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Soil Phosphorus Fractions in a Sandy Typic Hapludaft as Affected by Phosphorus Fertilization and Grapevine Cultivation Period

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The purpose of this study was to quantify the phosphorus (P) fractions in phosphate-fertilized sandy soils under grapevines. Soil was sampled from a grassland site and two vineyards (13 and 31 years old) in the State Rio Grande do Sul, Brazil, from the layers 0–5, 5–10, 10–15, 15–20, and 20–40 cm deep. The samples were prepared and P fractions were assessed by chemical fractionation. Phosphate fertilization of the vineyard soils increased P contents to a depth of 40 cm, especially in the more recalcitrant fractions but detectable in the more labile fractions as well, which are plant available and may increase the risk of environmental contamination. Phosphate fertilizers and the period of grapevine cultivation had little effect on the levels of organic P forms, whereas cover crops in vineyards could be an appropriate strategy to increase or maintain the levels of soil organic matter and the levels of organic P over the years.

Keywords Chemical phosphorus fractionation, inorganic phosphorus, organic phosphorus, *Vitis vinifera*

Introduction

In Brazil, the largest grape-producing area is in the State of Rio Grande do Sul (RS), which borders on Uruguay and Argentina, where grapevines are grown on approximately 31,000 ha (Lessa 2010) mainly in the Serra Gaúcha, a region in the northeast of the

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state. However, in view of the more suitable climatic conditions, grassland areas of the Campanha Gaúcha in the southeastern part of RS, formerly used for extensive grazing, were incorporated into the grape production system. Soils in this region are acidic and sandy with low fertility. Thus, prior to planting vineyards and when required over the years, soils are limed to raise the water pH to 6.5, and phosphate and potassium (K) fertilizers are applied to increase the soil levels of these nutrients. However, most grape producers do not use soil analysis and/or tissue analysis to determine the nutrient need and rate of the crops. Thus, phosphorus (P) accumulation in the surface layers and its redistribution in the soil profile, when the P adsorption sites of reactive soil particles become saturated, are to be expected. Consequently, there may be changes in the soil P fractions (Selles et al. 1997) that may affect the P availability to the grapevines and pose a risk of environmental contamination.

Soil P accumulation and fractions have been studied based on chemical fractionation as proposed by Hedley, Stewart, and Chauhan (1982). This technique uses chemical extractants that remove inorganic P (Pi) and organic P (Po) from the most available fractions with low binding energy to the most stable fractions with high binding energy. Anion exchange resin extracts labile Pi fractions, and 0.5 M sodium bicarbonate (NaHCO_3) extracts the labile Pi and Po fractions. Phosphorus extracted by these two extractants can directly contribute to the P supply to plants (Cross and Schlesinger 1995; Gatiboni et al. 2007). Phosphorus extracted by 0.1 M sodium hydroxide (NaOH) and 0.5 M NaOH corresponds to Pi bound to oxides and silicate clays with intermediate binding energy and Po of moderately labile fractions, which are only taken up by plants when little labile P is available (Cross and Schlesinger 1995; Guo and Yost 1998; Gatiboni et al. 2007). Phosphorus extracted by 1.0 M hydrogen chloride (HCl) corresponds to Pi contained in calcium phosphate and finally soil digestion with sulfuric acid (H_2SO_4) + hydrogen peroxide (H_2O_2) + magnesium chloride ($\text{MgCl}_2 \cdot 0$) extracts soil residual Pi and Po, called recalcitrant P, which can contribute to plant nutrition when soil P is extremely deficient (Guo and Yost 1998; Gatiboni et al. 2005).

The chemical fractionation showed that by successive phosphate fertilizer applications, especially to soils with annual crops, Pi and Po forms are accumulated in the soil with different degrees of binding energy, but generally in inorganic forms (Daroub, Pierce, and Ellis 2000; Conte, Anghinoni, and Rheinheimer 2003; Herlihy and McGrath 2007; Santos et al. 2008; Pavinato, Merlin, and Rosolen 2009; Linqvist, Ruark, and Hill 2010). The initially applied P is adsorbed at the most avid sites with higher binding energy and subsequently the remaining P is redistributed in lower energy fractions (Rheinheimer and Anghinoni 2001). The expected trend is therefore that as the amount of phosphate fertilizer applied is greater, the content of bioavailable P becomes greater because of the saturation of higher binding-energy sites. Thus, P applications over years can increase the amount of soluble P by increasing its availability to plants, because soluble or more labile fractions restore P in the soil solution (Gatiboni et al. 2007). On the other hand, this also increases the transfer of P to surface water sources and/or subsurface waters (Graetz and Nair 1995; Bergström and Kirchmann 2006; Gatiboni et al. 2008; Ceretta et al. 2010; Pingjin et al. 2010). Therefore, the purpose of this study was to quantify the P fractions accumulated in sandy soils of phosphate-fertilized vineyards in southern Brazil. The P distribution on the adsorption sites of reactive soil particles can be used to estimate the bioavailability of P to grapevines or to predict the contamination risk for the environment, especially for phosphate-fertilized sandy soils as in the case of sandy vineyard soils, on which studies of this nature are scarce or nonexistent.

Material and Methods

Study Area

The study was conducted in Santana do Livramento, in the region of Campanha Gaúcha, in the southeast of Rio Grande do Sul, southern Brazil. Two vineyards of different ages (vineyards 1 and 2) with a distinct history of phosphate fertilization (basically simple superphosphate) were studied, and a grassland area with no history of phosphate fertilization was used as reference. The soil was classified as Sandy Typic Hapludalf (Soil Survey Staff 1999).

In vineyard 1, cultivar Cabernet Franc, grafted onto rootstock SO₄, was planted in 1996 (latitude 30° 46' 58" S, longitude 55° 23' 12" W) at a density of 2875 plants per hectare (3.5 m × 1.0 m, in an espalier system. In vineyard 2, the same cultivar/rootstock combination was established in 1978 (latitude 30° 47' 22" S, longitude 55° 22' 22" W) at a density of 1429 plants per hectare (3.5 m × 2.0 m) in the same system. To the soil surface of both vineyards lime was applied and incorporated to raise the water pH to 6.5 before planting the grapevines. In addition, before planting the grapevines in vineyards 1 and 2, approximately 39.7 kg P ha⁻¹ and 145.8 kg K ha⁻¹ were incorporated into the soil. After planting, fertilizer was surface applied in both vineyards, but without incorporation. Table 1 shows the amounts of nitrogen (N), P, and K fertilizer sources applied as of 1993, because no fertilization records of the previous years in either vineyard were available. From the beginning, the soil was always covered by planted or spontaneous vegetation within and between the grapevine rows of both vineyards. *Lolium perenne*, *Paspalum notatum*, and *Trifolium repens* were predominant in this vegetation cover, which was cut three times a year and left on the soil surface within and between the plant rows. On the nearby grassland site (latitude 30° 47' 39" S, longitude 55° 22' 14" W), which had never been P fertilized, *Paspalum notatum*, *Paspalum plicatum*, *E. ciliatum*, and *Stylosanthes montevidensis* were predominant. The top growth of the species historically was not cut and the topography of the location was flat. The results of P fractions obtained in the layers of the grassland site were used as a reference throughout the study.

Soil Sampling, Chemical Analysis, and Chemical Phosphorus Fractionation

In April 2009, 13 years after the establishment of vineyard 1 and 31 years after planting vineyard 2, five trenches were randomly dug in each vineyard, beside six rows, where the greatest amount of P fertilizer had been applied over the years. There, soil was sampled from the layers 0–5, 5–10, 10–15, 15–20, and 20–40 cm deep. In the grassland, five trenches were also randomly dug and soil was sampled from the same layers. This material was air dried and sieved (2-mm mesh), and remaining plant residues were removed manually. Subsequently, the soil was ground in a mortar, sieved (1-mm mesh), and stored for analysis.

Soil texture was determined by the pipette method (Embrapa 1997). The water pH values (1:1), levels of exchangeable calcium (Ca) and magnesium (Mg) [by 1 M potassium chloride (KCl)], organic-matter content, and available P (by Mehlich 1) and available K (by Mehlich 1) were determined according to the methodology described by Tedesco et al. (1995) (Table 2). Hydrogen (H)⁺ aluminum (Al) was calculated using the equation adopted by CQFS-RS/SC (2004):

Table 1

Phosphorus, potassium, and nitrogen applied in Sandy Typic Hapludalf soil of vineyard 1 (Cabernet Franc, 13 years of age) and vineyard 2 (Cabernet Franc, 31 years of age)

Year of application	Source	Vineyard 1 (kg ha ⁻¹)			Vineyard 2 (kg ha ⁻¹)		
		N	P	K	N	P	K
1993	Urea	—	—	—	34.3	—	—
	Simple superphosphate	—	—	—	—	8.3	—
	5–20–20 NPK formula	—	—	—	4.0	7.9	8.8
1994	Urea	—	—	—	77.7	—	—
	Simple superphosphate	—	—	—	—	30.2	—
	Potassium chloride	—	—	—	—	—	20.5
1995	Urea	—	—	—	81.1	—	—
	Simple superphosphate	—	—	—	—	18.3	—
	Potassium chloride	—	—	—	—	—	14.6
	5–20–20 NPK formula	—	—	—	6.4	12.7	14.1
1996	Urea	69.3	—	—	70.5	—	—
	Triple superphosphate	—	—	—	—	19.6	—
	Potassium chloride	—	—	—	—	—	20.9
1997	Urea	38.4	—	—	67.4	—	—
	Potassium chloride	—	—	15.9	—	—	20.9
1998	Urea	77.0	—	—	64.9	—	—
	Potassium chloride	—	—	15.9	—	—	—
1999	Urea	81.4	—	—	82.3	—	—
	Potassium chloride	—	—	39.8	—	—	22.2
2000	Urea	98.5	—	—	62.0	—	—
2001	Urea	55.3	—	—	55.1	—	—
	Triple superphosphate	—	4.3	—	—	—	—
	Potassium chloride	—	—	47.8	—	—	20.9
2002	Urea	48.4	—	—	48.2	—	—
	Triple superphosphate	—	13.3	—	—	29.5	—
	Potassium chloride	—	—	43.9	—	—	41.4
2003	Urea	66.0	—	—	26.7	—	—
	Triple superphosphate	—	7.7	—	—	17.9	—
	Potassium chloride	—	—	—	—	—	30.2
2004	Urea	37.8	—	—	24.1	—	—
	Triple superphosphate	—	7.4	—	—	10.0	—
	Sodium chlodium	—	—	4.0	—	—	—
2006	Urea	—	—	—	22.0	—	—
	Ammonium phosphate	—	—	—	—	12.4	—
	Gafsa rock phosphate	—	5.9	—	—	—	—
2007	Triple superphosphate	—	—	—	—	10.0	—
2008	5–20–20 NPK formula	—	—	—	2.5	4.9	5.5
2009	5–20–20 NPK formula	7.5	14.8	16.5	2.5	4.9	5.5
Total		579.6	53.4	183.8	731.7	186.9	225.5

Table 2

Main physical and chemical characteristics in Sandy Typic Hapludalf soil in a grassland site near of the vineyards, vineyard 1 (Cabernet Franc, 13 years of age) and vineyard 2 (Cabernet Franc, 31 years of age), in five depth layers

Depth layer	Clay (g kg ⁻¹)	Sand (g kg ⁻¹)	Silt (g kg ⁻¹)	Organic matter (g kg ⁻¹)	pH	Available P (mg kg ⁻¹)	Available K (mg kg ⁻¹)	Exchangeable Ca (cmol _c kg ⁻¹)	Exchangeable Mg (cmol _c kg ⁻¹)	Exchangeable H + Al (cmol _c kg ⁻¹)
Grassland site	0-5	24.0	929.0	47.0	7.5	4.0	27.0	0.38	0.26	2.3
	5-10	29.0	923.0	48.0	7.0	4.0	27.0	0.31	0.21	2.1
	10-15	24.0	957.0	19.0	5.0	4.2	22.0	0.16	0.10	2.0
	15-20	31.0	927.6	41.4	4.9	4.2	22.0	0.13	0.08	2.0
	20-40	26.0	928.3	45.7	3.7	4.2	18.0	0.08	0.04	2.4
Vineyard 1	0-5	33.0	892.2	74.8	7.9	6.6	46.0	2.51	0.95	0.8
	5-10	36.0	892.7	71.3	2.9	6.4	51.0	1.52	0.76	0.9
	10-15	44.0	879.8	76.2	3.5	6.3	49.0	1.40	0.70	1.0
	15-20	49.0	870.8	80.2	2.9	6.1	54.0	1.41	0.65	1.1
	20-40	55.0	853.1	91.9	4.9	6.2	52.0	1.43	0.69	1.2
Vineyard 2	0-5	33.0	922.5	44.5	11.2	6.4	40.0	2.32	0.91	1.5
	5-10	36.0	918.6	45.4	5.3	6.5	23.0	1.36	0.74	1.9
	10-15	28.0	922.2	49.8	4.8	6.6	26.0	1.27	0.69	2.2
	15-20	35.0	912.3	52.7	4.7	6.4	24.0	1.28	0.70	2.6
	20-40	27.0	924.2	48.8	4.7	6.4	26.0	1.52	0.80	2.1

$$H + Al = e^{10.665 - 1.1483 \times \text{pH SMP}} / 10$$

where the soil concentration of H + Al in $\text{cmol}_c \text{ kg}^{-1}$ is estimated by the pH balance of the soil with the SMP solution (Shoemaker, Mclean, and Pratt 1961). The concentration of the SMP solution used was 1.78 M buffered at pH 7.5, consisting of triethanolamine, parani-trophenol, potassium chromate (K_2CrO_4), calcium acetate [$\text{Ca}(\text{CH}_3\text{COO})_2$], and calcium chloride ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$) (Tedesco et al. 1995).

The cation exchange capacity at pH 7.0 ($\text{CEC}_{\text{pH } 7.0}$) was calculated by the equation proposed by CQFS-RS/SC (2004):

$$\text{CEC}_{\text{pH } 7.0} = H + Al + (\text{Ca}^2 + \text{Mg}^2 + \text{K}^+)$$

where $\text{CEC}_{\text{pH } 7.0}$ is expressed in $\text{cmol}_c \text{ kg}^{-1}$.

Chemical P fractionation was performed according to the methodology proposed by Hedley, Stewart, and Chauhan (1982), with the modifications proposed by Condon, Goh, and Newman (1985). Phosphorus was extracted sequentially from dry 0.5-g soil samples in the following order: anion exchange resin strip (AR 103 QDP 434); 0.5 M sodium bicarbonate (NaHCO_3); 0.1 M sodium hydroxide (NaOH); 1.0 M hydrogen chloride (HCl); and 0.5 M NaOH. After the extractions, the remaining soil was oven dried and digested with $\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2 + \text{MgCl}_2$ (residual P). Inorganic P of the alkaline extracts (NaHCO_3 and NaOH) was determined by the method proposed by Dick and Tabatabai (1977). In these alkaline extracts, total P was determined by digestion with ammonium persulfate + sulfuric acid in an autoclave, and Po was calculated as the difference between total P and Pi . Phosphorus of the acid extracts was determined according to Murphy and Riley (1962). The P fractions determined by Hedley fractionation were grouped in geochemical P and biological P, according to the method proposed by Cross and Schlesinger (1995). The first was obtained by the sum of inorganic fractions (resin Pi + NaHCO_3 Pi + 0.1 M NaOH Pi + 0.5 M NaOH Pi + HCl Pi + residual P) and the second by the sum of organic fractions (NaHCO_3 Po + 0.1 M NaOH Po + 0.5 M NaOH Po).

Statistical Data Analysis

Data of P content in different layers of the same grassland or vineyard soil and the same layer of different grassland or vineyard soils were subjected to variance analysis (PROC GLM) using SAS 9 (SAS 2003). Phosphorus contents were used as dependent variable and soil depth and site were used as independent variables. The results of all analyses were tested for significance by the F test at probability ≤ 0.05 . Multiple comparisons for P contents, where the variance analysis result was statistically significant, were carried out using Tukey's honestly significant difference (HSD) test (0.05).

Results and Discussion

Soil Properties

The texture of the soil under grassland and the two Cabernet Franc vineyards (vineyard 1, established in 1996, 13 years old, and vineyard 2, established in 1978, 31 years old) was sandy throughout the profile (Table 2). The organic-matter contents of the grassland

and vineyard soils were similar and tended to decrease with increasing depth. However, water pH values in all soil layers under grassland were close to 4.0, whereas in vineyard 1 and 2 they were between 6.1 and 6.6. The greater pH values in the vineyard soils were a result of the liming before planting the grapevines, intended to raise the pH in the 0- to 40-cm layer to 6.5. Greater P levels (extracted by Mehlich 1) were found in all layers of vineyard 2 compared to soils under grassland and vineyard 1. This can be explained by the history of P fertilizer, incorporated in the 0- to 40-cm layer prior to the vineyard establishment and applied whenever required throughout the grapevine cycle, on the surface without incorporation (Table 1). In addition, the K levels (extracted by Mehlich 1) in the 0- to 5-cm layer of the vineyard 1 and 2 soils tended to be greater than in the same layer of the grassland soil, which is related to the application of K fertilizers over years (Table 1). It is noteworthy that the K contents in the soil layers at depths of 5–10, 10–15, 15–20, and 20–40 cm of vineyard 1 tended to be greater than those found in vineyard 2 and grassland soils, which is most likely related to the greater amount of K fertilizers applied as of 1993 (Table 1). The lower K contents in vineyard 2 can also be associated with the longer cultivation period and consequently the greater export of K in the bunches of grapes, as this organ is the main K drain of the plant (Mpelasoka et al. 2003; Brunetto et al. 2009). The vineyard soils also tended to have greater contents of exchangeable Ca and Mg in all soil layers than grassland soil. This was expected, because vineyard 1 and 2 soils were limed to correct soil acidity before planting grapevines. The cation exchange capacity ($\text{CEC}_{\text{pH } 7.0}$) in the layers of grassland and vineyard soils was similar, because these soils are sandy and have a low buffering capacity.

Phosphorus Fractions in Soils

The anion exchange resin extracting P_i and sodium bicarbonate (0.5 M NaHCO_3) extracting P_i and P_o are the first extractants in the fractionation method of Hedley, Stewart, and Chauhan (1982). They extract the forms of soil labile P that contribute to the nutrient supply to plants (Gatiboni et al. 2007). The greatest P_i levels extracted by anion exchange resin in soils under grassland and Cabernet Franc vineyards were found at the soil surface and decreased in the deeper layers (Table 3). The levels of resin P_i in all layers were greater in vineyard 1 and 2 soils, compared to the native grassland soil. Of the soils under grapevines, the resin P_i levels in the soil profile were greatest in vineyard 2, except for the 0- to 5-cm layer, probably because it is the oldest and has the longest history of phosphate fertilization. A greater content of resin P_i is common in phosphate-fertilized soils (Oberson et al. 1999; Dobermann, George, and Thevs 2002; Zhang et al. 2004; Martinazzo et al. 2007; Herlihy and McGrath 2007). Nevertheless, in this study the increase in the deeper soil layers of the vineyards was caused by the low amount of clay (Table 2) and iron and aluminum oxides in the soil profile and the presence of 1:1 clay minerals that reduce the P adsorption capacity of these soils.

After 31 years of cultivation of vineyard 2, the level of resin-extracted P in the 20- to 40-cm layer was $17.2 \text{ mg P kg}^{-1}$, which is more than the 2.3 mg P kg^{-1} content found in the reference soil under grassland (Table 3) and interpreted as the average concentration according to the Commission of Soil Chemistry and Fertility of Rio Grande do Sul and Santa Catarina (CQFS-RS/SC 2004). The increase of P content in the surface and also in deeper layers showed that fertilizations exceeded the required amount and thus increased the risk of element loss through runoff and/or leaching, which could result, for example, in eutrophication of surface waters (Graetz and Nair 1995; Bergström and Kirchmann 2006; Gatiboni et al. 2008; Ceretta et al. 2010).

Table 3

Resin inorganic P, NaHCO₃ inorganic P, and NaHCO₃ organic P fractions in Sandy Typic Hapludalf soil in a grassland site, vineyard 1 (Cabernet Franc, 13 years of age) and vineyard 2 (Cabernet Franc, 31 years of age), in five depth layers

P fractions	Depth Layer (cm)	Grassland site (mg kg ⁻¹)	Vineyard 1 (mg kg ⁻¹)	Vineyard 2 (mg kg ⁻¹)	CV (%)
Resin inorganic P	0–5	5.5 aC ^a	23.6 aA	16.5 bB	1.09
	5–10	5.5 aC	12.5 bB	15.0 cA	1.41
	10–15	2.6 bC	8.2 cB	16.6 bA	0.49
	15–20	1.9 cC	5.5 eB	11.6 dA	0.55
	20–40	2.3 bcC	7.4 dB	17.2 aA	2.75
	CV %	4.65	1.53	0.42	
NaHCO ₃ inorganic P	0–5	1.5 bC	5.0 aA	4.5 abB	3.21
	5–10	2.7 aC	3.6 bB	4.5 abA	5.83
	10–15	0.6 cC	2.1 cB	4.3 bA	5.12
	15–20	0.2 dC	1.5 dB	3.5 cA	5.03
	20–40	0.6 cC	1.8 dB	4.8 aA	1.32
	CV %	9.98	4.12	2.96	
NaHCO ₃ organic P	0–5	5.2 aA	2.2 bcB	2.5 aB	4.37
	5–10	2.9 bA	2.6 abA	1.7 bB	11.47
	10–15	2.0 cA	1.5 cB	1.2 cB	7.60
	15–20	2.7 bA	2.2 bcB	1.0 cC	7.77
	20–40	1.7 cBC	3.1 aA	1.0 cC	16.63
	CV %	6.28	13.78	9.26	

^aSignificant at probability values F test ($P < 0.05$), and mean values with the same lowercase letter in a column and same capital letter in the depth layer are not significantly different (Tukey test, $P < 0.05$).

The concentration of Pi extracted by 0.5 M NaHCO₃, similar to the fraction of resin Pi, decreased along the soil profile under grassland and vineyards 1 and 2 (Table 3). The greatest Pi contents were found in vineyard 2, except in the surface layer at 0–5 cm, probably due to the longer history of phosphate fertilizer application. Organic P extracted by 0.5 M NaHCO₃ tended to be greater, especially in the 0- to 5-cm layer of grassland soil. These greater Po contents in soil under grassland can be attributed to the plant residues left on the soil surface and also to root senescence and decomposition in the soil profile, contributing to increases in the soil organic-matter content (Table 2) and P cycling (Martinazzo et al. 2007). Moreover, in the vineyards, plant residues from pruning cuts had been removed from the area, which may have contributed to the lower content of labile organic matter and consequently the lower Po content extracted by 0.5 M NaHCO₃ from the vineyard soils. In addition, the control of cover crops and weeds near the crop row involved desiccation, which may have further limited the input of organic material on the soil surface in the areas under grapevines.

The fractions of P that represent P adsorbed with average binding energy to the surfaces of silicate clays and oxides, as well as Po found in moderately labile forms, are the Pi and Po fractions extracted by 0.1 M NaOH and 0.5 M NaOH (Cross and Schlesinger 1995; Gatiboni et al. 2007). The greatest Pi contents by 0.1 M NaOH were extracted from all layers in vineyard 2 (Table 4), similar to that observed for the levels of resin Pi and

Table 4

Inorganic and organic P extracted with 0.1 M NaOH and 0.5 M NaOH in a Sandy Typic Hapludalf soil in a grassland site, vineyard 1 (Cabernet Franc, 13 years of age) and vineyard 2 (Cabernet Franc, 31 years of age), in five depth layers

P fractions	Depth layer (cm)	Grassland (mg kg ⁻¹)	Vineyard 1 (mg kg ⁻¹)	Vineyard 2 (mg kg ⁻¹)	CV (%)
NaOH 0.1 M inorganic P	0–5	6.5 aC ^a	15.5 aB	16.6 aA	1.68
	5–10	6.3 aC	9.9 bB	11.6 dA	1.68
	10–15	5.5 bC	9.4 bB	12.1 cA	1.27
	15–20	5.5 bC	8.2 cB	9.6 eA	2.11
	20–40	5.8 bC	9.4 bB	13.9 bA	0.66
	CV %	2.79	2.31	1.11	
NaOH 0.1 M organic P	0–5	4.7 bcB	7.8 aA	8.4 aA	6.00
	5–10	5.2 aB	8.1 aA	3.2 aC	3.51
	10–15	4.5 bA	3.8 cB	1.5 bC	5.68
	15–20	4.4 bB	5.3 bA	0.9 cC	1.42
	20–40	5.7 aA	4.4 bcB	0.5 cC	2.94
	CV %	4.47	6.99	5.26	
NaOH 0.5 M inorganic P	0–5	5.4 eC	17.8 aA	16.2 aB	1.91
	5–10	11.6 aB	15.9 bcA	10.5 dC	1.45
	10–15	8.9 dC	15.3 cA	10.4 dB	0.17
	15–20	9.9 cC	16.1 bA	11.9 cB	0.39
	20–40	10.8 bB	13.1 dA	13.4 bA	1.41
	CV %	1.44	1.61	0.71	
NaOH 0.5 M organic P	0–5	7.5 aB	11.3 aA	6.4 aC	0.73
	5–10	1.1 bC	6.6 bA	4.4 bB	8.59
	10–15	0.7 bcC	2.6 dB	4.7 bA	8.70
	15–20	0.5 cdC	1.4 eB	3.6 cA	12.48
	20–40	0.3 dC	4.4 cA	2.6 dB	9.58
	CV %	7.36	6.69	4.23	

^aSignificant at probability values F test ($P < 0.05$) and mean values with the same lowercase letter in a column and same capital letter in the depth layer are not significantly different (Tukey test, $P < 0.05$).

0.5 M NaHCO₃ Pi (Table 3). Moreover, the content of Po extracted by 0.1 M NaOH tended to decrease in the deeper layers under grassland and in both vineyards. The lowest Po contents extracted by 0.1 M NaOH were found particularly in vineyard 2, except for the surface layer, 0–5 cm. This confirms these observations that as the P application to the soil is increased through phosphate fertilizers, the amount of soil P converted to the plant-available fractions will become greater. On the other hand, the greatest Pi contents extracted by 0.5 M NaOH in vineyard 1, down to a depth of 15–20 cm, were possibly due to the shorter period of grapevine cultivation and consequently the lower P input to the soil by fertilization. Nevertheless, the greatest Po contents extracted by 0.5 M NaOH were also found in vineyard 1, down to a depth of 5–10 cm, and in vineyard 2, in the layers 10–15 and 15–20 cm.

According to Cross and Schlesinger (1995), 1.0 M HCl extracts mainly P forms bound to Ca. The Pi levels extracted by 1.0 M HCl decreased in the deeper layers of grassland

Table 5

Inorganic P extracted by 1 M HCl and residual P fractions in a Sandy Typic Hapludalf soil in a grassland site, vineyard 1 (Cabernet Franc, 13 years of age) and vineyard 2 (Cabernet Franc, 31 years of age), in the five depth layers

P fractions	Depth layer (cm)	Grassland (mg kg ⁻¹)	Vineyard 1 (mg kg ⁻¹)	Vineyard 2 (mg kg ⁻¹)	CV (%)
HCl inorganic P	0–5	5.5 aC ¹	20.3 aB	26.9 aA	1.54
	5–10	5.4 aC	12.6 bB	22.5 bA	1.93
	10–15	4.6 bC	7.6 cB	14.2 cA	1.69
	15–20	4.3 cC	6.4 dB	7.6 eA	4.82
	20–40	4.4 bcC	7.6 cB	8.5 dA	2.36
	CV %	2.40	2.69	1.52	
Residual P	0–5	29.4 bC	44.4 bB	50.6 aA	1.38
	5–10	26.5 cC	49.4 aA	47.9 bB	1.57
	10–15	24.9 dC	38.1 cB	41.5 dA	1.28
	15–20	22.3 eC	44.6 bA	37.4 eB	0.73
	20–40	37.5 aB	45.0 bA	44.6 cA	1.33
	CV %	1.02	1.15	1.59	

¹Significant at probability values F test ($P < 0.05$) and mean values with the same lowercase letter in a column and same capital letter in the depth layer are not significantly different (Tukey test, $P < 0.05$).

and vineyard soils (Table 5). However, the Ca-bound P extracted by 1.0 M HCl is usually derived from primary apatite minerals, although in the moderately weathered soils of southern Brazil, the occurrence of apatite is rare, refuting this hypothesis. Also, in no-tillage soils in southern Brazil, Rheinheimer and Anghinoni (2001) found increasing quantities of Ca-bound P in the surface soil layer with the application of phosphate fertilizer. Probably, a similar phenomenon occurred in these vineyards, where the addition of phosphates, including reactive rock phosphate (Table 1), to the topsoil and low nutrient export by crops resulted in P accumulation in various forms, allowing the preservation of part of Ca-bound P from phosphate itself (such as apatite, dicalcium, and octacalcium phosphate) or from lime added to correct soil acidity. However, the greater Pi extraction by 1.0 M HCl from vineyard than from grassland soil, especially in topsoil, can be associated with the fertilization of phosphate rock, as applied to vineyard 1 (Table 1), throughout the grapevine cycle.

The most recalcitrant P fraction is extracted by residual P ($\text{H}_2\text{SO}_4 + \text{H}_2\text{O}_2 + \text{MgCl}_2$) and is resistant to the access by selective chemical extractants in the soil. This P fraction can only contribute to plant nutrition in situations of extreme P deficiency. The contents of residual P in all analyzed layers showed no difference between the soils of vineyard 1 and 2, because the differences in the soil layers were apparently random. This was reflected in the sum of all P fractions of the five layers (Figure 1), where P in the recalcitrant fraction (i.e., the residual fraction) was predominant. Similar results were reported by Santos et al. (2008), who applied soluble phosphate fertilizer (triple superphosphate) to an Ultisol soil to meet the requirement of corn plants, resulting in P accumulation in the residual fraction in the long term. Similarly, Song, Han, and Tang (2007) found P accumulated in lower lability fractions in Udic Mollisols under crop rotation and phosphate fertilization. Thus, one can infer that much of the P applied in the vineyards over the years tends to be accumulated

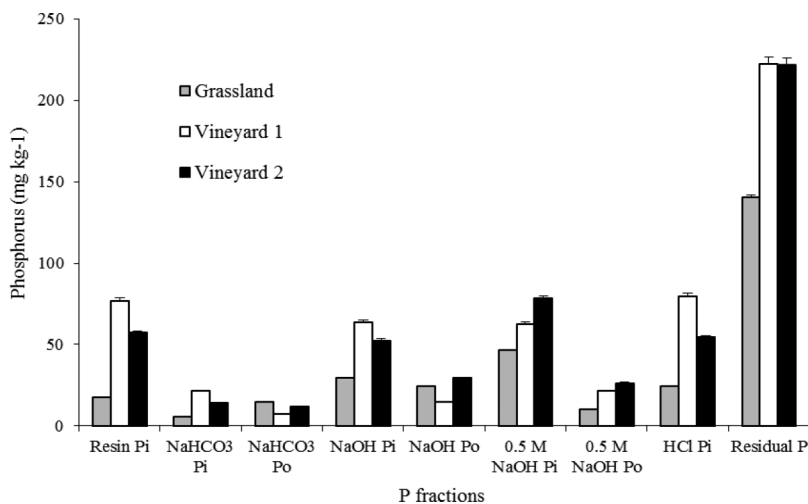


Figure 1. Sum of P fractions in the five depth layer, in a Sandy Typic Hapludalf soil in a grassland site, Vineyard 1 (Cabernet Franc with 13 years of age) and Vineyard 2 (Cabernet Franc with 31 years of age). Vertical bars represent standard errors.

in the soil in a form practically unavailable to plants. On the other hand, a positive aspect of the increase in soil residual P is that it can minimize the losses of the element to the environment.

In the soil of vineyard 2, the amount of the labile resin Pi was greater than in vineyard 1 (Table 3). In addition, a greater accumulation of Pi extracted by 0.1 M NaOH and 0.5 M NaOH (Table 4), adsorbed with average binding energy to the surfaces of silicate clays and oxides (Cross and Schlesinger 1995; Gatiboni et al. 2007), was found in vineyard 2 than in vineyard 1 soil. However, in vineyard 1, the levels of Po extracted by 0.5 M NaOH were greater than those in vineyard 2 (Table 4). Thus, even if most P applied to vineyard soils is accumulated in the residual P fraction, part of P, to a lesser extent, can be found in labile fractions (resin Pi) or with average binding-energy particles (0.1 M NaOH- and 0.5 M NaOH-extracted Pi fractions), especially in vineyards with a long-term fertilization history. Similar observations were reported by Pavinato, Merlin, and Rosolen (2009), who stated that in crop soils subjected to phosphate fertilization, labile P is accumulated, which can be used by plants during the cycle.

The geochemical P contents were greater in the layers of the vineyard soils than in soil under grassland. The accumulation of geochemical P in soil layers of the vineyards can be attributed to application of phosphate fertilizers over the years and the migration of part of the P in the soil profile, reflected in the increase of the total P content. Moreover, the levels of geochemical P in all layers of grassland and vineyard soils were greater than those of biological P (Table 6). The biological P content was greater in all layers of vineyard 1, except in the 10- to 15-cm layer, than in the grassland and vineyard 2 soils. This could be attributed to the cultivation of cover crops within and between grapevine rows planted in both vineyards and the residues left on the soil surface, probably in greater amounts in vineyard 1 than in vineyard 2. However, it is reported that the biological P concentration decreased throughout the soil profile, especially because of the decrease in organic-matter content (Table 2).

The levels of total P in all layers of the vineyard soils were greater than those found in soil under grassland, with a pattern similar to the geochemical P content (Table 6). The

Table 6
Geochemical and biological P pools and sum of all P fractions in a Sandy Typic Hapludalf soil in a grassland site, vineyard 1 (Cabernet Franc, 13 years of age) and vineyard 2 (Cabernet Franc, 31 years of age), in the five depth layers

P fractions	Depth layer (cm)	Grassland (mg kg ⁻¹)	Vineyard 1 (mg kg ⁻¹)	Vineyard 2 (mg kg ⁻¹)	CV (%)
Geochemical P	0–5	53.7 cC ^a	126.6 aB	131.2 aA	0.44
	5–10	58.0 bC	103.9 bB	112.3 bA	1.11
	10–15	47.2 dC	80.7 dB	99.1 dA	0.69
	15–20	43.9 eC	82.3 dA	81.5 eB	0.16
	20–40	61.4 aC	84.2 cB	102.5 cA	0.55
	CV %	0.77	0.66	0.69	
Biological P	0–5	17.5 aB	21.3 aA	17.3 aB	2.20
	5–10	9.2 bB	17.4 bA	9.3 bB	3.48
	10–15	7.3 cA	8.0 dA	7.3 cA	5.85
	15–20	7.6 cB	8.8 dA	5.5 dC	5.00
	20–40	7.7 cB	12.0 cA	4.1 eC	5.33
	CV %	2.64	4.55	2.95	
Sum of all P	0–5	71.2 aB	147.9 aA	148.5 aA	0.23
	5–10	67.2 cB	121.3 bA	121.6 bA	0.99
	10–15	54.5 dC	88.7 eB	106.4 cA	0.61
	15–20	51.5 eC	91.1 dB	87.0 dA	0.47
	20–40	69.1 bC	96.2 cB	106.6 cA	0.60
	CV %	0.62	0.72	0.56	

^aSignificant at probability values F test ($P < 0.05$) and mean values with the same lowercase letter in a column and same capital letter in the depth layer are not significantly different (Tukey test, $P < 0.05$).

levels of total down to the 10- to 15-cm layer were greatest in vineyard 2 soil. These results show that in spite of the high affinity of P for adsorption to reactive functional groups of soil particles, when applied over the years in the form of phosphate fertilizers, some P may migrate into the soil profile, reaching a depth of 40 cm.

The application of phosphate fertilizer to the soil under grapevines increased P contents down to a depth of 40 cm, especially in the more recalcitrant fractions, but this was also noticeable in the more labile fractions, which are plant available and increase the risk of environmental contamination. The phosphate fertilization and grapevine cultivation periods had little effect on the Po levels, but cover crops planted between or even in the grapevine rows and the maintenance of their residues on the soil surface may be an appropriate strategy to increase or maintain the levels of soil organic matter over years and, consequently, the content of Po.

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