecossistemas tropicais e subtropicais. 2ª ed. Porto Alegre: Metrópole; 2008. p. 243-61.

Cambui CA, Svennerstam H, Gruffman L, Nordin A, Ganeteg U, Nasholm T. Patterns of plant biomass partitioning depend on nitrogen source. Plant Soil. 2011;6:192-211.

Cassol PC, Costa AC, Ciprandi O, Pandolfo CM, Ernani PR. Disponibilidade de macronutrientes e rendimento de milho em Latossolo fertilizado com dejeto suíno. R Bras Ci Solo. 2012;36:1911-23.

Empresa Brasileira de Pesquisa Agropecuária - Embrapa. Sistema brasileiro de classificação de solos. 3ª ed. Rio de Janeiro: Empresa Brasileira de Pesquisa Agropecuária; 2013.

Giacomini SJ, Aita C. Cama sobreposta e dejeto líquido de suínos como fonte de nitrogênio ao milho. R Bras Ci Solo. 2008;32:195-205.

Giacomini SJ, Aita C, Jantalia CP, Urquiaga S, Santos GF. Imobilização do nitrogênio amoniacal de dejetos líquidos de suínos em plantio direto e preparo reduzido do solo. R Bras Ci Solo. 2009;33:41-50.

González-Prieto SJ, Carballas T. Composition of organic N in temperate humid region soils (NW Spain). Soil Biol Biochem. 1991:23:887-95.

González-Prieto SJ, Jocteur-Monrozier L, Hétier JM, Carballas T. Changes in the soil organic N fractions of a tropical Alfisol fertilized with ^{15}N-urea and cropped to maize or pasture. Plant Soil. 1997;195:151-60.

Instituto Brasileiro de Geografia e Estatística - IBGE. Brasil em números - Brazil in figures. Rio de Janeiro: Instituto Brasileiro de Geografia e Estatística; 2013 [cited 2014 Jan 20]. Available at: http://biblioteca.ibge.gov.br/visualizacao/periodicos/2/bn_2013_v21.pdf.

Komarova NY, Thor K, Gubler A, Meier S, Dietrich D, Weichert A, Suter Grotemeyer M, Tegeder M, Rentsch D. AtPTR1 and AtPTR5 transport dipeptides in plant. Plant Physiol. 2008;148:856-69.

Lara Cabezas WAR, Trivelin PCO, Kondörfer GH, Pereira S. Balanço da adubação nitrogenada sólida e fluida de cobertura na cultura do milho em sistema plantio direto no Triângulo Mineiro (MG). R Bras Ci Solo. 2000;24:363-76.

Lourenzi CR, Ceretta CA, Silva LS, Girotto E, Lorensini F, Tiecher TL, Conti L, Trentin G, Brunetto G. Nutrients in soil layers under no-tillage after successive pig slurry applications. R Bras Ci Solo. 2013;37:157-67.

Müller C, Laughlin RJ, Christie P, Watson CJ. Effects of repeated fertilizer and slurry applications over 38 years on N dynamics in a temperate grassland soil. Soil Biol Biochem. 2011;43:1362-71.

Näsholm T, Kielland K, Ganeteg U. Uptake of organic nitrogen by plants. New Phytol. 2009;182:31-48.

Nguyen TA, Shindo H. Effects of different levels of compost application on amounts and distribution of organic nitrogen forms in soil particle size fractions subjected mainly to double cropping. Agric Sci. 2011;2:213-19.

Oliveira PAV. Manual de manejo e utilização dos dejetos de suínos. Concórdia: Empresa Brasileira de Agropecuária; 1993. (Documentos, 27).

Passos RR, Ruiz HA, Mendonça ES, Cantarutti RB, Souza AP. Substâncias húmicas, atividade microbiana e carbono orgânico lábil em agregados de um Latossolo Vermelho distrófico sob duas coberturas vegetais. R Bras Ci Solo. 2007;31:1119-29.

Paungfoo-Lonhienne C, Visser J, Lonhienne TGA, Schmidt S. Past, present and future of organic nutrients. Plant Soil. 2012;359:1-18.

Plaza C, Murias DC, Fernández JM, Polo A, Simpson AJ. Physical, chemical, and biochemical mechanisms of soil organic matter stabilization under conservation tillage systems: A central role for microbes and microbial by-products in C sequestration. Soil Biol Biochem. 2013;57:124-34.

Schirmann J, Aita C, Giacomini SJ, Pujol SB, Giacomini DA, Gonzatto R, Olivo J. Inibidor de nitrificação e aplicação parcelada de dejetos de suínos nas culturas do milho e trigo. R Bras Ci Solo. 2013;37:271-80.

Soper FM, Paungfoo-Lonhienne C, Brackin R, Rentsch D, Schmidt S, Robinson N. *Arabidopsis* and *Lobelia anceps* access small peptides as a nitrogen source for growth. Funct Plant Biol. 2011;38:788-96.

Stevenson FJ. Humus chemistry - genesis, composition, reactions. 2nd ed. New York: Willey; 1994.

Tedesco MJ, Gianello C, Anghinoni I, Bissani CA, Camargo FAO, Wiethölter S, editores. Manual de adubação e de calagem para os estados do Rio Grande do Sul e Santa Catarina. Porto Alegre: Sociedade Brasileira de Ciência do Solo; 2004.

Tedesco MJ, Volkweiss SJ, Bohnen H, Gianello C, Bissani CAA. Análise de solos, plantas e outros materiais. 2a ed. Porto Alegre: Universidade Federal do Rio Grande do Sul; 1995. (Boletim técnico de solo, 5).

Tegeder M, Rentsch D. Uptake and partitioning of amino acids and peptides. Molec Plant. 2010;3:997-1011.

Yonebayashi H, Hattori T. Improvements in the method for fractional determination of soil organic nitrogen. Soil Sci Plant Nutr. 1980;32:189-200.

Zhang JB, Zhu TB, Cai ZC, Qin SW, Müller CM. Effects of long-term repeated mineral and organic fertilizer applications on soil nitrogen transformations. Eur J Soil Sci. 2011;63:75-85.