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SPECTROSCOPIC QUANTIFICATION OF SOIL PHOSPHORUS FORMS BY ^{31}P-NMR AFTER NINE YEARS OF ORGANIC OR MINERAL FERTILIZATION(1)

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SUMMARY

Long-standing applications of mineral fertilizers or types of organic wastes such as manure can cause phosphorus (P) accumulation and changes in the accumulated P forms in the soil. The objective of this research was to evaluate the forms of P accumulated in soils treated with mineral fertilizer or different types of manure in a long-term experiment. Soil was sampled from the 0-5 cm layer of plots fertilized with five different nutrient sources for nine years: 1) control without fertilizer; 2) mineral fertilizer at recommended rates for local conditions; 3) 5 t ha^{-1} year^{-1} of moist poultry litter; 4) 60 m^3 ha^{-1} year^{-1} of liquid cattle manure and 5) 40 m^3 ha^{-1} year^{-1} of liquid swine manure. The ^{31}P-NMR spectra of soil extracts detected the following P compounds: orthophosphate, pyrophosphate, inositol phosphate, glycerophosphate, and DNA. The use of organic or mineral fertilizer over nine years did not change the soil P forms but influenced their concentration. Fertilization with mineral or organic fertilizers stimulated P accumulation in inorganic forms. Highest inositol phosphate levels were observed after fertilization with any kind of manure and highest organic P concentration in glycerophosphate form in after mineral or no fertilization.

Index terms: glycerophosphate, manure, phytate, organic phosphorus, organic fertilization.

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RESUMO: *QUANTIFICAÇÃO ESPECTROSCÓPICA DE FORMAS DE FÓSFORO DO SOLO POR ^{31}P-RNM APÓS NOVE ANOS DE USO DE FERTILIZANTES ORGÂNICOS OU MINERAIS*

Aplicações sucessivas de estercos de animais no solo por longos períodos podem acumular fósforo no solo e modificar as formas de fósforo acumuladas no solo. O objetivo deste estudo foi avaliar as formas de acumulação de fósforo em solos que receberam adubação com fertilizantes minerais ou com diferentes tipos de estercos, em um experimento de longa duração. Amostras de solo foram coletadas da profundidade de 0-5 cm de parcelas de um experimento fertilizado, durante nove anos com diferentes fontes de nutrientes: testemunha sem fertilização; adubação mineral NPK, de acordo com as recomendações regionais; 5 t ha^{-1} ano^{-1} de cama de aviário; 60 m^3 ha^{-1} ano^{-1} de esterco líquido de bovinos; e 40 m^3 ha^{-1} ano^{-1} de esterco líquido de suínos. O fósforo das amostras de solo foi extraído com NaOH+EDTA e os extratos foram submetidos à análise de ressonância nuclear magnética de fósforo (^{31}P-RNM). Os espectros de ^{31}P-RNM revelaram a presença dos seguintes compostos: ortofosfato, pirofosfato, fosfatos de inositol, glicerofosfatos e DNA. O uso de fertilizantes orgânicos ou mineral durante nove anos não modificou as formas de fósforo encontradas no solo, mas provocou modificação nas concentrações de cada forma. A fertilização com qualquer tipo de fertilizante estimulou o acúmulo de fósforo, principalmente em formas inorgânicas. Observou-se o aumento da concentração de fosfatos de inositol, quando o solo foi fertilizado com qualquer um dos fertilizantes orgânicos testados, enquanto nos tratamentos testemunha e na adubação mineral a elevação de fósforo orgânico ocorreu na forma de glicerofosfato.

Termos de indexação: glicerofosfato, estercos, fitato, fósforo orgânico, fertilizantes orgânicos.

INTRODUCTION

Much of the organic waste generated on rural properties is potential fertilizer. In the recent past, the use of agricultural residues has become more attractive because of the increasing importance of the organic agriculture model, which recommends the use of non-industrialized and low energy-cost nutrient sources (Mäder et al., 2002). Of the waste generated on farms, the most common is animal manure, widely used as fertilizer. However, these materials must be used with caution, because if applied excessively, soil nutrients can be accumulated, particularly nitrogen (N) and phosphorus (P), above the soil adsorption or plant uptake capacity, posing contamination risks as nonpoint pollution sources with high nutrient transfer capacity to aquatic systems (Sharpley et al., 1995).

When added to soil, manure enhances P levels, both in organic and inorganic forms, although the increase in inorganic forms is always higher (Koopmans et al., 2003; Hansen et al., 2004). The reason is mainly that most P in manure is in inorganic forms (Koopmans et al., 2003; McDowell & Stewart, 2005), which in cattle manure represents about 70 % of total P (Cassol et al., 2001). Organic P is not directly available to plants but can be converted to inorganic P through hydrolysis or mineralization. Although at low proportions, soil organic P is a nutrient reservoir that can become available to plants and soil microbial biomass (Gatiboni et al., 2008). Additionally, e.g., in inorganic forms, organic P can also be transferred from soil to water bodies and contribute to the nutrition of aquatic organisms, including cyanobacteria, which can cause eutrophication (Sharpley et al., 1995).

Several studies have shown that organic P forms in soil are sensitive to the different management practices (Condron et al., 1990; Guggenberger et al., 1996; Rheinheimer et al., 2002). When inorganic P is added in sufficient quantities to maintain plant requirements, soil organic P is preserved. On the other hand, when P applications are below plant requirements, the stock of organic P is reduced, especially the diester forms (Gatiboni et al., 2005).

Phosphorus added by fertilizers is distributed in the soil in inorganic and organic forms, which are subdivided in labile, moderately labile and non-labile forms (Negassa & Leinweber, 2009). This separation depends on the P fractionation method; however, most of these methods provide no information on the structural composition of soil P compounds (Guggenberger et al., 1996). The technique of nuclear magnetic resonance of phosphorus (^{31}P-NMR) is a spectroscopic method by which structural forms of P can be identified in soil extracts (Cade-Menun & Preston, 1996).

Koopmans et al. (2003) evaluated the P forms assessed by ^{31}P-NMR in soils treated with soluble fertilizers or different manure types for 11 years. They reported P accumulation mainly in inorganic forms, and monoester Po was accumulated only in soil treated with pig slurry. Similar results were found by Hansen et al. (2004) in a soil that had been treated with cattle manure application over 10 years. The accumulation of monoester Po in soil occurs because the most animals are unable to digest phytic acid (inositol hexaphosphate), which accumulates in manures and, consequently, in soils treated with this waste. In animal feed, this kind of organic P dramatically reduces the efficiency of P absorption by animals and creates an environmental problem due the P-richness of the manure (Golovan et al., 2001).

Since different organic phosphate compounds can be decomposed differently in the soil, it is important to know the possible forms of P accumulation after the application of different fertilizers to understand the preferred way of accumulation and the possible availability to plants and transfer to aquatic systems. The objective of this research was to evaluate the P forms accumulated in soils treated with mineral fertilizer or different manure types in a long-term experiment.

MATERIAL AND METHODS

Site and experiment description

Samples were collected in 2003, from soils with annual applications of nutrient sources since 1994. The experiment was established at the EPAGRI Experimental Station located in Campos Novos (27° 24´ S; 51° 13´ W), a county in the state of Santa Catarina, Brazil, with subtropical climate, 970 m asl, and annual rainfall of 1,300 to 2,100 mm. The mean air temperature is between 15.8 and 17.9 °C. The studied soil was a Typic Haplorthox with 700 g kg^{-1} clay and was used for nine years with no-tillage crop rotation in three year-cycles. The first cycle included triticale (*Triticum secale* Wittimack)/soybean (*Glycine max* L. Merrill)/common vetch (*Vicia sativa* L.)/maize (*Zea mays* L.)/black oat (*Avena strigosa* Schreb.)/ common bean (*Phaseolus vulgaris* L.)/and wheat (*Triticum aestivum* L.). In the second and third cycles triticale was substituted by rye (*Secale cereale* L.) and wheat by oilseed radish (*Raphanus sativus* L.). Nitrogen and potassium fertilizers were added according to the local Soil Fertility Commission (CFSRS/SC, 1994).

Soil samples were collected from the four replications, from the 0-5 cm layer of plots fertilized for nine years with five different nutrient sources: 1) Control: without fertilizer; 2) Mineral: mineral fertilizer according to technical recommendations for each crop (140 kg ha^{-1} N, 70 kg ha^{-1} P_2O_5 and 100 kg ha^{-1} K_2O for maize; 0 kg ha^{-1} N, 60 kg ha^{-1} P_2O_5 and 90 kg ha^{-1} K_2O for soybean; 85 kg ha^{-1} N, 20 kg ha^{-1} P_2O_5 and 40 kg ha^{-1} K_2O for common bean); 3) Poultry: 5 t ha^{-1} year^{-1} of moist poultry litter; 4) Cattle: 60 m^3 ha^{-1} year^{-1} of liquid cattle manure, and 5) Swine: 40 m^3 ha^{-1} year^{-1} of liquid swine manure. In the mineral fertilizer treatment, the P source was triple superphosphate. Based on the average P levels in the different organic wastes, estimated by CFSRS/SC (1994), the total P applied after nine years was 0 kg ha^{-1} to the control, 197 kg ha^{-1} to the mineral fertilizer, 560 kg ha^{-1} to the poultry litter, 189 kg ha^{-1} to the cattle manure and 377 kg ha^{-1} to the swine manure treatment.

Soil chemical analysis

Available P was extracted by ion exchange resin membranes saturated with $NaHCO_3$; total P was determined in $H_2O_2 + H_2SO_4$ digestion and organic P by the ignition method (Kuo, 1996); and organic carbon was analyzed by the Walkley-Black method (Nelson & Sommers, 1996). Exchangeable Ca, Mg and Al were extracted with 1 mol L^{-1} KCl; K was extracted by anion exchange resin; Cu and Zn were extracted by 0.1 mol L^{-1} HCl; and H+Al was extracted by 1 mol L^{-1} $Ca(CH_3COOH)_2$, as described by Embrapa (1997). Exchangeable Ca, Mg, Cu, and Zn were determined by atomic absorption spectrometry, K by flame emission spectrometry and Al and H+Al by titration with NaOH. Results of the chemical analyses are presented in table 1.

^{31}P-NMR analysis

For the ^{31}P-NMR analysis, 2.0 g dried soil samples were transferred to centrifuge tubes and 40 mL of 0.25 mol L^{-1} NaOH + 50 mmol L^{-1} EDTA was added (Cade-Menun & Preston, 1996). The tubes were shaken for 4h in an end-over-end shaker at 25 °C, and after centrifugation at 2,500 rpm for 15 min, the extracts were frozen and lyophilized to complete dryness. The lyophilized extract was dissolved in 2.7 mL of 0.25 mol L^{-1} NaOH + 50 mmol L^{-1} EDTA. An amount of 0.3 mL D_2O was added and the mixture vortex-stirred for 5 min. After standing for 120 min, the supernatant was separated by centrifugation (2,500 rpm for 15 min), filtered through a 0.22 µm membrane and transferred to 5 mm NMR tubes (Cade-Menun, 2005). The ^{31}P spectra were obtained in a Bruker Advance DPX 400 spectrometer at a frequency of 162 MHz with proton decoupling. A pulse angle of 90° was used at 20 °C with 0.2 s of acquisition time and 15 s of relaxation time (McDowell et al., 2006). The average number of scans was 12,000. The peak area was obtained by electronic integration at a baseline of 1Hz. The chemical shifts were obtained in comparison with external 85 % orthophosphoric acid and interpreted according to Doolette et al. (2009) as follows: orthophosphate (6.2 ppm), pyrophosphate (-4.07 to -4.18 ppm), *myo*-inositol hexakisphosphate (4.91 - 4.95 ppm; 4.55 to 4.58 ppm; 4.44 - 4.47 ppm), *scyllo*-inositol hexakisphosphate (4.07 - 4.11 ppm), α-glycerophosphate (5.17 - 5.21 ppm), β-glycerophosphate (4.84 - 4.88 ppm) and DNA (-0.22 ppm). Software MestRe-C v 2.3a was used for peak area integration.

Table 1. Accumulated crop yield and soil characteristics after nine years of cultivation on no tillage soil with application of mineral fertilizer and different kinds of organic fertilizers

Variable	Treatment				
	Control	Mineral	Cattle	Swine	Poultry
Accumulated crop yield (t ha^{-1})$^{(1)}$	16.9	25.9	25.8	32.0	32.7
Accumulated dry matter of cover crops(t ha^{-1})$^{(2)}$	25.1	34.0	32.2	38.1	38.9
Total organic carbon (g kg^{-1})	24.1	25.9	26.8	26.6	26.6
pH (H_2O) (1:1)	5.1	4.9	5.2	5.0	5.4
Available P (mg kg^{-1})$^{(3)}$	3.2	9.5	5.0	15.8	17.3
Available K (mg kg^{-1})	136	251	268	98	215
Exchangeable Al (cmol$_c$ kg^{-1})	0.37	0.63	0.13	0.23	0.07
Exchangeable Ca (cmol$_c$ kg^{-1})	6.2	6.3	6.6	7.4	10.3
Exchangeable Mg (cmol$_c$ kg^{-1})	3.1	2.6	3.4	3.5	3.5
H + Al (cmol$_c$ kg^{-1})	7.74	9.74	6.15	7.74	5.49
Available Zn (mg kg^{-1})	1.5	1.8	3.0	20.7	7.3
Available Cu (mg kg^{-1})	4.1	5.3	5.0	9.9	3.8

(1) Accumulated crop yield of soybean, maize or common bean of nine growing seasons; (2) Accumulated cover crop yield (triticale, common vetch, black oat or rye) of nine growing seasons; (3) Anion exchange resin method.

RESULTS AND DISCUSSION

Crop yield and soil characteristics after nine years of fertilization

The accumulated crop yield and cover crop dry matter were greater in the fertilized than in the control treatments (Table 1). Also, an increase in P availability after fertilization was confirmed. This indicates that the amount of P added was higher than the P exported by crops, promoting accumulation in the soil. It can also be observed (Table 1) that the levels of available P in the soil were related with P added via organic and mineral fertilizers because in treatments with higher P addition, more available P was found after nine years of experiment. Total organic carbon increased very little after manure addition (Table 1) in the no-tillage system, as also observed by Calegari et al. (2008). Therefore, a reduced P immobilization in organic forms was expected. Higher acidification (lower pH, higher exchangeable Al and H+Al) was observed in the mineral fertilizer treatment and pH increase or no acidification was observed after organic fertilization. This effect can be explained by organic compounds that can complex hydrogen and aluminum in the soil solution or by the existence of carbonates in the residues (Silva et al., 2008). Higher Zn and Cu soil levels were observed in the liquid swine manure treatment, probably because of the addition of these elements in pig feeding.

^{31}P-NMR Spectra

To identify the compounds detected by ^{31}P-NMR, the peaks were interpreted as suggested by Doolette et al. (2009), although the chemical shifts of resonances were a little different from their findings, since chemical shifts vary with differences in pH and electrolyte concentration, as emphasized by the authors. The chemical shifts of resonances (Table 2) were almost identical in each spectrum. The variation in chemical shifts was largest for pyrophosphate resonance (peak P in Figure 1), with a shift of 0.11 ppm. The other eight resonances (peaks O and A-G in Figure 1) shifted by no more than 0.04 ppm between treatments. In this work no spike was used in the samples with known organic P compounds, as recommended by Doolette et al. (2009), but in the monoester organic P region it is possible to clearly identify: *myo*-inositol hexakisphosphate (*myo*-IHP), *scyllo*-inositol hexakisphosphate (*scyllo*-IHP), α-glycerophosphate and β-glycerophosphate. Figure 1 shows that the P categories found in our soil samples were: orthophosphate, pyrophosphate, *myo*-IHP, *scyllo*-IHP, α-glycerophosphate, β-glycerophosphate, and DNA. No P was detected in the DNA category in the mineral fertilizer treatment nor phytate (*myo*-IHP) in the control and mineral fertilizer treatments (Figure 1).

Even when using a pulse angle of 90° and a very high relaxation time (15 s) (Cade-Menun, 2005; McDowell et al., 2006), no P was detected in the polyphosphate and phosphonate categories in our soil samples. Similar results were obtained by Rheinheimer et al. (2002) and Gatiboni et al. (2005), with soils from the same Brazilian region. It is known that phosphonates are very resistant to microbial or chemical degradation (Benitez-Nelson et al., 2004) while polyphosphates seem to be more readily degradable (Cade-Menun et al., 2006). As the presence of EDTA decreases the decomposition of polyphosphates during extraction (Cade-Menun, 2005) and the spectra were obtained immediately after

Table 2. Assignment of prominent resonances of the NaOH + EDTA extracts from soil under no tillage after application of mineral fertilizer and different kinds of organic fertilizer for nine years

Phosphorus category	Control	Mineral	Cattle	Swine	Poultry
	Peak identification, ppm				
Orthophosphate (O)(1)	6.20	6.20	6.20	6.20	6.20
α-glycerophosphate (A)	5.21	5.21	5.17	5.17	5.17
β-glycerophosphate (C)	4.88	4.88	4.84	4.84	4.84
Myo-inositol hexakisphosphate (B)	nd(2)	nd	4.95	4.95	4.91
Myo-inositol hexakisphosphate (E)	nd	nd	4.58	4.58	4.55
Myo-inositol hexakisphosphate (F)	nd	nd	4.47	4.47	4.44
Scyllo-inositol hexakisphosphate (G)	4.07	4.11	4.11	4.11	4.07
DNA (D)	-0.22	nd	-0.22	-0.22	-0.22
Pyrophosphate (P)	-4.18	-4.14	-4.11	-4.14	-4.07

(1) Letters in parentheses indicate the NMR peaks in figure 1; (2) nd: not detected.

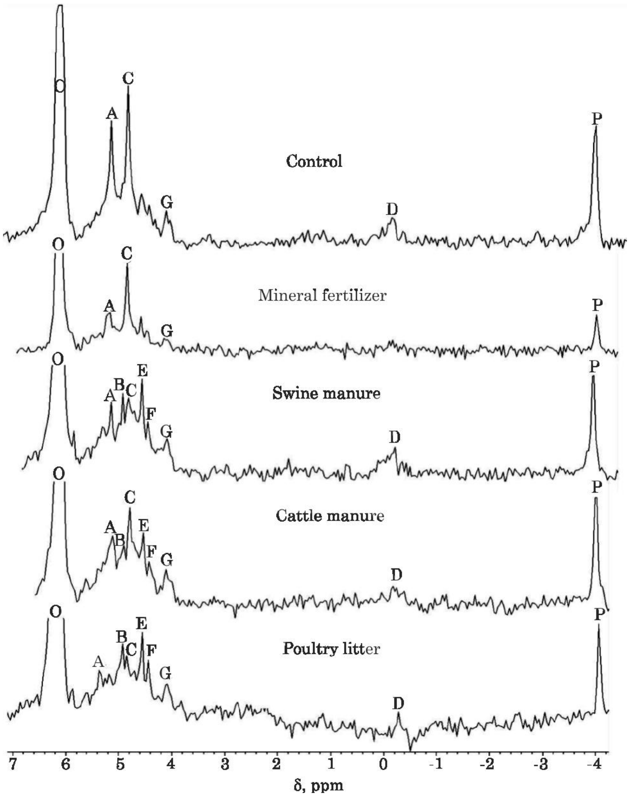

Figure 1. Solution ^{31}P-NMR spectra of NaOH - EDTA extracts of soil cultivated for nine years without fertilization and fertilized with cattle manure, swine manure, poultry litter and mineral fertilizer. Resonances O, P and A through G correspond to the peaks identified in table 2.

extraction, as recommended by Turner et al. (2003), it is likely that the concentrations of these compounds were not detectable in the soil by NMR. Dai et al. (1996) and Makarov (1997) warned that these compounds are only found when microbial activity is very low or the soil organic matter content is very high, which was not the case in this experiment.

Inorganic orthophosphate P and organic monoester P were the main P categories in all treatments (Figure 1). Some works analyzing Brazilian soils also reported orthophosphate Pi and monoester Po as major P components (Rheinheimer et al., 2002; Cardoso et al., 2003; Gatiboni et al., 2005), because orthophosphate Pi is adsorbed with high bounding energy to the soil mineral components. Likewise, some kinds of monoester Po, especially inositol hexaphosphate (*myo* and *scyllo*), are also accumulated in soils because of their high residual charge, promoting stabilization of the soil mineral components (Anderson et al., 1974). The P forms detected by ^{31}P-NMR in this work were similar of those described by Koopmans et al. (2003), Hansen et al. (2004), He et al. (2008) and Dou et al. (2009) in soils treated with long-term fertilization or animal manure applications.

Accumulated phosphorus forms

The distribution of the inorganic P forms measured by chemical methods and NMR was similar to the amount of P added by treatments (Figure 2). A positive correlation (Pearson correlation) was found between the amount of P added by treatments and soil-available P content (0.940*). A positive correlation was also observed between soil-available P and Total soil P by acid digestion (0.941*) and with Total P extracted by NaOH + EDTA (0.987**). The concentration of NaOH-EDTA-extractable P ranged from 388.7 to 620.6 mg kg^{-1}, representing 47-60 % of the total soil P. Evaluating the P categories measured by NMR analysis, soil-available P was positively correlated with P orthophosphate (0.981**) and inorganic P (orthophosphate + pyrophosphate) (0.980**). On the other hand, there was no significant correlation (-0.602^{ns}) between soil-available P and the sum of organic P forms detected by NMR (*myo*-IHP + *scyllo*-IHP + α-glycerophosphate + β-glycerophosphate + DNA).

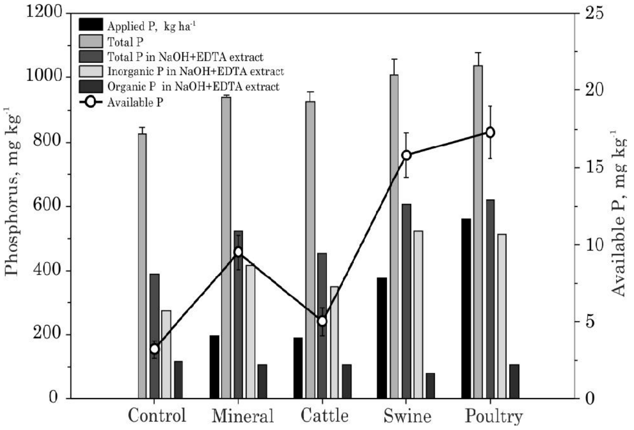

Figure 2. Amount of phosphorus added by fertilizers, total and available P by chemical methods and orthophosphate P and inorganic P by ^{31}P-NMR analysis in soil after nine years of cultivation under no tillage with application of mineral fertilizer and different kinds of organic fertilizers. Error bars represent the standard error of analytical replicates.

The results in table 3 show the P concentrations and percentage of P categories detected by ^{31}P-NMR. In all treatments, the major P form was orthophosphate Pi (62.2-81.8 % of NaOH+EDTA extracted P). The sum of inorganic P forms (orthophosphate + pyrophosphate) ranged from 70.3 to 86.6 %. This shows that even in the organic fertilizer treatments, P was accumulated mainly in inorganic forms, due to the high inorganic P levels that are already present in animal manures (Cassol et al., 2001; Koopmans et al., 2003; McDowell & Stewart, 2005) or because of the rapid enzymatic hydrolysis after soil application (He et al., 2008). Ajiboye et al. (2007) showed that in alkali extract of cattle manure, 92 % of total P belonged to the orthophosphate category and only 5 % to monoester and 3 % to the diester category. In poultry litters, orthophosphate accounted for only 43 % of total P, monoester for 51 % (47 % in phytic acid) and diester for only 6 %. He et al. (2008) also showed similar results of P pools in poultry litter (only 17.9 % of inorganic P, and 60.9 % of *myo*-IHP), while Leinweber et al. (1997) found that orthophosphate accounted for 77 % of total P, monoester for 13 % and diester for 9 %.

The addition of mineral or organic fertilizers changed the distribution of organic P/inorganic P rates in relation to the control treatment (Table 3). While in the control treatment organic P forms accounted for 29.7 % of P in the NaOH+EDTA extract, in fertilized treatments the organic P ranged from 13.4 to 23.3 %, as also observed by Koopmans et al. (2003). However, these results are based on relative proportions, and in terms of the concentration of each P category in the NaOH+EDTA extract, all fertilizer types increased the inorganic P concentration extracted by NaOH+EDTA and had no or a low reduction effect on the concentration of organic P forms (Table 3). Similar results were found by He et al. (2008), who showed that the pools of both labile and stable inorganic P in the soil increased after 20 years of poultry litter application, whereas repeated poultry litter applications induced no significant accumulation of hydrolysable organic P.

Of the two forms of inorganic P, orthophosphate increased P concentration most after organic and mineral fertilization, while the P concentration in pyrophosphate was similar in the treatments. These results were reported by several authors because when P is fertilized, inorganic P is the main form of P accumulation. For inositol phosphates, the phytate concentration was higher in the organic fertilizer treatments and no P was detected in this category in the control and mineral fertilizer treatments. On the other hand, *scyllo*-IHP was detected at similar concentrations in all treatments. After organic fertilizer applications, α-glyceraphosphate and β-glyceraphosphate were detected at lower concentrations than in the control and mineral fertilizer treatments. Concentrations of P in the DNA category were similar, except in the mineral fertilizer treatments, where no P was detected in this category.

The P categories detected by NMR analysis were grouped by similarity (Table 4). The inorganic P forms (orthophosphate plus pyrophosphate) were grouped and called "Inorganic phosphorus". P accumulation was observed in this group after fertilization, as previously discussed. A second group was called "Inositol phosphates" (*myo*-IHP plus *scyllo*-IHP), with a supposedly high charge density and high adsorption to soil. In this group, accumulation was greater in the organic fertilizer treatments (cattle, poultry and swine), which may be due to the addition of these compounds by organic fertilizers and the low degradability of these compounds in the soil. Therefore, inositol phosphates could be used as tracers of organic fertilizer. On the other hand, Doolette et al. (2010, 2011) demonstrated that *myo*-inositol hexakisphosphate can be mineralized quickly in some soils. However, as the fertilizers were applied repeatedly over nine years in the current experiment, we assume that the high concentration of inositol phosphates in the cattle, poultry and swine manure treatments occurred because these slowly degradable compounds were present in the annually reapplied manures, permitting Po accumulation as inositol phosphates.

The DNA compounds, α-glycerolphosphate and β-glyceraphosphate were grouped (Table 4) and called "Diester phosphate and its decomposition products". This group was established because it seems that these compounds have the same origin, since Doolette et al. (2009) showed that α-glycerolphosphate and β-glyceraphosphate are monoester P compounds originated from the degradation of phospholipids

Table 3. Total extractable P by NaOH + EDTA and P concentration in each category identified by ^{31}P-NMR in extracts from soil under no tillage after application of mineral fertilizer and different kinds of organic fertilizer for nine years

Variable	Control	Mineral	Cattle	Swine	Poultry
	P (mg kg^{-1})				
Orthophosphate	241.8 (62.2)(3)	397.2 (76.0)	317.3 (69.9)	494.3 (81.8)	493.3 (79.5)
Pyrophosphate	31.5 (8.1)	17.8 (3.4)	30.9 (6.8)	29.0 (4.8)	19.9 (3.2)
Myo-inositol hexakisphosphate	0.0 (0.0)	0.0 (0.0)	40.0 (8.8)	33.8 (5.6)	61.4 (9.9)
Scyllo-inositol hexakisphosphate	10.5 (2.7)	11.0 (2.1)	8.6 (1.9)	7.3 (1.2)	13.0 (2.1)
α-glycerophosphate	42.0 (10.8)	32.4 (6.2)	26.3 (5.8)	14.5 (2.4)	9.9 (1.6)
β-glycerophosphate	52.5 (13.5)	64.3 (12.3)	17.7 (3.9)	18.1 (3.0)	13.0 (2.1)
DNA	10.5 (2.7)	0.0 (0.0)	13.2 (2.9)	7.3 (1.2)	9.9 (1.6)
Sum of Inorganic P categories(1)	273.3	415.0	348.2	523.3	513.2
Sum of Organic P categories(2)	115.5	107.7	105.8	81.0	107.2
Total P extractable by NaOH-EDTA	388.7	522.6	454.0	604.2	620.6

(1) Sum of inorganic P categories = orthophosphate + pyrophosphate; (2) Sum of organic P categories = *myo*-IHP + *scyllo*-IHP + glycerophosphates + DNA; (3) Figures in parentheses indicate the percentage of each P category in NMR analysis.

Table 4. Phosphorus groups formed by similar compounds detected by ^{31}P-NMR in extracts from soil under no tillage after application of mineral fertilizer and different kinds of organic fertilizer for nine years

Variable	Control	Mineral	Cattle	Swine	Poultry
	P (mg kg^{-1})				
Inorganic phosphorus(1)	273.3	415.0	348.2	523.3	513.2
Inositol phosphates(2)	10.5	11.0	48.6	41.1	74.4
Diester phosphate and its decomposition products(3)	105.0	96.7	57.2	39.9	32.8

(1) Sum of inorganic P categories = orthophosphate + pyrophosphate; (2) Sum of inositol P categories = m*yo*-IHP + s*cyllo*-IHP; (3) Sum of other P categories = α-glycerophosphate + β-glycerophosphate + DNA.

(diester P) and this degradation may occur during P extraction with NaOH + EDTA for NMR analysis or phospholipids can be degraded previously in the soil by microbial biomass. In the control and mineral fertilizer treatments, the P contents in the group "Diester phosphate and its decomposition products" were almost twice as high as in the organic fertilizer treatments (Table 4). In treatments with manure application, even though this readily mineralizable organic P was added together with a carbon source (manures), this kind of organic P was probably rapidly mineralized by microbial biomass, because nutrients and energy for microorganisms were present in the waste. In the control and mineral fertilizer treatments, no carbon source was added, which may have slowed down the diester P decomposition. According to Doolette et al. (2009), one of the probable origins of glycerophosphate is soil microorganism tissue, but in this experiment it was hypothesized that these compounds were originated from plant tissue, once it is known that phospholipids can be released from plant roots as mucilage (Dunbabin et al., 2006) or from plant tissue during the first stages of straw decomposition (Ha et al., 2007). In this experiment, the soil was cultivated under no-tillage, the crop residues were left on the soil surface and additionally only the topsoil layer (0-5 cm) was sampled, where the effect of straw applied to the soil surface is strongest. Therefore, it is suggested that the highest concentrations of α-glycerolphosphate and β-glyceraphosphate in control and mineral fertilizer treatments were due to the low input of easily mineralizable carbon, reducing microbial activity and delaying the degradation of these phosphates in the soil.

CONCLUSIONS

1. The ^{31}P-NMR spectra of soil extracts indicated the presence of phosphorus (P) in the forms orthophosphate, pyrophosphate, inositol phosphates, glycerophosphate, and DNA in all treatments analyzed.

2. The fertilization with mineral or organic fertilizers stimulated P accumulation only in the inorganic P forms detected in NaOH+EDTA extract.

3. The treatments modified the distribution of organic P input in the preexisting forms of organic P in the soil. An increase in inositol phosphate concentration was observed when soil was fertilized with any kind of manure and a higher P concentration in form of glycerophosphate in the mineral fertilizer treatment or without fertilization.

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