

# Organic compounds from plant extracts and their effect on soil phosphorus availability

Paulo Sergio Pavinato<sup>(1)</sup>, Alexandre Merlin<sup>(2)</sup> and Ciro Antonio Rosolem<sup>(2)</sup>

<sup>(1)</sup>Universidade Tecnológica Federal do Paraná, Estrada para Boa Esperança, Km 04, CEP 85660-000 Dois Vizinhos, PR, Brazil. E-mail: pavinato@utfpr.edu.br <sup>(2)</sup>Universidade Estadual Paulista, Departamento de Produção Vegetal, Faculdade de Ciências Agrônomicas, CEP 18603-970 Botucatu, SP, Brazil. E-mail: amerlin@fca.unesp.br, rosolem@fca.unesp.br

**Abstract** – The objective of this work was to evaluate the effect of organic compounds from plant extracts of six species and phosphate fertilization on soil phosphorus availability. Pots of 30 cm height and 5 cm diameter were filled with Typic Hapludox. Each pot constituted a plot of a completely randomized design, in a 7x2 factorial arrangement, with four replicates. Aqueous extracts of black oat (*Avena strigosa*), radish (*Raphanus sativus*), corn (*Zea mays*), millet (*Pennisetum glaucum*), soybean (*Glycine max*), sorghum (*Sorghum bicolor*), and water, as control, were added in each plot, with or without soluble phosphate fertilization. After seven days of incubation, soil samples were taken from soil layers at various depths, and labile, moderately labile and nonlabile P fractions in the soil were analysed. Plant extracts led to an accumulation of inorganic phosphorus in labile and moderately labile fractions, mainly in the soil surface layer (0–5 cm). Radish, with a higher amount of malic acid and higher P content than other species, was the most efficient in increasing soil P availability.

**Index terms:** *Raphanus sativus*, cover crops, crop rotation, labile phosphorus, low-molecular-weight organic acids, phosphorus fractionation.

## Compostos orgânicos de extratos de plantas e seus efeitos na disponibilidade de fósforo no solo

**Resumo** – O objetivo deste trabalho foi avaliar o efeito de compostos orgânicos de extratos de plantas de seis espécies e da fertilização fosfatada na disponibilidade de fósforo no solo. Tubos de 30 cm de altura e 5 cm de diâmetro foram preenchidos com Latossolo Vermelho-Amarelo. Cada tubo constituiu uma parcela, em delineamento completamente casualizado, em arranjo fatorial 7x2, com quatro repetições. Extratos líquidos de aveia-preta (*Avena strigosa*), nabo forrageiro (*Raphanus sativus*), milho (*Zea mays*), milheto (*Pennisetum glaucum*), soja (*Glycine max*), sorgo forrageiro (*Sorghum bicolor*) e água (testemunha) foram aplicados em cada parcela, com ou sem fertilização com fosfato solúvel. Após sete dias de incubação, amostras de solo foram coletadas em várias profundidades, e foram analisadas as formas lábil, moderadamente lábil e não lábil de fósforo no solo. Houve acúmulo de fósforo inorgânico nas frações lábil e moderadamente lábil do solo, como consequência da adição dos extratos de plantas, principalmente na camada superficial (0–5 cm). O nabo forrageiro, com maior concentração de ácido málico e maior conteúdo de P no tecido do que outras espécies, foi o mais eficiente em incrementar a disponibilidade de P no solo.

**Termos para indexação:** *Raphanus sativus*, culturas de cobertura, rotação de culturas, fósforo lábil, ácido orgânico de baixa massa molecular, fracionamento de fósforo.

### Introduction

Tropical soils are generally very poor in available phosphorus (P), and adequate levels of this plant nutrient are essential for optimal grain yield. Furthermore, P fixation is very high in tropical soils, mainly due to their mineralogy (Novais & Smith, 1999). The presence of organic compounds in rhizosphere may increase or decrease nutrient availability, depending on the characteristics of each organic compound and

their interactions with the soil (Franchini et al., 2003; Moraes et al., 2007).

The study of low-molecular-weight organic acids (LMWOA), and the ability of their carboxylate functional groups to interact with soil, by occupying anion adsorption sites and competing with phosphate, has an expressive importance to increase available P in soils (Guppy et al., 2005). The role of carboxylates, particularly in relation to P availability, has been clearly established in the literature (Guppy et al., 2005); it is

regulated by organic acids concentration, number of carboxylate groups, soil sorption capacity, and persistence in soil, although many of these LMWOA are degraded in hours or days (Stevenson, 1967). In tropical acidic soils, pH change in rhizosphere and LMWOA presence may increase organic P solubility by complexing Fe and Al oxides, besides the competition for adsorption sites promoted by the carboxylate groups (Sposito, 1989; Jones, 1998).

The main source of organic acids in soil is organic matter decomposition, but some root and microbial exudates are also important. Generally, organic acids concentration in soils is very low ( $10^{-3}$  to  $10^{-5}$  mol L<sup>-1</sup>). Conversely, LMWOA concentration can be high during residue decomposition, immediately after its deposition on soil surface (Stevenson, 1967). Otherwise, LMWOA are rapidly degraded or complexed in soil, after residues deposition (hour our days) (Franchini et al., 2003).

Plant species normally present root morphological and physiological processes for adapting to low soil P availability conditions, but the relative importance of these processes may differ among plant species (Gahoonia et al., 2000). Organic acids from plants can increase available P by decreasing adsorption of P and by increasing dissolution of relatively insoluble P compounds (Bolan et al., 1990), including the effect of root and shoot release of LMWOA on break-up of Ca, Fe and Al phosphates in soil.

Information about the action of LMWOA effect in P availability on Brazilian soils is sparse. The main idea to run this kind of experiment was based on works from Franchini et al. (2003) and Amaral et al. (2004), whose research data showed that organic acids promote changes on cations availability in soil. Information about phosphorus is important for elucidating some factors involving plant and soil reactions, which may increase the release and P uptake from less soluble fractions, in soil.

Fractionation of P pools in soil, with various extractors, is an interesting way to investigate its availability and solubility, and to evaluate the effect of treatments over a short time. The P fractionation procedure, developed by Hedley et al. (1982) and adapted by Cross & Schlesinger (1995), has been one of the most useful procedures over the last 15 years; it allows the extraction of both inorganic P (Pi) and organic P (Po) fractions, with labile P being extracted first, and the more resistant forms later (Gatiboni et al., 2007).

Considering that cover crops have different amounts of LMWOA which can be rapidly released to soil, after deposition (Stevenson, 1967; Franchini et al., 2003), the objective of this study was to evaluate the effects of organic compounds, present in plant extracts of six species, and phosphate fertilization on soil phosphorus availability.

## Materials and Methods

The experiment was carried out in greenhouse, in Botucatu, São Paulo, Brazil, in 2005. The plant species used were: black oat (*Avena strigosa*), radish (*Raphanus sativus*), corn (*Zea mays*), millet (*Pennisetum glaucum*), soybean (*Glycine max*), and sorghum (*Sorghum bicolor*), grown for 60 days after germination, when the shoot was collected.

Franchini et al. (2003) reported that the amount of nutrients and organic acids in plant tissue changes with plant age, and higher concentrations are generally found in about 60-day-old plants. Plant shoots, dried in a forced-air oven at 65°C for 72 hours, were weighed and ground to pass through a 0.1 cm sieve, and then analysed for phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg), using wet acid digestion and induced coupled plasma. Nitrogen was analysed using sulfuric acid digestion and distillation by micro-Kjeldahl. Organic carbon (C) was analyzed by Walkley-Black method, as described in Raij et al. (2001). Water pH of the dry matter (1 g dry matter: 10 mL water) was also determined.

Water-extractable LMWOA was determined (Franchini et al., 2003) with some modifications, as described: the extracts were obtained by horizontally shaking of 4 g dry matter (DM) with 100 mL distilled water, at 220 rev min<sup>-1</sup>, for one hour; the solution was filtered using a filter paper; 20 mL of the filtrate were taken in triplicate, acidified with HCl (pH 2.5), and successively extracted (three times) with 75 mL of ethyl acetate, by hand shaking for one minute. The total volume of ethyl acetate (approximately 225 mL) was evaporated under low pressure, and then was reduced to 0.5–1.0 mL. The remaining solution was re-dissolved in H<sub>2</sub>SO<sub>4</sub> 0.005 mol L<sup>-1</sup>. Then, subsamples were filtered using 0.45 µm nitrocellulose membrane, for chromatographic injection. The analysis of LMWOA was carried out by high-performance liquid chromatography (HPLC), using an ion-exchange column (HPX-87H, Bio-rad) with H<sub>2</sub>SO<sub>4</sub> 0.005 mol L<sup>-1</sup>

as mobile phase. The flow rate was 0.6 mL min<sup>-1</sup>, and detection was performed at 210 nm. The identification and quantification of organic compounds were carried out for time retention, and the area of spectra was compared to the one of reference standards.

Pots were made of PVC tubes (5 cm diameter and 30 cm long), with the capacity 0.65 kg of dry soil. The soil chosen for this experiment was a Typic Hapludox (Soil Survey Staff, 1999), with 222 g kg<sup>-1</sup> clay; 40 g kg<sup>-1</sup> silt and 738 g kg<sup>-1</sup> sand, with the following chemical characteristics (Raij et al., 2001): pH CaCl<sub>2</sub>, 5.60; OM, 24.7 g dm<sup>-3</sup>; resin-P, 4.6 mg dm<sup>-3</sup>; with 0.7, 33 and 13.3 mmol<sub>c</sub> dm<sup>-3</sup> K, Ca, and Mg, respectively; and 72% base saturation. The soil was homogeneously compacted inside the tube, to simulate field conditions. A net was fixed at the tube bottom to prevent soil loss. Each pot constituted a plot.

Plant extracts were obtained in a similar way for organic acid analysis, by shaking 4 g DM with 100 mL of distilled water at 220 rev min<sup>-1</sup> for one hour. Then, the solutions were filtered using a filter paper. The treatments consisted of plant filtered extracts application, equivalent to 20 Mg ha<sup>-1</sup> of dry matter, which corresponded to 87 mL of extract in each plot. It is important to emphasize that this amount is similar to the expected one to be released by harvested and cover crops in a two-year crop rotation, but in the present work the extracts were applied in a few minutes, in order to maximize the possible effects of LMWOA in soil. The amount applied was much higher than that from residues decomposition, but this amount was adopted based on the possibility to rapidly measure the effects, and based on literature research about nutrient availability under plant residues deposition (Franchini et al., 2003; Amaral et al., 2004; Moraes et al., 2007).

The treatments used were: black oat, millet, corn, radish, soybean, sorghum, and distilled water, as control, with the chemical characteristics described in

Table 1. These seven treatments were conducted with and without phosphate fertilization using, as P source, simple superphosphate (100 kg ha<sup>-1</sup> of P) applied in soil surface, immediately before plant extracts application, totalizing fourteen treatments. Soil columns were previously wet and soil humidity was kept close to water field capacity, throughout the seven-day experiment. Plastic film inside the PVC tube was used for a better contact between soil and tube, and to avoid preferential water flux through the internal surface of the column. The 87 mL of the extract applied to each plot contained nutrients in the quantities shown in Table 1. Temperature inside the greenhouse, during the seven-day experiment, was from 22 to 32°C.

After one hour of plant extracts application, the solution that percolated through the soil column was collected. The amount percolated was approximately 80 mL in each column, almost all the extract volume added. A similar collect was repeated on the seventh day after extract application. By this time, 50 mL of distilled water was applied, and approximately 40 mL of the percolated solution was collected in each column. The solutions were analyzed for P, K, Ca, and Mg, by inductive couple plasma. On the seventh day, the columns were destroyed and the soil samples were taken in the layers of 0–5, 5–10, 10–15 e 15–30 cm. Soil samples were oven-dried at 65°C for 48 hours, ground, and sieved (0.2 cm).

Phosphorus pools in soil were determined in soil samples, using P fractionation (Hedley et al., 1982), with some modifications (Cross & Schlesinger, 1995) as follows: sequential extraction by anion-exchange resin, 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub>, 0.1 mol L<sup>-1</sup> NaOH, 1 mol L<sup>-1</sup> HCl, 0.5 mol L<sup>-1</sup> NaOH and residual digestion with HCl + HNO<sub>3</sub> + H<sub>2</sub>SO<sub>4</sub>. Inorganic and residual P fractions were analyzed using blue molybdate-ascorbic acid (Murphy & Riley, 1962). The organic fractions extracted by 0.5 mol L<sup>-1</sup> NaHCO<sub>3</sub>, 0.1 mol L<sup>-1</sup> NaOH and 0.5 mol L<sup>-1</sup>

**Table 1.** Chemical characteristics of plant dry matter and total amount of nutrients applied in each soil column (plot) via plant extracts.

Plant species	pH H <sub>2</sub> O (1:10)	C	N	P	K	Ca	Mg	P	K	Ca	Mg	
		-----Element contents in dry matter (g kg <sup>-1</sup> )-----						-----Amount applied (mg plot <sup>-1</sup> )-----				
Black oat	6.05	429	15.7	1.3	20.3	3.9	1.5	1.5	76.6	7.0	4.5	
Millet	5.82	411	22.5	1.5	22.7	6.3	3.5	2.9	80.8	8.0	10.6	
Corn	5.72	416	15.8	0.8	19.2	2.9	2.2	1.9	62.4	5.9	6.9	
Radish	5.81	377	26.0	2.2	30.5	22.1	3.9	5.9	108.3	31.5	12.8	
Soybean	6.31	418	41.3	2.7	25.9	21.0	4.2	4.6	77.3	21.3	11.2	
Sorghum	5.78	422	16.6	1.1	19.8	7.1	3.0	1.0	64.5	11.1	9.4	

NaOH were determined by digestion with ammonium persulfate and sulfuric acid, in autoclave at 120°C, for two hours. And then, the organic P was measured in the same way as described in case of inorganic P.

The experimental design was a 7x2 factorial with 4 replicates. ANOVA was carried out using SAS 8.2 (SAS Institute, 2001), and means were compared using the t test, at 5% probability.

## Results and Discussion

Each plant species had a different nutrient content in shoot dry matter (Table 1). Soybean and radish presented higher contents of N, P, K, and Ca. Consequently, these plant species were able to return a higher amount of these nutrients to soil, when their extracts were applied, leading to a higher nutrient availability. The maximum amount of P applied via plant extract (radish) was 5.9 mg per soil column. The amounts of K were high in all the plant extracts, and the amounts of Ca and Mg added via extracts were considered low, compared to the original soil contents.

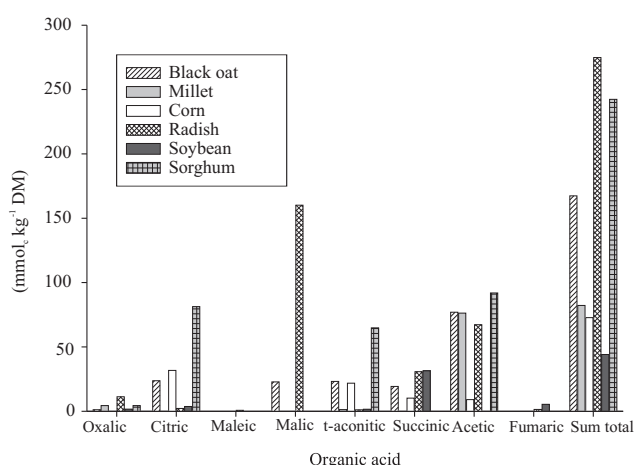
There was a large variation in organic acid contents among species, and some acids were very specific to each plant species (Figure 1). Radish had the highest quantity of malic and oxalic acids, which explain the highest quantity of total accumulated LMWOA of 275 mmol kg<sup>-1</sup> of DM. Soybean presented the lowest content of LMWOA, with a total accumulated of 44 mmol kg<sup>-1</sup>, with predominance of succinic acid. In all crops, citric, t-aconitic, and acetic acids were found predominantly in black oat, millet, corn, and sorghum. Accordingly, radish extract could play an important role in increasing soil nutrient availability, since this crop normally presents high amount of malic acid and other LMWOA in plant tissue (Franchini et al., 2003; Moraes et al., 2007). In a similar study, Amaral et al. (2004) observed that citric and malic acids are predominant in radish tissue.

The type and amount of carboxylates from organic compounds produced or accumulated by plants are responsible for different interactions with soil nutrients, promoting adsorption and complexation reactions (Jones, 1998; Guppy et al., 2005). Monocarboxylic organic acids, such as acetic acid, have a limited effect in competing with ions for adsorption sites in soil. Conversely, malic and citric acids, which present two and three carboxylates, respectively, plus additional

hydroxyl groups, are stronger competitors for soil adsorption sites (Guppy et al., 2005). Otherwise, some LMWOA may promote changes in soil physical properties, like penetration resistance and water disperse clay content, as mentioned by Correa et al. (2008), who evaluated the application of citric and oxalic acids in soil.

Despite the high amounts of LMWOA applied through plant extracts, they were not detected in solution percolated through the soil columns, analysed by HPLC, possibly due to a rapid interaction with soil colloids, retention in soil functional groups (clay and oxides), or degradation by microorganisms in soil (van Hees et al., 2005; Moraes et al., 2007). Similar results were found by Amaral et al. (2004), after the application of black oat and radish residues in soil. However, Pires et al. (2007) found a reasonable quantity of organic acids in the leachates, but only when the rhizosphere zone solution was extracted from the soil, which revealed the presence of acetic, lactic and oxalic acids and trace concentrations of butyric, citric, fumaric, gluconic, malic, succinic, and tartaric acids.

The nutrient percolations were very low compared with the amounts applied, and there was no effect of P fertilization on nutrients percolation from plant extracts through the soil columns, in both (0 and 7 days) solution samplings (Tables 2 and 3). One hour after extract application, P, K, and Ca percolation through soil columns seemed to be inhibited by the presence of plant extracts, since the leached nutrient content was higher in water control than in treatments (Table 2).



**Figure 1.** Low-molecular-weight organic acid (LMWOA) present in the dry matter of plant species, and detected by HPLC.

Seven days after the application, the amount of nutrients percolated through the soil columns showed a large variation (Table 3), some plant extracts seemed to inhibit, whereas others accelerated nutrient percolation in soil. Millet and radish were the most effective species in reducing P concentration in the leached solution, in both samplings (0 and 7 days), possibly due to complexation between organic compounds from plant residues and phosphate, in soil profile (Gahoonia et al., 2000).

Although phosphorus was the focus of the work, some important cations were also evaluated in percolated solution. Potassium, calcium, and magnesium concentrations in leached solution were considerably increased after seven days, for all plant extracts (Table 3), when compared to the solution collected one hour after application. This increment was attributed to the amount of K, Ca, and Mg applied via plant extract (Table 1), which, after seven days, were available in soil solution and were susceptible to leaching. Otherwise, interactions among organic carboxylates and soil cations could favour ion binding to soil colloids, which could be a consequence of reduced leaching in some plant species.

The extracts of all species led to an increase in resin-extractable P in the 0–5-cm layer, with radish and soybean showing the highest increase (Figure 2 A).

The amount of P present in radish and soybean extracts (Table 1) contributed to a large extent for the increment observed in available P in soil, since 5.9 and 4.6 mg of P were applied to those columns, respectively. If most of this P remain in the surface layer, the increment in available P could be significant. Following this idea, literature information shows that high amounts of malic acid and total LMWOA present in plant extracts affect P availability, reacting and promoting complex formation between phosphate and organic compounds in soil (Gahoonia et al., 2000).

In general, grass species presented lesser potential to make P available than soybean and radish, in surface layer 0–5 cm (Figure 2 A). However, in deeper layers, there was no effect of plant extracts on P availability. When phosphate fertilizer was applied, plant extracts presented potential to immobilize P, reducing its availability, when compared to control treatment, possibly by a complexation between carboxylates and phosphate (Figure 2 B) mentioned in literature (Gahoonia et al., 2000). This complexation was more evident in the 0–5 and 5–10-cm soil layers, highlighted when grass extracts were used.

The inorganic P extracted by bicarbonate ( $0.5 \text{ mol L}^{-1} \text{ NaHCO}_3$ ) was increased in all soil depths with all extracts, but the increase was significant only in the 0–5 and 5–10-cm layers (Figure 2 C), similarly

**Table 2.** Nutrients percolated in soil solution, collected one hour after plant extracts application.

Plant extract	P ( $\mu\text{g L}^{-1}$ )	K ----- ( $\text{mg L}^{-1}$ )	Ca ----- ( $\text{mg L}^{-1}$ )	Mg ----- ( $\text{mg L}^{-1}$ )
Without P application				
Black oat	0.22	10.1	18.5	15.8
Millet	0.12	9.9	19.3	15.9
Corn	0.18	9.5	18.0	14.5
Radish	0.13	9.3	19.4	15.8
Soybean	0.12	9.5	18.5	14.7
Sorghum	0.31	10.0	19.4	16.1
Water	0.64	21.8	47.2	16.0
LSD <sup>(1)</sup>	0.24	2.0	2.4	ns
With application of $100 \text{ kg ha}^{-1}$ of P				
Black oat	0.25	9.3	18.5	15.4
Millet	0.14	9.6	19.7	16.5
Corn	0.10	9.4	19.6	16.4
Radish	0.20	9.3	18.9	15.8
Soybean	0.13	10.6	19.1	15.7
Sorghum	0.14	9.1	19.4	15.3
Water	0.57	20.8	44.5	15.3
LSD	0.12	1.9	5.1	ns

<sup>(1)</sup>LSD: least-significance difference, by Student's test, at 5% of probability.

<sup>ns</sup>Nonsignificant.

**Table 3.** Nutrients percolated in soil solution, collected seven days after plant extracts application.

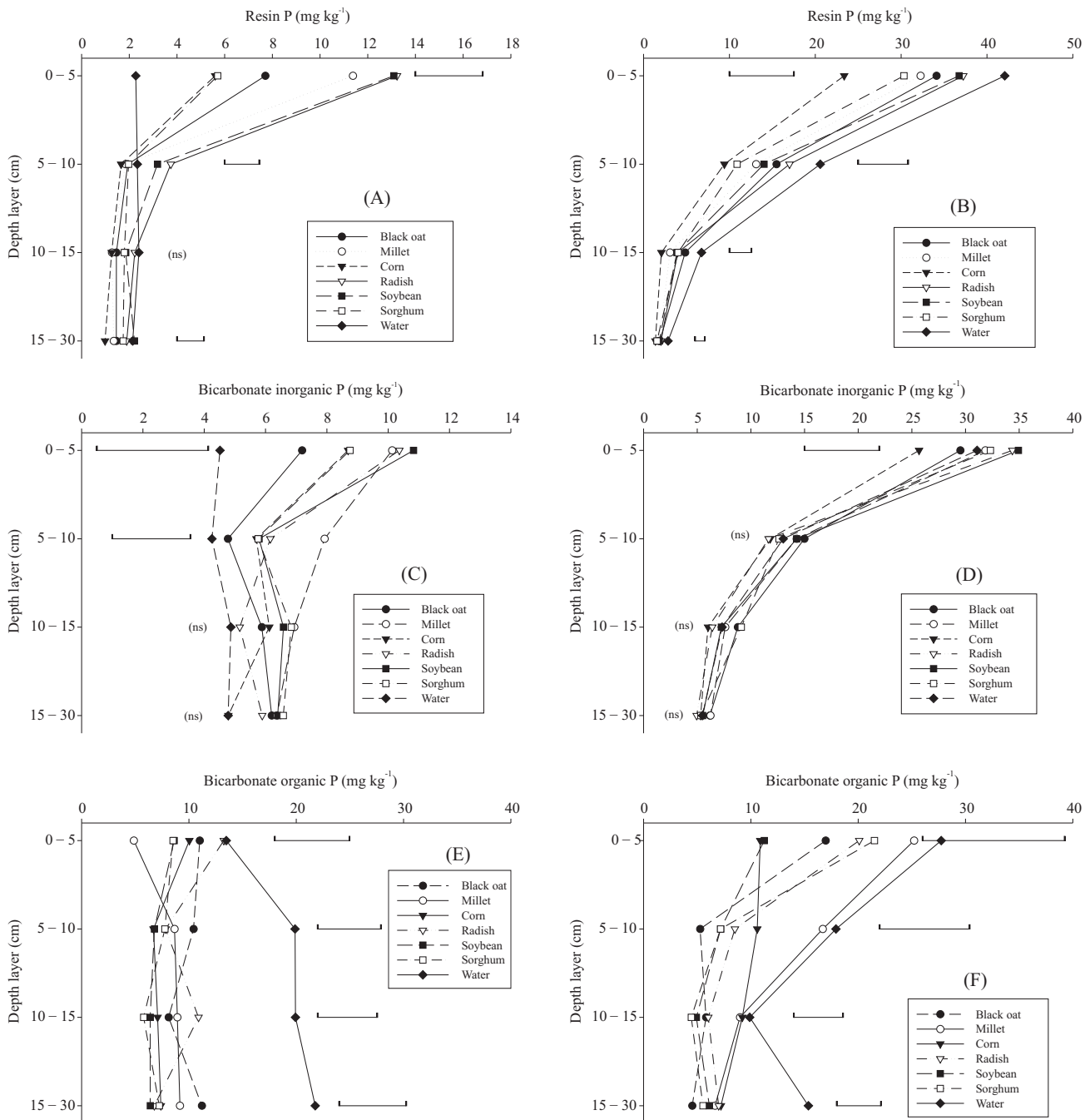
Plant extract	P ( $\mu\text{g L}^{-1}$ )	K ----- ( $\text{mg L}^{-1}$ )	Ca ----- ( $\text{mg L}^{-1}$ )	Mg ----- ( $\text{mg L}^{-1}$ )
Without P application				
Black oat	0.25	24.0	56.8	52.0
Millet	0.14	16.4	45.7	43.2
Corn	0.27	25.8	63.1	52.3
Radish	0.15	20.9	47.1	42.3
Soybean	0.30	16.6	43.8	44.1
Sorghum	0.16	17.8	39.6	36.2
Water	0.26	26.8	41.9	15.7
LSD <sup>(1)</sup>	ns	5.1	12.8	15.5
With application of $100 \text{ kg ha}^{-1}$ of P				
Black oat	0.22	22.0	52.6	48.3
Millet	0.13	18.2	39.0	33.2
Corn	0.25	19.6	58.0	48.2
Radish	0.16	26.6	61.8	57.9
Soybean	0.39	20.8	50.5	47.6
Sorghum	0.25	25.0	54.7	51.2
Water	0.16	23.2	37.9	14.5
LSD	ns	4.4	14.0	13.6

<sup>(1)</sup>LSD: least-significance difference, by Student's test, at 5% of probability.

<sup>ns</sup>Nonsignificant.

to the resin fraction. The higher increases in surface layers were obtained with millet, radish, and soybean extracts. When phosphate fertilizer was applied, the

amount of inorganic P in bicarbonate fraction increased substantially (Figure 2 D). The organic P fraction extracted by bicarbonate was lower in plant extracts

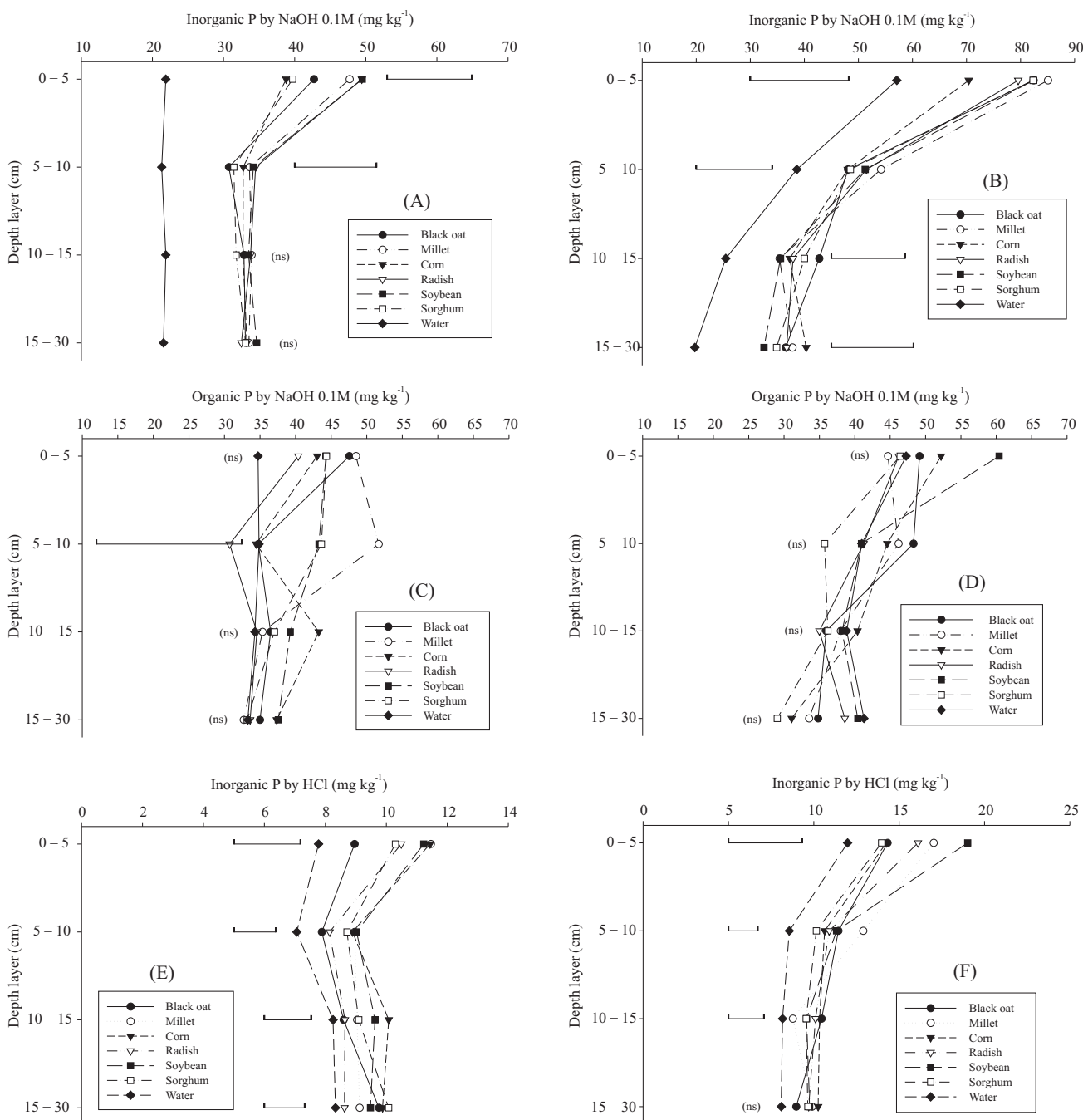


**Figure 2.** Fractions of labile P, extracted by resin (A and B), bicarbonate inorganic (C and D) and bicarbonate organic (E and F). A, C and E, without fertilizer; B, D and F, with 100 kg ha<sup>-1</sup> of P. Bars: least significant difference, at 5% of probability. <sup>ns</sup>Nonsignificant.

than in control, regardless of phosphate fertilization (Figure 2 E and F).

Plant extracts increased inorganic P extracted by 0.1 mol L<sup>-1</sup> NaOH at all depth layers (Figure 3 A), with

higher values in the surface layer 0–5 cm for millet, soybean and radish, which may also be attributed to the amount added in plant extract, since phosphorus is a component of organic compounds in plant



**Figure 3.** Fractions of moderately labile P, extracted by NaOH 0.1 mol L<sup>-1</sup>, inorganic (A and B) and organic (C and D), and nonlabile P extracted by HCl (E and F). A, C and E, without fertilizer; B, D and F, with 100 kg ha<sup>-1</sup> of P. Bars: least significant difference, at 5% of probability. <sup>ns</sup>Non-significant.



tissue. Taking into account all the previous fractions, plant extracts seem to be acting as chelating agents, maintaining P in forms less available in soil. However, this P cannot be considered unavailable over a long term, as it can become available as soon as the solution P is depleted (Novais & Smyth, 1999; Gahoonia et al., 2000). When phosphate fertilizer was applied, the amount of inorganic P in this fraction was significantly increased, in all soil layers (Figure 3 B), which indicates that the total P applied via fertilizer does not remain only in labile forms in soil, and may also be part of the moderately available fraction.

Organic P extracted by 0.1 mol L<sup>-1</sup> NaOH allowed no conclusive discussion (Figure 3 C and D). Similar results for both organic and inorganic P extracted by 0.1 mol L<sup>-1</sup> NaOH were obtained by Gatiboni (2003) and Gatiboni et al. (2007), in clayey soils from Southern Brazil. Organic compounds present in plant extracts reduced the organic available P, extracted by bicarbonate, possibly by complexation of phosphate or by formation of organo-metallic bonds (Gahoonia et al., 2000; Guppy et al., 2005). Other experiments showed that P applied as fertilizer or from plant and animal manure normally promotes accumulation of P in inorganic fractions in soil, with small or no changes in organic P availability (Friesen et al., 1997; Gatiboni, 2003).

The fraction of inorganic P extracted by 1 mol L<sup>-1</sup> HCl was affected by plant extracts in almost all soil layers, which promoted higher values than the control, with small differences between plant species (Figure 3 E). When fertilizer was applied, significant increment in P extracted by HCl was observed in almost all soil layers, similarly as without fertilizer, although the fertilizer promoted increments in this P fraction only at 0–5-cm layer (Figure 3 F).

The extraction of inorganic P by 0.5 mol L<sup>-1</sup> NaOH was similar to those obtained for 0.1 mol L<sup>-1</sup> NaOH, with higher values in the presence of plant extracts, pointing to a possible P immobilization of available fractions, as a result of extracts applied, although most data were not significant (Figure 4 A and B). The organic P fraction presented a wide variation among treatments and depths, turning difficult any clear conclusion (Figure 4 C and D). No effect of phosphate fertilizer was observed on P fractions extracted by NaOH 0.5 mol L<sup>-1</sup>, which followed the same behaviour observed in data presented by Gatiboni et al. (2007).

Residual P fraction remained stable with the application of plant extracts and fertilizer (Figure 4 E and F). No significant difference was observed between plant extracts, although it was possible to detect some variations in soil profile. The residual fraction accounted for approximately 60% of total P present in soil; similar results were obtained by Gatiboni et al. (2007). These authors studied a clayey soil in Southern Brazil, and mentioned that although the residual amount was high, even this fraction may be depleted after successive cultivated crops without fertilizer, which shows that residual P can become available when a long time frame is considered.

Considering all data from P fractionation, there was an increase in labile pool, which could be a consequence of the quantity added via plant extract or of the interaction between organic compounds and soil, being more expressive with soybean and radish extracts. However, when inorganic P was applied, in the form of fertilizer, plant extracts seemed to immobilize P, complexing it with organic compounds and retaining it in less available forms (Gahoonia et al., 2000). Although the inorganic labile P was increased by application of plant extracts, the highest increase was observed in inorganic moderately labile P. Considering these labile and moderately labile fractions together, it seems that plant extracts made P less available immediately after addition to the soil but, possibly, it may turn available later, as soil/solution reactions occur. The increase in moderately labile P pool could be considered a positive aspect in a long term, because, supposedly, as soon as the available pool is depleted, the less available pool can become available.

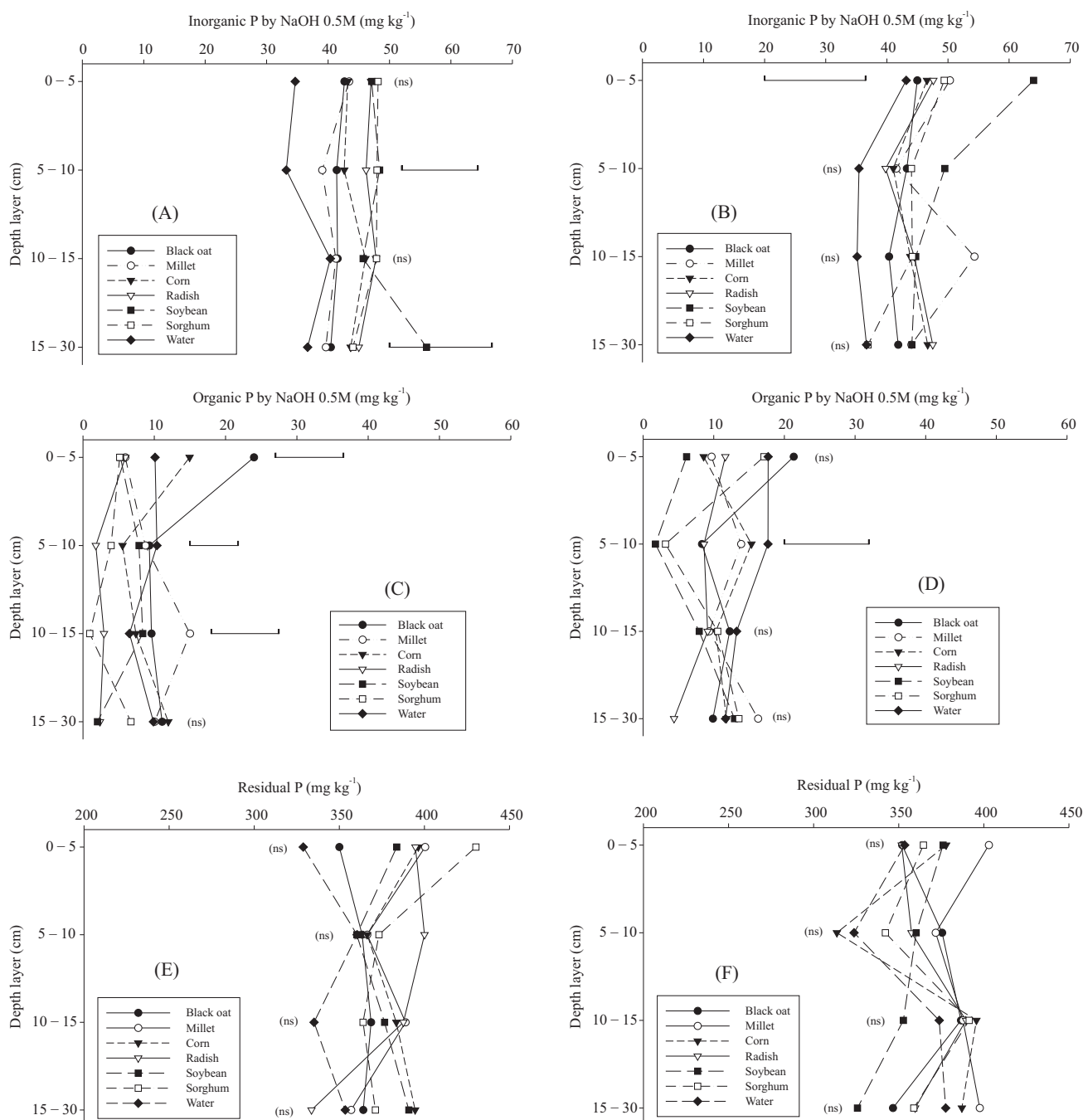
Evaluating all data from Hedley fractionation, it is possible to infer that the addition of plant extracts increased most of the inorganic soil P fractions, without significant changes in organic fractions. However, it is important to emphasize that the amount of plant extract applied was much higher than what normally occurs in cultivated areas. The observed changes were more evident in the surface layer 0–5 cm, which may be in part attributed to the amount applied via plant extracts. Small changes were also observed in deeper layers, such as 5–10 and 10–15 cm.

Many studies have demonstrated the effect of LMWOA on the availability of soil nutrient (Franchini et al., 2003; Amaral et al., 2004; Moraes et al., 2007). However, in most of them, high rates of LMWOA



and high rates of fertilizers were used, which is not observed in farms. In the present study, the amount of plant extracts applied were equivalent to a two-year crop rotation (about 20 Mg ha<sup>-1</sup> of DM), but the extracts were applied in a short time in soil columns.

This fact makes the extrapolation to field conditions difficult, since in these conditions soil reaction and microbial activity are slower, and the results would possibly be different from the obtained in greenhouse pots.



**Figure 4.** Fractions of nonlabile P, extracted by NaOH 0.5 mol L<sup>-1</sup>, inorganic (A and B) and organic (C and D), and residual P (E and F). A, C and E, without fertilizer; B, D and F, with 100 kg ha<sup>-1</sup> of P. Bars: least significant difference, at 5% of probability. <sup>ns</sup>Nonsignificant.

## Conclusions

1. Plant extracts lead to an accumulation of inorganic phosphorus in labile and moderately labile fractions, mainly in the soil surface layer 0–5 cm.

2. Among the plant species used, radish and soybean are the most efficient in increasing available phosphorus fractions in soil.

3. The labile and moderately labile inorganic phosphorus fractions are increased by phosphate fertilization down to 10–15-cm soil layer.

## Acknowledgements

To Fundação de Amparo à Pesquisa do Estado de São Paulo, for the financial support.

## References

- AMARAL, A.S.; ANGHINONI, I.; DESCHAMPS, F.C. Resíduos de plantas de cobertura e mobilidade dos produtos da dissolução do calcário aplicado na superfície do solo. **Revista Brasileira de Ciência do Solo**, v.28, p.115-123, 2004.
- BOLAN, N.S.; WHITE, R.E.; HEDLEY, M.J. A review of the use of phosphate rocks as fertilizers for direct application in Australia and New Zealand. **Australian Journal of Experimental Agriculture**, v.30, p.297-313, 1990.
- CORREA, M.M.; ANDRADE, F.V. de; MENDONÇA, E. de S.; SCHAEFER, C.E.G.R.; PEREIRA, T.T.C.; ALMEIDA, C.C. Ácidos orgânicos de baixo peso molecular e ácidos húmicos e alterações em algumas propriedades físicas e químicas de Latossolos, Plintossolo e Neossolo Quartzarênico. **Revista Brasileira de Ciência do Solo**, v.32, p.121-131, 2008.
- CROSS, A.F.; SCHLESINGER, W.H. A literature review and evaluation of the Hedley fractionation: applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. **Geoderma**, v.64, p.197-214, 1995.
- EMBRAPA. **Sistema brasileiro de classificação de solos**. Brasília: Embrapa Produção da Informação; Rio de Janeiro: Embrapa Solos, 1999. 412p.
- FRANCHINI, J.C.; HOFFMANN-CAMPO, C.B.; TORRES, E.; MIYAZAWA, M.; PAVAN, M.A. Organic composition of green manure during growth and its effect on cation mobilization in an acid Oxisol. **Communications in Soil Science and Plant Analysis**, v.34, p.2045-2058, 2003.
- FRIESEN, D.K.; RAO, I.M.; THOMAS, R.J.; OBERSON, A.; SANZ, J.I. Phosphorus acquisition and cycling in crop and pasture systems in low fertility tropical soils. **Plant and Soil**, v.196, p.289-294, 1997.
- GAHOONIA, T.S.; ASMAR, F.; GIESE, H.; GISSEL-NIELSEN, G.; NIELSEN, N.E. Root-released organic acids and phosphorus uptake of two barley cultivars in laboratory and field experiments. **European Journal of Agronomy**, v.12, p.281-289, 2000.
- GATIBONI, L.C. **Disponibilidade de formas de fósforo do solo às plantas**. 2003. 231p. Tese (Doutorado) - Universidade Federal de Santa Maria, Santa Maria.
- GATIBONI, L.C.; KAMINSKI, J.; RHEINHEIMER, D. dos S.; FLORES, J.P.C. Biodisponibilidade de formas de fósforo acumuladas em solo sob sistema plantio direto. **Revista Brasileira de Ciência do Solo**, v.31, p.691-699, 2007.
- GUPPY, C.N.; MENZIES, N.W.; MOODY, P.W.; BLAMEY, F.P.C. Competitive sorption reactions between phosphorus and organic matter in soil: a review. **Australian Journal of Soil Research**, v.43, p.189-202, 2005.
- HEDLEY, M.J.; STEWART, J.W.B.; CHAUHAN, B.S. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. **Soil Science Society of America Journal**, v.46, p.970-976, 1982.
- JONES, D.L. Organic acids in the rhizosphere: a critical review. **Plant and Soil**, v.205, p.25-44, 1998.
- MORAES, M.F.; CANTARELLA, H.; QUAGGIO, J.A.; COSCIONE, A.R. Mobilidade de íons em solo ácido com aplicação de calcário, ácido orgânico e material vegetal em superfície. **Revista Brasileira de Ciência do Solo**, v.31, p.673-684, 2007.
- MURPHY, J.; RILEY, J.P. A modified single solution method for the determination of phosphate in natural waters. **Analytica Chimica Acta**, v.27, p.31-36, 1962.
- NOVAIS, R.F.; SMYTH, T.J. **Fósforo em solo e planta em condições tropicais**. Viçosa: UFV, 1999. 399p.
- PIRES, A.M.M.; MARCHI, G.; MATTIAZZO, M.E.; GUILHERME, R.L.G. Organic acids in the rhizosphere and phytoavailability of sewage sludge-borne trace elements. **Pesquisa Agropecuária Brasileira**, v.42, p.917-924, 2007.
- RAIJ, B. van; ANDRADE, J.C.; CANTARELLA, H.; QUAGGIO, J.A. **Análise química para avaliação da fertilidade de solos tropicais**. Campinas: Instituto Agrônomo, 2001. 285p.
- SAS INSTITUTE. **SAS user's guide: statistics**, ver. 8.2. Cary: SAS Institute, 2001.
- SOIL SURVEY STAFF. **Soil taxonomy: a basic system of soil classification for making and interpreting soil surveys**. 2<sup>nd</sup> ed. Pittsburgh: USDA, 1999. 871p.
- SPOSITO, G. **The chemistry of soils**. New York: Oxford University Press, 1989. 304p.
- STEVENSON, F.J. Organic acids in soil. In: MCLAREN, A.D.; PETERSON, G.H. (Ed.). **Soil biochemistry**. New York: Marcel Decker, 1967. p.119-146.
- VAN HEES, P.A.W.; JONES, D.L.; NYBERG, L.; HOLMSTRÖM, S.J.M.; GODBOLD, D.L.; LUNDSTRÖM, U.S. Modelling low molecular weight organic acid dynamics in forest soils. **Soil Biology and Biochemistry**, v.37, p.517-531, 2005.

Received on May 19, 2008 and accepted on September 12, 2008