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Phosphorus fractions and microbiological indicators in vineyards soils of a tropical semiarid setting in Brazil

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ABSTRACT: Brazilian semi-arid tropical is responsible for the production and export of fine table grapes, but the demand for phosphate fertilizers and other agricultural inputs increases the cost of production and threatens local water bodies by leaching of phosphorus (P). The objectives of this study were to quantify the organic and inorganic fractions of P and to determine chemical and microbiological attributes related to its mineralization in soils cultivated with vines in São Francisco Valley. It was hypothesized that P fractions and microbial activity would increase in areas fertilized with phosphate. Soil samples from vines were collected in cultivated areas and in fallow systems. P fractionation, macro-elements analysis, microbial biomass phosphorus (Pmic), and phosphatase enzyme activity were determined and correlated with each other and with the total proportion of synthesized glomalin. In both treatments, the inorganic P fractions exceeded the organic ones, and the total phosphorus available in those soils was associated with the most recalcitrant fractions, reducing risks of leaching of this element and contamination of water bodies. Pmic was higher in the winery areas due to the presence of phosphate fertilizers and organic matter, hence the activity of alkaline phosphatase enzymes and the production of total glomalin increased respectively in three and four evaluated areas, indicating that P-mineralized bacteria and arbuscular mycorrhizal fungi have been positively affected by the current agricultural system.

Key words: São Francisco Valley, *Vitis labrusca* L., phosphatase, glomalin.

INTRODUCTION

The São Francisco Valley is in the semi-arid setting of Northeast Brazil and it is responsible for the production and export of fine table grapes. The climate of this region favors the development of vines and provides more than two harvests of different types of grapes for one year, boosting the demand for phosphate fertilizers and agricultural inputs (Arata et al. 2017). Some studies have already determined the concentrations of P in that vineyard's soils and have asserted that the available fraction of this element is one of the highest ever reported in a tropical environment, and this can threaten the water bodies by leaching and eutrophication, consequently (Silva et al. 2012, Preston et al. 2016, Preston et al. 2017).

Phosphorus is represented by the organic (Po), and inorganic (Pi) conditions in the soil, and it is mainly associated to silicate clays and Fe and Al oxides (Pi), or in the presence of inositol phosphate (Po) (Damon et al. 2014). Assessing the potential for transferring the accumulated P to surface or groundwater, studies also should consider the fractions of greater or lesser lability. In this sense, Hedley's fractionation method (1982) has been suitable for sequentially extracting P in organic and inorganic fractions, thus obtaining the total proportion of this element in the soil to optimize the management of phosphate fertilizers, providing greater agricultural productivity, and less environmental damage (Gatiboni et al. 2008, Schmitt et al. 2014).

It is known that soil microorganisms perform essential activities in the cycling of P and are directly responsible for maintaining soil fertility and carbon storage (Richardson and Simpson, 2011, Tian et al. 2021). Soil microbial biomass can be used as an indicator of the soil quality even in saturated phosphate fertilizers agricultural systems, because of the activity of microorganisms drives P-available to the plants (Schloter et al. 2018). In addition, phosphatase enzymes have been responsive to changes in agricultural management and can indicate an effective P-mineralizing microbial activity in the soil (Luo et al. 2016, Wei et al. 2017).

Arbuscular mycorrhizal fungi (AMF) efficiently act on the soil availability of P, mainly by producing a thermostable hydrophobic glycoprotein known as glomalin. Hence, after release into the soil, this protein becomes prone to microbial decomposition due to stabilization with organic matter (Chen et al. 2018). So, the presence of glomalin in fertilized and cultivated soils provides evidence of fungal activity in which plant-arbuscular mycorrhizal fungi symbiosis occurs (Wang et al. 2017).

Since the vine growing in the Brazilian semi-arid region depends on the high concentration of phosphate fertilizers, this work aimed to quantify the fractions of inorganic and organic P and to determine the chemical and microbial attributes directly responsible for P-mineralization in soils with vineyard cultivation and in interrupted agricultural production (fallow system). It was hypothesized that P fractions and microbial activity would increase in areas fertilized with phosphate.

MATERIALS AND METHODS

Site description and sampling strategy

Soil samples were collected in vineyards from São Francisco Valley, Pernambuco state, Brazil. Local climate is Bswh', according to Köppen classification. The average annual temperature and rainfall are 26 °C and 578 mm, respectively. Soils of all areas were classified as Typic Quartzipsamment, with textures ranging from loam to sandy loam in the surface layer (0-20 cm).

Six study areas were selected with cultivation of grape varieties: Arra-15, Itália Moscat, Vitória, Crimsom, and Iris in the companies Santo Antônio, Brasil Fruit, Galdino, Andorinha, Frutinalli, and Vale Verde, respectively. These plots are cultivated with vineyards for at least 10 years. Adjacent areas, with the same soil type and fallowed for five years, were selected to evaluate the contents and fractions of P. The soils had a history of liming with dolomitic limestone, and areas 1, 4 and 5 were fertilized with magnesium sulfate. The main source of P used in all areas is monoammonium phosphate (MAP), with annual application varying between 100 and 200 kg·ha^{-1} of P_2O_5.

Sampling was performed in the row of cultivated areas (CA) and in the fallow area (FA) at 0-20 cm depth. The experiment was based on a randomized design, and these areas were divided into three equal plots, in which the soil was collected in triplicate samples and stored at -20 °C until their conduction to laboratory. Before chemical and microbiological analyses, the samples were air-dried and sieved through 2-mm mesh for disaggregate the bulk soil.

Chemical and physical analyses

Chemical and physical characterization was performed according to Embrapa (2011). The pH was measured at a soil: water ratio of 1:2.5. The determination of soil organic carbon was based on the Walkley-Black chromic acid wet oxidation method (Bowman 1998). The exchangeable contents of Ca^{2+} and Mg^{2+} were extracted with 1 mol·L^{-1} KCl solution and determined by complexometric titration.

The available contents of P and K+ were extracted by Mehlich-1 and analyzed by colorimetry (P) and flame photometry (K), respectively. H + Al was determined by extraction with 0.5 mol·L^{-1} of calcium acetate adjusted to pH 7, followed by titration. Cation concentrations were calculated and used in base saturation and cation exchange capacity. The determination of the physical characteristics of soil granulometry and density were carried out by the densimeter method (Teixeira et al. 2017).

The chemical fractionation of P was performed according to Condron et al. (1985), in which 0.5 g of soil were subjected to sequential extraction with 0.5 $mol \cdot L^{-1}$ $NaHCO_3$, 0.5 $mol \cdot L^{-1}$ NaOH, and 1 $mol \cdot L^{-1}$ HCl. Each extraction was carried out for 16 hours in a horizontal shaker at 120 rpm, followed by centrifugation at 3,500 rpm for 10 minutes, reserving the supernatant, and adding 5 mL of 0.5 $mol \cdot L^{-1}$ NaCl to the remaining soil in the tubes, followed by a new centrifugation at 3,500 rpm for 10 minutes. Supernatant being placed in the same containers as the previous extract. The remaining soil was oven-dried and subjected to digestion with $H_2SO_4 + H_2O_2$ + saturated $MgCl_2$ for 3 hours at 200 °C to obtain residual P.

After the extractions, inorganic-P (Pi) was determined, and 5-mL aliquot was taken from each of the extracts–0.5 $mol \cdot L^{-1}$ $NaHCO_3$ (pH 8.5), 0.5 $mol \cdot L^{-1}$ NaOH, 1.0 mol HCl L^{-1}–and submitted to a nitroperchloric digestion to obtain total P-(Pt). The total-P, inorganic-P and residual-P fractions of all extracts were quantified according to Murphy and Riley (1962). Organic-P (Po) was obtained by the difference between the concentration of Pt (Pi + Po) and inorganic P (Pi) in each extract.

Microbiological analyses

Phosphorus of soil microbial biomass (P-mic) was estimated by the irradiation-extraction method (Mendonça and Matos 2017). Acid and alkaline phosphatase activities were measured by spectrophotometry (400 nm) of p-nitrophenol released from 1 g of soil after incubation for 60 minutes at 37 °C with a 0.025 $mol \cdot L^{-1}$ of p-nitrophenyl phosphate substrate (Tabatabai 1994). The extraction and quantification of glomalin in the soil of vines followed Bradford's method (Wright and Upadhyaya 1998). The extraction of the easily extractable glomalin fraction was performed from 1 g of soil in 8 mL of sodium citrate [20 mM (pH 7)], with a single digestion in an autoclave at 121 °C for 60 minutes, and the determination was performed by colorimetry according to Bradford (1976).

Data analysis

Data normality and homoscedasty were evaluated, and, when necessary, transformations were performed. The results of the variables were submitted to analysis of variance (ANOVA) ($p < 0.05$). The average values of chemical and microbiological parameters evaluated in the two collection environments (cultivated soils and FA) were compared using the Tukey's test ($p < 0.05$). Correlation analysis was used to investigate the relationship among microbiological attributes and parameters of fertility and phosphorus speciation of CA with vines and FA. All statistical analyses were performed using Statistica 10.1 software.

RESULTS AND DISCUSSION

Chemical and physical analyses

Organic carbon (OC) contents were significantly higher ($p < 0.05$) in CA compared to FA, except for vineyard 6 (Table 1), and these high values were due to organic fertilization used in that plots (Couto et al. 2016). Because of sandy texture of these soils, the increase in OC is extremely important for nutrient accumulation and soil structuring, thus improving the chemical soil quality (Preston et al. 2017). The same trend was observed for the availability of Ca, K and Na, while Mg levels were higher in V1, V4 and V5 than other areas, probably due to magnesium sulfate-based fertilization (Silva et al. 2014).

Available P (Table 1) was significantly higher ($p < 0.05$) in all CA comparing to FA (428.4 to 1,026.8 and 101.6 to 422.9 $mg \cdot dm^{-3}$ in CA and FA, respectively). There was in CA a cumulative effect of high P rates applied under fertigation, and these values were 178 times higher than the reference areas (native dry soil forest) reported by Preston et al. (2017). This indicates that, even in area without fertilizers for at least five years, the boost in P contents is prone to leaching and bodies water eutrophication subsequently (Ding et al. 2014). There is also the structural effect of the soil, as a high presence of sand and a low presence of clay (i.e., sandy soils) reduce the adsorption of P by Fe and Al oxides, making even more P available in that environment (Bünemann 2015).

Table 1. Averages fertility soil attributes and physical analyses evaluated of cultivated areas (CA) with grape and fallow area (FA) in São Francisco Valley*.

	V1		V2		V3		V4		V5		V6	
	CA	FA	CA	FA	CA	FA	CA	FA	CA	FA	CA	FA
pH	6.93a	5.50b	6.53a	6.70a	6.63a	6.60a	7.07a	6.60a	6.83a	6.40a	6.91a	6.90a
OC ($g \cdot kg^{-1}$)	18.23a	10.82b	15.08a	7.22b	15.23a	10.22b	12.98a	8.12b	14.24a	10.22b	14.18a	14.42a
OM ($g \cdot kg^{-1}$)	31.42a	18.65b	25.99a	12.44b	26.26a	17.62b	22.24a	14.00b	24.54a	17.61b	24.45a	24.85a
P ($mg \cdot dm^{-3}$)	428.4a	162.9b	584.3a	101.6b	610.5a	374.6b	521.7a	276.4b	1026.8a	422.9b	792.5a	344.5b
Ca^{2+} ($cmol_c \cdot dm^{-3}$)	7.70a	3.08b	6.20a	2.48b	5.64a	2.26b	4.64a	1.86b	7.39a	2.96b	8.29a	3.31b
Na^{+} ($cmol_c \cdot dm^{-3}$)	0.22a	0.08b	0.16a	0.07b	0.63a	0.08b	0.17a	0.04b	0.30a	0.10b	0.24a	0.21b
K^{+} ($cmol_c \cdot dm^{-3}$)	0.48a	0.52a	0.55a	0.61a	1.01a	0.60b	0.84a	0.20b	0.73a	0.25b	1.58a	0.13b
Mg^{2+} ($cmol_c \cdot dm^{-3}$)	1.84a	1.54b	1.92a	1.78a	2.32a	1.87a	1.72b	0.65a	3.91a	1.92b	1.68a	1.88a
H+ Al ($cmol_c \cdot dm^{-3}$)	1.05a	0.83a	1.38a	0.50b	1.16a	0.83a	0.99a	0.58b	1.57a	1.16b	1.63a	1.32a
SB ($cmol_c \cdot dm^{-3}$)	9.94a	5.52b	8.81a	4.94b	9.61a	4.81b	6.30a	3.83b	12.32a	5.23b	11.79a	5.53b
CEC_{total} ($cmol_c \cdot dm^{-3}$)	10.98a	6.35b	10.18a	5.44b	10.76a	5.64b	7.29a	4.41b	13.89a	6.40b	13.42a	6.85b
V (%)	90.53a	86.93a	85.71b	90.75a	89.02a	85.29a	86.42a	86.85a	88.69a	81.81b	87.22a	80.73a
ESP (%)	2.05a	1.26b	1.68a	1.29b	5.98a	1.42b	2.43a	1.20b	2.17a	1.56b	1.77a	6.29b
Sand ($g \cdot kg^{-1}$)	872	857	808	832	828	857	880	867	863	884	785	733
Silt ($g \cdot kg^{-1}$)	49	35	73	41	55	33	10	66	54	28	62	83
Clay ($g \cdot kg^{-1}$)	79	108	119	128	117	110	110	67	83	88	153	206

V1–V6: vineyards located in the irrigated area from Petrolina (PE), Brazil; OC: organic carbon; OM: organic matter; CEC: cation exchange capacity; SB: sum of bases, V (%): base saturation; ESP: exchangeable sodium percentage; *means followed by same letter (between cultivated areas and fallow areas for the same attribute) not differ significantly by Tukey's test ($P < 0.05$).

P fractionation

Pi contents extracted by 0.5 $mol \cdot L^{-1}$ of $NaHCO_3$ were significantly higher or equal ($p < 0.05$) in CA compared to FA (Fig. 1). This kind of P is considered labile, i.e., available for plant absorption or mobile with percolation in the soil profile, leading an effective damage with groundwater contamination (Pizzeghello et al. 2011, Schmitt et al. 2013). We emphasize that, in addition to high contents of labile Pi due to excessive use of phosphate fertilizers, the sandy texture of these soils can contribute to more P-lost (Guardini et al. 2012, Couto et al. 2016).

Po contents extracted by 0.5 $mol \cdot L^{-1}$ of $NaHCO_3$ contents were higher ($p < 0.05$) in CA 2 and 5 than in FA (Fig. 1). In V1, the labile Po contents were higher in FA, showing that this fraction is driven by the activity of microorganisms that mineralize P in soil organic matter more effectiveness than other treatments (Gatiboni et al. 2008, Turner et al. 2013). In addition, organic P hardly accumulates in the labile condition, as it is a very dynamic fraction in the soil, where P can be mineralized or converted into other more stable compounds (Darilek et al. 2010, Bünemann 2015).

The levels of Pi extracted with NaOH 0.5 $mol \cdot L^{-1}$ were higher ($p < 0.05$) in CA, except for V6 (Fig. 1) probably due to the lower proportion of organic residues in that soil in the moment of sampling. This kind of P is considered moderately labile, i.e., represents inorganic and organic P physically protected within the aggregates and bounds to more resistant organic compounds, with intermediate binding energy (Cross and Schlesinger 1995). In Southern Brazil, a sandy vineyard soil and fertilized with P for 30 years also had a moderately labile Pi accumulation compared to the reference soil (Schmitt et al. 2013), although these results are roughly 100 times lower compared to our data. On the other hand, the levels of Po extracted by NaOH 0.5 $mol \cdot L^{-1}$ were higher ($p < 0.05$) in FA 1, 3 and 4 than in CA (Fig. 1). This fraction is mainly constituted by humic and fulvic acids, and the latter can be considered more labile and with greater P availability (Schroeder and Kovar 2006).

In general, the contents of Pi and Po extracted by HCl 1.0 $mol \cdot L^{-1}$ were significantly higher ($p < 0.05$) in CA (Fig. 1). These fractions are non-labile and represent the inorganic forms of P associated with Ca (calcium phosphate) or strongly adsorbed to soil colloids (Ceretta et al. 2010, Schmitt et al. 2014). Due to the low content of Fe oxides in soil of both CA or FA, the increase in this fraction is related to the high application of fertilizers based on P_2O_5 and to the neutral-alkaline pH (Table 1), which favors the precipitation of this compound (Vu et al. 2008). Over time, the tendency is to decrease the labile forms of P, increasing the inorganic fractions (Pavinato et al. 2010), and this can reduce greater risks of P leaching to the water bodies of the São Francisco basin.

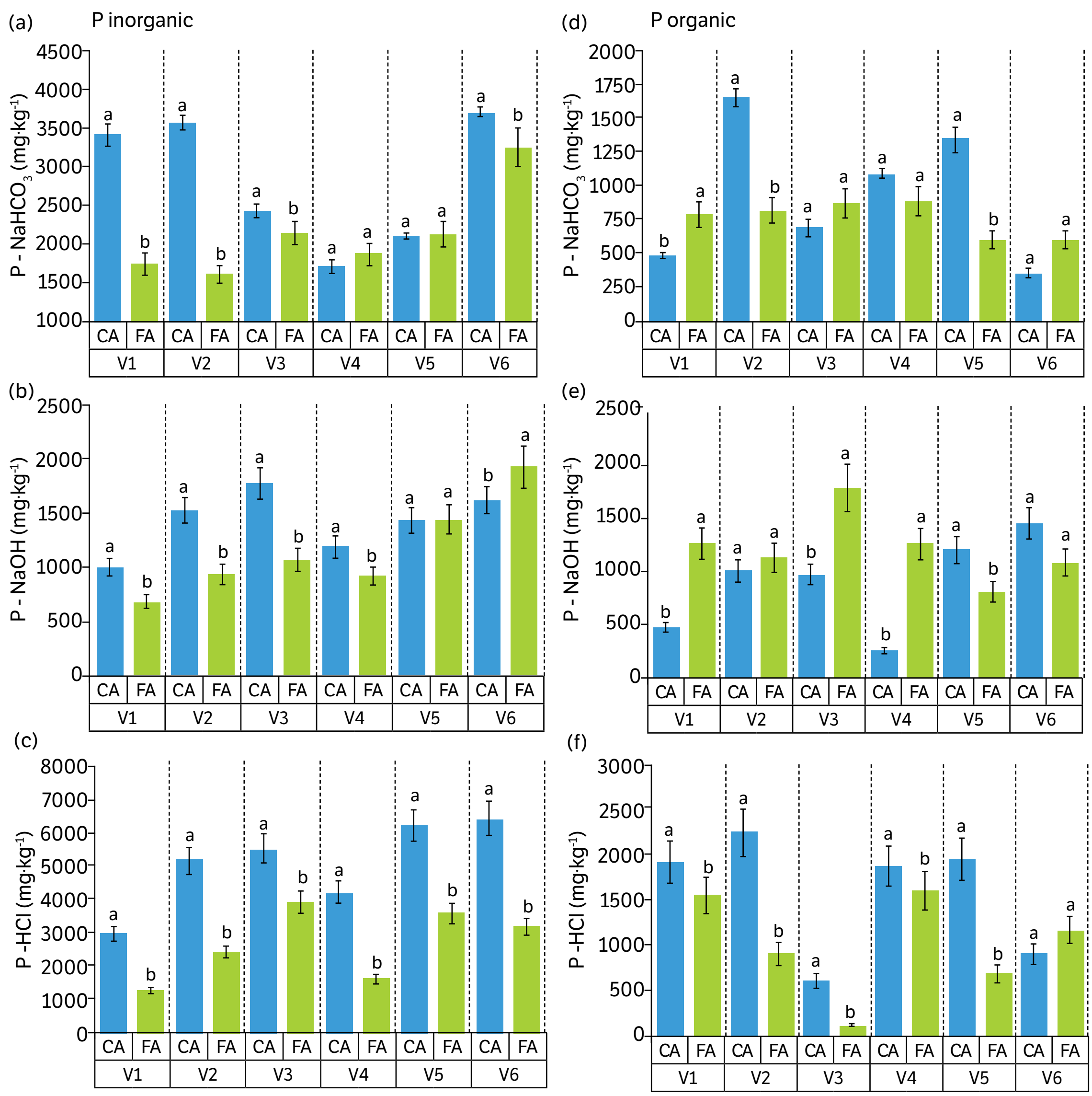

Figure 1. Fractionation of phosphorus Hedley from soils cultivated with grapes (CA) and in fallow areas with five years without cultivation (FA) in the São Francisco Valley.

Residual P contents were higher ($p < 0.05$) in FA (Fig. 2), but a small increase in this fraction was observed, reaching 2.6 and 3% of the total-P in CA and FA, respectively, indicating that the P added via fertilization did not influence on this fraction. Residual P represents the inorganic and organic forms of recalcitrant P, of low soil lability, which does not actively participate in the availability of P to the plants (Cross and Schlesinger 1995, Gatiboni et al. 2008). Soil residual P content depends on the content and type of clay, i.e., more clayey soils show higher P retention of this fraction (Ceretta et al. 2010, Guardini et al. 2012). Particularly, the presented clay contents below 20% (Table 1), and this justifies the low percentage of residual P between the areas with and without cultivation.

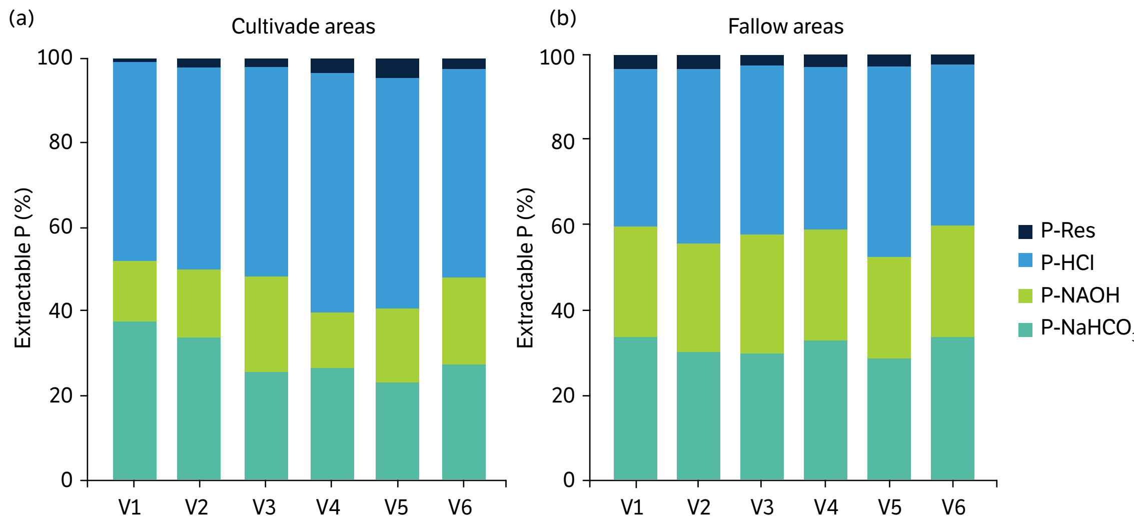

Figure 2. Extractable phosphorus in areas cultivated with grapes (CA) and in fallow with five years without cultivation (FA) in the São Francisco Valley.

In general, there was an increase ($p < 0.05$) in total P contents in CA than in FA. The predominant forms of organic P in CA were also non-labile (45% of total Po), followed by labile (29.5%) and moderately labile Po (25.5%). Similar condition was observed in FA, where the main forms of Pi were non-labile (44.8% of total Pi), labile (35.7%), and moderately labile (19.5%). In CA, the predominant forms of Pi were non-labile (55.1% of total Pi), followed by labile forms (29.5%), with lower participation of moderately labile Pi (15.5%) (Fig. 2). The predominant forms of organic P were moderately labile (41% of total Po), non-labile (33.7%) and labile (25.3%). These results were justified by the excess of phosphate fertilizers that increased the levels of Po and Pi in all soil fractions.

The fractionation results showed that the inorganic P in CA contributed with 70.5% of the total P, while 26.9% was in the organic form. In FA, inorganic P represented 64.6% of the total P accounted for 32.5%. Residual P was similar in both treatments with and without cultivation (2.6% and 3%, respectively). The inorganic fractions surpassed the organic fractions of P, similarly to Preston et al.'s (2016) report. This indicates that the level of inorganic P depends on its stock in the soil, which can be increased through fertilization used, while the contents of organic P in the soil depend on other environmental factors that promote its mineralization.

Microbiological indicators

P of microbial biomass (P-mic) ranged from 6.45 to 8.44 $mg{\cdot}kg^{-1}$ in CA and 3.38 to 6.54 $mg{\cdot}kg^{-1}$ in FA (Fig. 3) and was higher in vineyards 1, 2, 3 and 4 comparing to the other one ($p < 0.05$), where it was positively correlated with soil pH, available P, sum of bases, soil organic carbon, Pi of the labile and non-labile fraction and with Po of the moderately labile fraction (Table 2). P-mic represents a very dynamic fraction of soil total-P, it is influenced by temperature, humidity, and carbon availability, and its release occurs as orthophosphate and in organic forms, which are rapidly mineralized in the soil (Achat et al. 2010). Particularly, in sandy soils there is a low adsorption of organic-P, which is significantly increased by its rapid mineralization (Oehl et al. 2001).

Soil glomalin contents (EE-GRSP) ranged from 0.044 to 0.052 $mg{\cdot}g^{-1}$ in CA and 0.024 to 0.038 $mg{\cdot}g^{-1}$ in FA (Fig. 3). Vineyards 1, 2 and 3 showed significantly higher contents of EE-GRSP ($p < 0.05$) comparing to FA. In general, EE-GRSP was positively correlated to available P, sum of bases, soil organic matter, Pi of labile and non-labile fraction, Po of moderately labile and non-labile fraction (Table 2). However, it was smaller than the results reported in cultivated and fertilized (Banegas et al. 2022), degraded (Luna et al. 2016) and revegetated (Yu et al. 2017) tropical semi-arid soils, probably due to the high concentrations of P in that soil, since phosphate fertilization reduces the biomass of mycorrhizal fungi, which are directly responsible for the availability of P to plants (Qin et al. 2015).

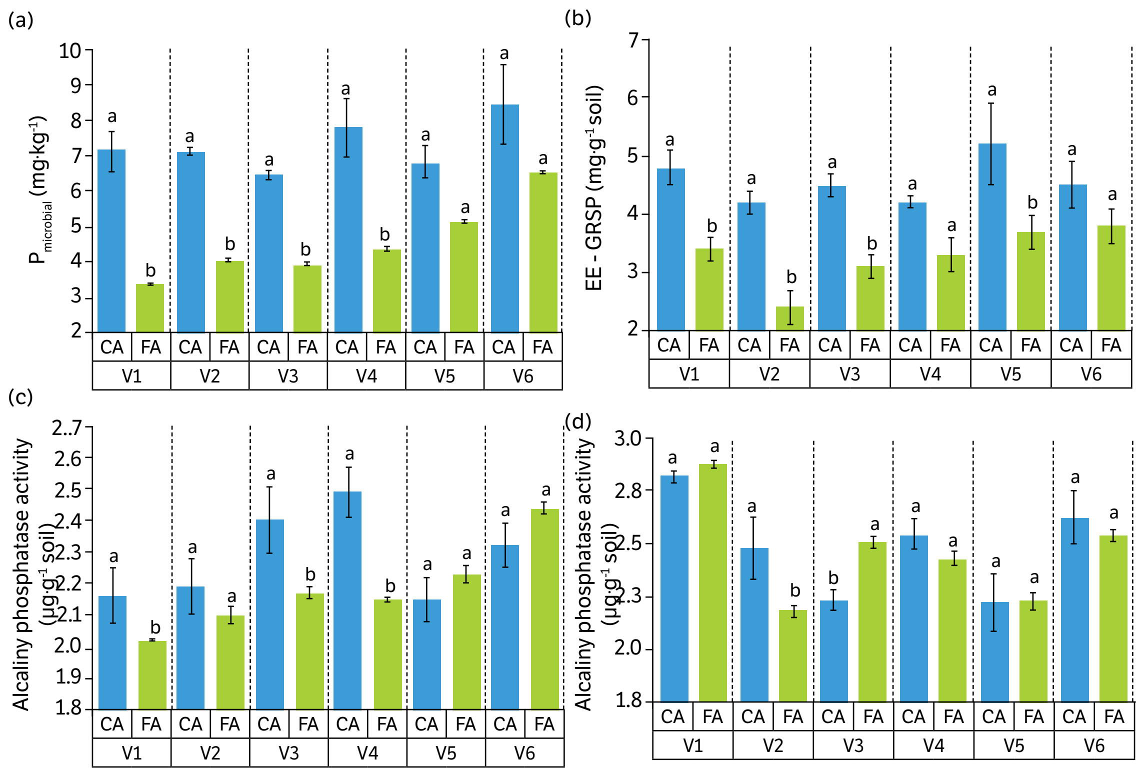

Figure 3. Microbiological indicators of soils in areas cultivated with grapes (CA) and in fallow with five years without cultivation (FA) in the São Francisco Valley.

Table 2. Pearson's linear correlation matrix between microbiological variables indicating soil quality and fertility parameters, the environmentally available levels of metals and phosphorus speciation of cultivated areas (CA) with grape and fallow area (FA) in Vale do São Francisco*.

	P-mic	Acid phosphatase	Alkaline phosphatase	EE-GRSP
pH	0.57	-0.36	0.41	0.27
P	0.61	-0.33	0.14	0.72
SB	0.62	-0.06	0.03	0.76
OM	0.74	0.29	0.38	0.81
ESP	0.42	-0.10	0.44	0.36
$P_i - NaHCO_3$	0.65	0.26	0.43	0.57
$P_o - NaHCO_3$	-0.03	-0.23	-0.23	0.11
$P_i - NaOH$	-0.47	-0.04	-0.26	-0.33
$P_o - NaOH$	0.66	-0.33	0.69	0.42
$P_i - HCl$	0.65	-0.43	0.28	0.57
$P_o - HCl$	0.29	0.17	-0.14	0.45
P_{res}	0.12	-0.49	-0.16	0.20
Cu	0.39	0.46	0.20	0.27
Pb	-0.13	0.00	0.17	-0.32
Cd	-0.15	-0.03	-0.13	-0.08
Cr	0.22	0.11	0.49	-0.11
Zn	0.48	-0.01	0.31	0.25

OM: organic matter; SB: sum of bases; ESP: exchangeable sodium percentage; EE-GRSP: glomalin-related soil protein easily extractable; *values in bold and italics indicate significant correlation at 5% probability.

The values of acid phosphatase activity in V4 (2.43 and 2.46 μg p-nitrophenol·g^{-1} soil·h^{-1} in CA and Fa, respectively) showed a negative and significant correlation ($p < 0.05$) with pH, P available, Pi of the non-labile fraction, and P residual (Table 2). Alkaline phosphatase (2.23 and 2.19 μg p-nitrophenol·g^{-1} soil·h^{-1} in CA and FA, respectively) showed a positive and significant correlation ($p < 0.05$) with pH, soil organic matter, Pi of the labile fraction and Po of the moderately labile fraction. The activity of both phosphatases can be used as an indicator of the potential for mineralization of organic P in the soil, as the primary producers of acid phosphatase are plant roots and soil microorganisms, while alkaline phosphatase is produced only by microorganisms' activities (Spohn and Kuzyakov 2013).

Alkaline phosphatase activity can be significantly affected by different long-term fertilization regimes, increasing with the addition of organic matter (Zhang et al. 2014, Luo et al. 2017) and decreasing with the presence of mineral P (Spohn et al. 2015). Regardless of the vineyard with or without cultivation, the activity of both phosphatases in the soil was much lower than that reported by other studies in semi-arid regions (Tian et al. 2016, Silva et al. 2018), probably due to the high concentrations of P from fertilization.

CONCLUSION

The management conditions and the use of phosphate fertilizers in soils with vines in the tropical semi-arid region increased soil fertility comparing to FA. In addition, the inorganic P fractions surpassed the organic fractions, as the total P contents are mainly associated with the non-labile fraction, reducing greater risks of P leaching to the water bodies of the São Francisco basin.

P of microbial biomass in vine cultivation was higher than in fallow soils, and the high demand for phosphate inputs positively affect the biological activity of phosphatase enzymes and the total availability of glomalin, considerably affecting the biological quality of these soils.

AUTHORS' CONTRIBUTION

Conceptualization: Fracetto, G. G. M., Freitas, E. M., Nascimento, C. W. A. and Silva, D. J.; **Methodology:** Freitas, E. M., Fracetto, G. G. M., Fracetto, F. J. C., Buzó, L. H. N. and Medeiros, E. V.; **Investigation:** Freitas, E. M., Fracetto, G. G. M., Fracetto, F. J. C. and Buzó, L. H. N.; **Formal Analysis:** Freitas, E. M., Silva, W. R. and Silva, F. B. V.; **Funding:** Fracetto, G. G. M., Nascimento, C. W. A. and Silva, D. J.; **Project Administration:** Fracetto, G. G. M. and Nascimento, C. W. A.; **Supervision:** Fracetto, G. G. M. and Nascimento, C. W. A.; **Writing – Original Draft:** Fracetto, G. G. M., Freitas, E. M., Nascimento, C. W. A., Fracetto, F. J. C. and Silva, W. R.; **Writing – Review and Editing:** Fracetto, G. G. M., Nascimento, C. W. A., Fracetto, F. J. C., Silva, W. R. and Silva, F. B. V.

DATA AVAILABILITY STATEMENT

All dataset were generated and analyzed in the current study.

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