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Phosphorus fractions in soil cultivated with vineyards after 62 years of poultry litter addition

Abstract – The objective of this work was to evaluate changes in phosphorus fractions in a Humic Cambisol cultivated with vineyards, after 62 years of additional fertilization with poultry litter. Soil samples were collected from two vineyards (V1 and V2) and a forested area adjacent to them. A total of 1,365 and 910 kg ha⁻¹ P were applied to V1 and V2, respectively, throughout the past 62 years. Samples were collected at 0–10, 10–20, 20–30, and 30–40-cm soil depths for the analysis of soil physicochemical properties. The addition of poultry litter to the soil of the vineyards increased the contents of inorganic P (P_i) in all fractions, except in the residual fraction (residual-P), mainly at the 0–10-cm soil depth, in comparison with the forested area. The addition of poultry litter as fertilizer does not affect the organic P (P_o) stock in the Humic Cambisol, it only increases the P_i contents of the soil.

Index terms: *Vitis labrusca*, chemical fractionation of P, grapevine, organic phosphorus, organic residue.

Frações de fósforo em solo cultivado com vinhedos após 62 anos de adição de cama-de-aves

Resumo – O objetivo deste trabalho foi avaliar as modificações de frações de fósforo em um Cambissolo Húmico cultivado com vinhedos, após 62 anos de fertilização adicional com cama-de-aves. Amostras de solo foram coletadas de dois vinhedos (V1 e V2) e de uma área de floresta (F) adjacente a eles. Foram aplicados 1.362 e 910 kg ha⁻¹ de P ao V1 e ao V2, respectivamente, durante os últimos 62 anos de cultivo. Amostras de solo foram coletadas a 0–10, 10–20, 20–30 e 30–40 cm de profundidade, para análises das propriedades físico-químicas do solo. A adição de cama-de-aves ao solo dos vinhedos aumentou os teores de P inorgânico (P_i) em todas as frações, exceto na fração residual, principalmente à profundidade de 0–10 cm, em comparação à área de floresta. A adição de cama-de-aves como fertilizante não afeta os estoques de P orgânico (P_o) do Cambissolo Húmico, apenas aumenta os teores de P_i do solo.

Termos para indexação: *Vitis labrusca*, fracionamento químico de P, videira, fósforo orgânico, resíduo orgânico.

Introduction

In the subtropical region of Brazil, many vineyard management decisions are based on empirical knowledge, without technical standards or soil testing. As a consequence, vineyard soils are often poor in nutrients. When agricultural liming and fertilization requirements

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are diagnosed, mainly by soil analyses, lime and other nutrients including phosphorus (P) are often prescribed (Silva et al., 2016).

Phosphorus is highly reactive in the soil – both in alkaline soils (Eriksson et al., 2015) and weathered acid soils (Boitt et al., 2018). When the applications of organic fertilizers are carried out above the exportation by the plants, P accumulation takes place in several fractions (Boitt et al., 2018). Phosphorus accumulations are usually more pronounced in inorganic fractions (He et al., 2008; Boitt et al., 2018) due to part of the organic fertilizer showing Pi (Turner & Leytem, 2004), and also due to the mineralization of part of organic P of the soil (Steffens et al., 2010).

Current technical recommendation for fertilization before grapevine planting is to apply the fertilizers and incorporate them into the soil to approximately 20 cm depth (Hanlon et al., 2005). For side dressing, the fertilizers are only spread on top of the soil, without manual incorporation (Skinner et al. 1988). However, in older vineyards, phosphate fertilizers were often added without technical criteria, and at doses exceeding the requirements for the grapevine (*Vitis* sp.) crop; these issues were especially common for side dressing (Schmitt et al., 2013b).

Since P is poorly absorbed by grapevine, only a small quantity of P is transported to grape bunches (Tecchio et al., 2011), which can lead to an accumulation of P fractions in the soil layers (Wang et al., 2015), and increase the risk of environmental losses of the more labile P fractions (Gatiboni et al., 2015) by surface runoff (Sharpley et al., 1996); surface runoff is a risk for vineyard on steeper slopes. Therefore, frequent monitoring of soil P levels is required.

Simple P extraction methods such as Olsen, Bray, anion-exchange resin, Mehlich-1, and Mehlich-3 only extract part of the labile P from the soil, masking the occluded P and mineral-bound P, which hampers the understanding of P soil dynamics (Yang & Post, 2011). However, in the chemical P fractionation method proposed by Hedley et al. (1982), P is extracted from the soil in stages with different extractors, which allows of a more accurate quantification of P.

There is a lack of research addressing the accumulation and distribution of P fractions in clayey vineyard soils on steep slopes, with high-P adsorption capacity and a long history of organic residue application. Under these conditions, P₁ and P₀ contents of the moderately labile P fractions are

expected to be greater in vineyards with lesser slopes, especially in the near-surface soil. For this reason, successive applications of poultry litter to the vineyard soil surface are supposed to cause P accumulation in different P pools, mainly the most labile forms and in the near-surface soil.

The objective of this work was to evaluate changes in P fractions in a Humic Cambisol cultivated with vineyards, after 62 years of the addition of poultry litter as fertilizer.

Materials and Methods

The experiment was carried out in the municipality of Videira, in the midwestern of Santa Catarina state, Southern Brazil, in two vineyard (V1 and V2) areas settled in 1950, and in a forested area (F) located next to the vineyards, as a reference. The vineyards were 62 years old, had approximately 2 ha each, and were located at as follows: V1, 26°57'14"S and 51°12'39"W; V2, 26°57'14"S and 51°12'29"W; and F, 26°57'09"S and 51°12'25"W. The V1 and V2 areas had a mean slope of 16 and 26%, respectively. The climate of the region is humid mesothermic with mild summers, according to the Köppen-Geiger's classification (Cfb). The mean annual temperature is 16.5°C, and the mean annual rainfall is 1,400 mm. Soil type of both vineyards, according to Santos et al. (2013), is Cambissolo Húmico, i.e. Humic Cambisol (FAO, 1998), with a predominance of kaolinite in the clay fraction.

Vitis labrusca 'Isabel' was grown on ungrafted rootstock in both vineyards, in pergola vine training systems with plants spaced 2.0 m apart, and rows spaced 3.5 m apart (1,525 plants ha⁻¹). V1 and V2 soils, were subjected to 6 and 4 Mg ha⁻¹ poultry litter addition once every 5 year, respectively. Poultry litter was applied at 78 and 52 Mg ha⁻¹ to the soil surface both in and between rows, without incorporation to the soil, in V1 and V2, respectively. Poultry litter was obtained from raising 6-8 batches of birds, and each application contained 17.5 g kg⁻¹ P on average (Silva et al., 2016). Thus, over the course of 62 years of grapevine cultivation, total P amounts at 1,365 and 910 kg ha⁻¹ P were added to V1 and V2, respectively. No other nutrient source was used in the management of the vineyards except for leaf applications of the Bordeaux mixture for disease control, which added Ca, S, and Cu to the system.

In March 2012, five years after the last application of poultry manure, six trenches (60 x 60 cm) were randomly dug in the F, V1 and V2 areas. Soil samples were collected at 0–10, 10–20, 20–30, and 30–40 cm depth, in six replicates. The soil was air-dried and organic coarse material were removed manually. The soil was then ground in an agate mortar and pestle before being stored for analysis.

Soil samples from each depth were analysed by the pipette method for distribution of particle size soil constituents (Claessen, 1997). Organic matter (OM) content was determined by the Walkley-Black method (Claessen, 1997), and soil pH was measured using a 1:1 v/v soil:water suspension test, after 30 min of equilibration time. Available P and exchangeable K were extracted from the soil samples with the Mehlich-1 solution (0.05 mol L⁻¹ HCl + 0.0125 mol L⁻¹ H₂SO₄), and characterized by spectrophotometry and flame photometry, respectively (Tedesco et al., 1995).

The exchangeable Ca, Mg, and Al were extracted from the soil samples with 1 mol L⁻¹ KCl solution; Ca and Mg were characterized by atomic absorption spectrometry, and Al was characterized by titration with 0.0125 mol L⁻¹ NaOH (Tedesco et al., 1995). The effective cation exchange capacity (CEC_e) was calculated by Al + Ca + Mg + K. The cation exchange capacity at pH 7.0 (CEC_{pH7.0}) was calculated as the sum of exchangeable bases (Ca + Mg + K) plus the estimated H + Al, using an SMP buffer solution (Silva et al., 2016). Base saturation (V) was calculated as the sum of Ca + Mg + K divided by CEC_e and expressed as a percentage. The remaining soil was subjected to chemical fractionation of P, according to the methodology of Hedley et al. (1982), with modifications proposed by Condon & Goh (1989).

Soil samples of 0.5 g were subjected to sequential extraction with anion-exchange resin (AER-P_i) and then 0.5 mol L⁻¹ sodium bicarbonate, which extracted inorganic and organic labile P (NaHCO₃-P_i and NaHCO₃-P_o, respectively). Then, the samples were treated with 0.1 mol L⁻¹ sodium hydroxide (to extract NaOH I-P_i and NaOH I-P_o), 1 mol L⁻¹ hydrochloric acid (to extract HCl-P_i), and 0.5 mol L⁻¹ sodium hydroxide (to extract NaOH II-P_i and NaOH II-P_o). Phosphorus extracted by 0.1 and 0.5 mol L⁻¹ represents inorganic P linked to oxides and silicate clays with intermediate binding energy and the organic P of moderately labile fractions (Gatiboni et al., 2013). HCl-P_i extracts

inorganic P contained in the calcium phosphates and strongly adsorbed phosphates. The content of HCl-P_i was evaluated only in the inorganic fraction because the amount of P_o extracted by HCl in weathered soils is usually small. After all extractions had been performed, the remaining soil was oven-dried at 45°C, and subjected to acid digestion with concentrated H₂SO₄ + H₂O₂ + MgCl₂ to measure the residual P.

P_i extracts resulting from the alkaline extractants (NaHCO₃-P_i, NaOH I-P_i, and NaOH II-P_i) were determined by the method proposed by Dick & Tabatabai (1977). In these alkaline extracts, total P was determined by digestion in an autoclave with ammonium persulfate and sulfuric acid ((NH₄)₂S₂O₈ + H₂SO₄), and P_o was calculated as the difference between total P and P_i in the extract. Phosphorus in the acid extracts was determined according to Murphy & Riley (1962).

For the statistical analysis, the P pool was treated as the dependent variable, and the soil layer, the independent one. Phosphorus pools of soil samples from the F and vineyard areas were subjected to analysis of variance, and the significant effects were compared by the Tukey's test, at 5% probability, using the Sisvar 5.3 software (Ferreira, 2011).

Results and Discussion

The contents of labile P pools (AER-P_i, NaHCO₃-P_i, and NaHCO₃-P_o), and moderately labile P pools (NaOH I-P_i, NaOH I-P_o, NaOH II-P_i, NaOH II-P_o) increased during the grapevine cultivation, mainly at 0–10 cm soil depths (Figures 1 and 2). In comparison to the forested area, the greatest increases of labile and moderately labile P contents in V1 and V2 were observed in the P_i fractions at 0–10 cm soil depths. In the forested area, the labile P contents were similar throughout the soil profile.

The highest levels of AER-P_i and NaHCO₃-P_i, as well as of NaOH I-P_i and NaOH II-P_i, at 0-10 cm soil depths of V1 and V2 can be attributed to the poultry litter added to the soil over the 62 years of cultivation – 1,365 and 910 kg ha⁻¹ P, respectively. In addition, P accumulation in the labile and moderately labile fractions may be more pronounced in vineyards with longstanding cultivation, which can be attributed to pruning residues and senescent leaves. Furthermore, weeds or cover crops can take P from deeper soil layers

and store it in the shoot tissues, which is later deposited on the soil surface (Brunetto et al., 2011). These plant residues and organic fertilizers are mineralized by soil microorganisms, increasing the P_i content (Damon et al., 2014), especially in the topsoil (Rubaek et al., 2013). High-P levels in the near-surface soil of vineyards are further elevated due to small quantities of P transported to grape bunches (Tecchio et al., 2011). In general, approximately 4 kg ha^{-1} P per year are transported to grape bunches, considering an average annual yield of 25 Mg ha^{-1} grapes per year (Tecchio et al., 2011). This low value of P removed from the vineyards reflects the difficulty of establishing a critical P level for fruit species (Brunetto et al., 2015), as mineralized P_o may be more than sufficient to supply the plant demand (Damon et al., 2014), and total P supply can easily exceed the crop requirement.

In vineyard soil at 0–10 cm depths, high-P contents, both in the labile and the moderately labile fractions extracted by Mehlich-1 (Table 1) exceed the critical P level of 18 mg kg^{-1} for the clay class between 21 and 40% (Silva et al., 2016).

The increase of NaOH I- P_i and NaOH II- P_i (Figure 2) is an indication that the P applications exceeded the uptake capacity of the grapevines and any intercropped cover species, resulting in P accumulation in all fractions (Schmitt et al., 2013a, 2013b). However, the distribution of accumulated P varies with soil type. For instance, in clayey soils, P is high in the fractions associated with Fe and Al oxyhydroxides extracted by sodium hydroxide (Gama-Rodrigues et al., 2014), while in sandy soils, labile fractions of P are predominant (Schmitt et al., 2013a, 2013b).

At all study sites, contents of P in the HCl- P_i pool were low than in other P pools, as determined from the Hedley's procedure. The HCl- P_i pool is related to Ca-P forms which are expected to be very low in weathered soils (Tiecher et al., 2018), such as those in the present study. The HCl- P_i contents at 0–10 cm soil depths were 207 and 52% higher in the V1 and V2 areas than in the forested area, respectively (Figure 2). However, the HCl- P_i fraction in the soils decreased with depth. Higher-HCl- P_i contents at 0–10 cm soil depth of both vineyards were related to the P application rate throughout cultivation, and to higher-pH and Ca levels (Table 1), which increased the P_i fraction (Borda et al., 2014). Conversely, the residual-P content (Figure 2), which was the greatest P fraction in the soils, was not

influenced by either the grapevine cultivation, poultry litter applications, or the vineyard slopes. Moreover,

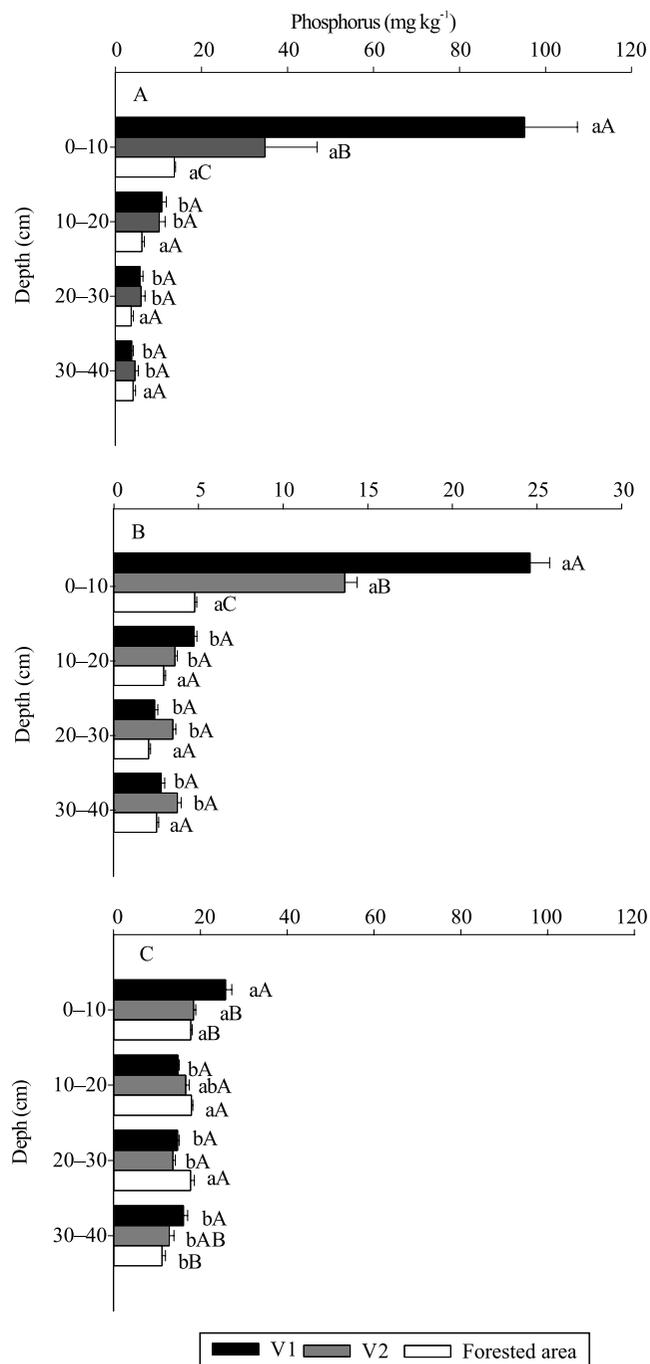


Figure 1. (A) AER- P_i , (B) NaHCO₃- P_i , and (C) NaHCO₃- P_o from the soils of two vineyards (V1 and V2) and forested area. Uppercase letter compare areas within the same depth, and lowercase ones compare the depths within the same area. Bars followed by equal letters do not differ, by Tukey's test, at 5% probability. Error bars indicate standard error of the mean.

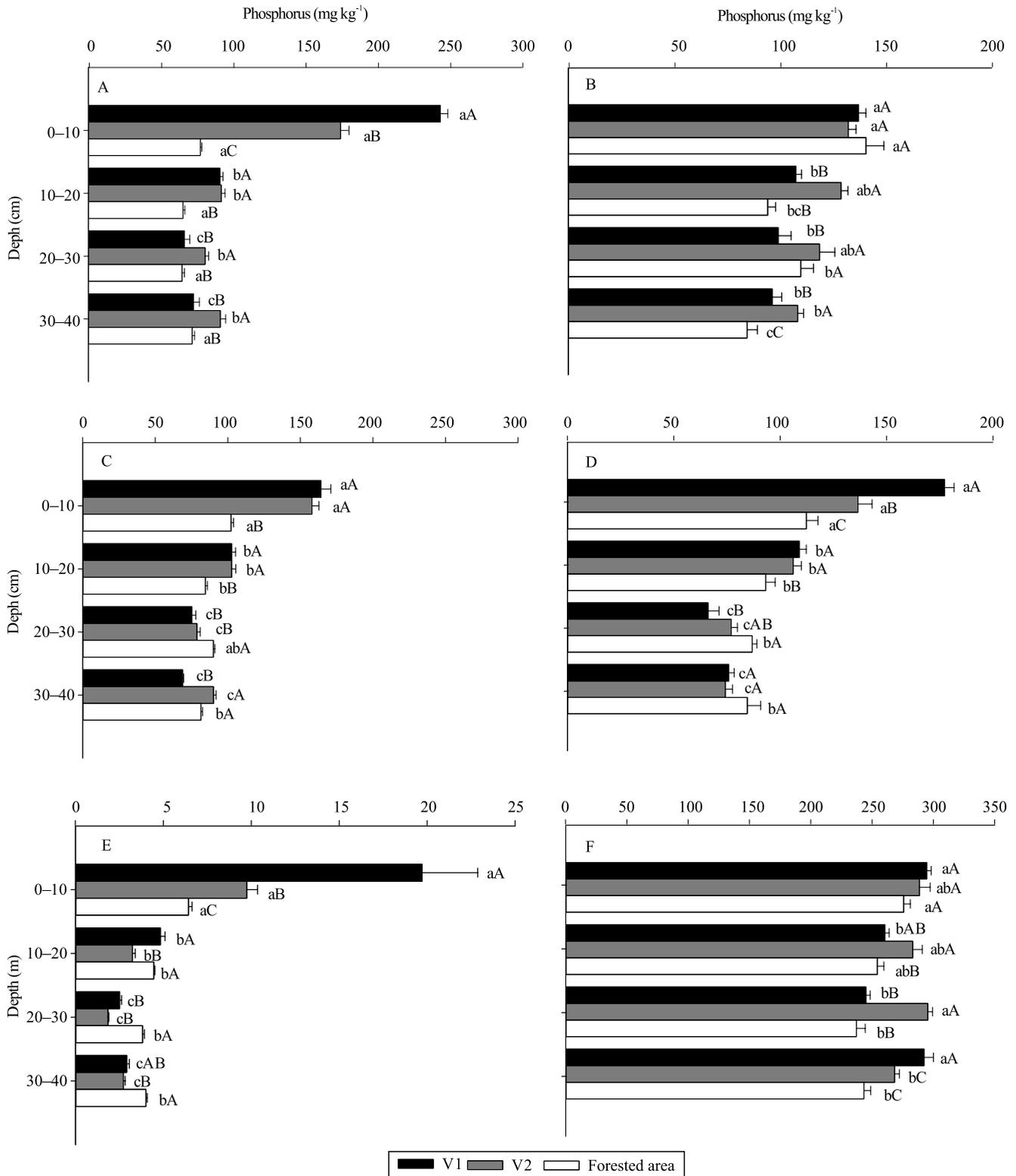


Figure 2. (A) NaOH I-P_i, (B) NaOH I-P_o, (C) NaOH II-P_i, (D) NaOH II-P_o, (E) HCl-P_i, and (F) residual-P from the soils of two vineyards (V1 and V2) and forested area. Uppercase letters compare areas within the same depth, and lowercase ones compare the depths within the same area. Bars followed by equal letters do not differ, by Tukey's test, at 5% probability. Error bars indicate the standard error of the mean.

compared to the forest site, residual P content in both vineyards increased in deeper soil layers, in contrast to the other fractions, which decreased.

The sum of all P fractions measured at 0–10 cm soil depths of V1, V2, and forested area were approximately 1,200, 1,080, and 790 mg kg⁻¹ P, respectively. At 10–20 cm soil depth, in V1, V2, and forested area measured P amounts were 780, 650, and 730 mg kg⁻¹ P, respectively; the difference in total P of this layer and at 0–10 cm

soil depths indicates a P accumulation at the surface layer.

Additionally, in comparison to the P_i fractions in the forest soil, those in the vineyards changed throughout cultivation (Figure 3); however, P_o remained stable, despite the organic fertilizer applications. This indicates that the poultry litter and other plant residues were mineralized and transformed into P_i (Borda et al., 2014). This study indicates that the entire P_o – added as

Table 1. Soil physicochemical properties from depth intervals 0–10, 10–20, 20–30, and 30–40 cm, of a Cambissolo Húmico (Humic Hapludox) in two vineyards (V1 and V2) and a forested area.

Location	Depth (cm)	Clay ----- (g kg ⁻¹) -----	Silt	Sand	OM	pH -1:1-	Ca ---- (cmol _c kg ⁻¹) ----	Mg	P ---- (mg kg ⁻¹) ----	K	CEC _{ef} ---- (cmol _c kg ⁻¹) ----	CEC _{pH7.0}	V (%)
V1	0–10	269dAB	498aA	233aA	66aA	6.5aA	12.5aA	5.4aA	26.8aA	186aA	18aA	20aA	90aA
	10–20	331cB	513aAB	156bA	28bA	6.1bA	8.8bA	4.9aA	5.5bA	160abA	14bA	17abA	85bA
	20–30	440bB	420bA	140bB	23bA	5.7cA	6.8cB	4.2bA	3.2bA	135bcA	11bA	14bB	80cA
	30–40	512aA	378bB	110cB	21bA	5.7cA	9.2bA	4.6abA	2.9bA	117cA	14bA	17abB	82dA
V2	0–10	321dA	524aA	156aB	45aC	6.3aA	12.3aA	3.9aB	22.6aA	134aB	17aA	20aA	84aB
	10–20	421cA	465bB	113bB	32bA	6.1abA	9.2bA	3.6aB	3.4bA	118abAB	13bA	17abA	79bB
	20–30	554aA	364cB	82bC	22bcA	5.8bA	7.5bAB	3.1aB	2.7bA	89bcB	11bA	15bB	74cB
	30–40	483bA	429bA	88bB	17cA	5.3cA	8.0bA	3.0aB	2.7bA	64cB	11bA	15bB	73cB
Forested area	0–10	245bB	545aA	210aA	75aA	6.3aA	13.7aA	4.2aB	3.5aB	201aA	18aA	21abA	86aB
	10–20	254bC	558aA	188aA	40bA	6.0aA	11.2abA	4.1aAB	1.4aA	136bB	16abA	19bA	81bB
	20–30	342aC	463bA	195aA	29bcA	5.6bA	9.8bA	3.4aAB	1.2aA	100bcAB	14bA	22abA	62cC
	30–40	379aB	428bA	193aA	27cA	5.6bA	10.7bA	3.3aB	1.2aA	82cAB	14bA	24 aA	59cC

Clay, sand, and silt contents were measured by the pipette method; OM, organic matter; pH, pH in water 1:1; Ca and Mg extracted by KCl 1 mol L⁻¹; P and K extracted by Mehlich-I; CEC_{ef}, effective cation exchange capacity; CEC_{pH7.0}, cation exchange capacity at pH 7.0; V, base saturation. Uppercase compare the locations within the same depth and, lowercase, the depths within the same location. Means followed by equal letters do not differ, by Tukey's test, at 5% probability.

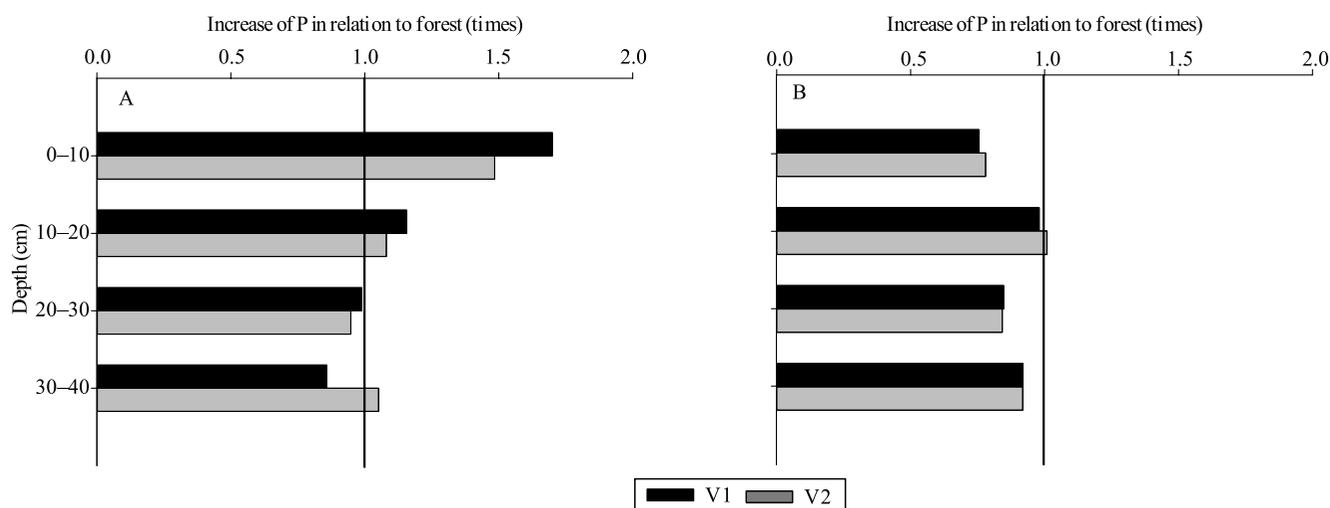


Figure 3. (A) Increase of P_i, (B) P_o contents in the soils of two vineyards (V1 and V2), in comparison with the forested area. The line represents the relationship between P_i and P_o in the forest area.

poultry litter and field residues – was transformed into P_i . In comparison to the P_i amount in the forest soil, P_i contents in the topsoil (0–10 cm depths) increased by approximately 1.6 and 1.5 times in the vineyards V1 and V2, respectively. However, no differences were observed in the P_o fractions between the vineyard and forest soils.

Conclusions

1. The addition of poultry litter as fertilizer does not affect the organic P (P_o) stock in a Humic Cambisol; however, it increases the P_i contents in the soil.

2. Inorganic P is the main form of P accumulation in a Humic Cambisol, even with the long-term use of organic fertilizer based on poultry litter.

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