FERTILIZER-INDUCED GEOCHEMICAL FRACTIONS OF PHOSPHORUS IN FRUIT ORCHARDS

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Abstract: Phosphorus (P) is essential to all known life forms because it is a key element in many physiological and biochemical processes. In this study, different physiographic units of Atabey Plain have developed soils with varying characteristics. The variability in land use and fertilizer input management causes changes in the availability and geochemical fractions of phosphorus. In this study, 29 surface soil samples (0-20 cm) were taken from various fruit orchards to investigate the plant cover-related inter-fraction mobility and availability of P as a function of soil characteristics. Sequentially extracted geochemical P fractions were: sodium bicarbonate [$NaHCO_3$-P (organic Po, inorganic Pi, and Total Pt)], sodium hydroxide [NaOH-P (Po, Pi, and Pt)], citrate bicarbonate dithionite (CBD-P), hydrochloric acid (Ca-P), and residual (Res-P) phosphorus. In order to study the relationship of phosphorus fractions with soil properties, chemometric analyses were performed. According to the results, the average concentrations of the fractions in descending order were: Ca-P (200-1029 mg kg^{-1}) > Res-P (55.3-449 mg kg^{-1}) > CBD-P (56.5-204 mg kg^{-1}) > NaOH-Pi (26.0-206 mg kg^{-1}) > $NaHCO_3$-Pi (4.38-93.4 mg kg^{-1}) > NaOH-Po (0.55-30.0 mg kg^{-1}) > $NaHCO_3$-Po (0.26-7.56 mg kg^{-1}). The added fertilizer P was likely to be converted to NaOH-P in the short term and Ca-P and/or CBD-P for a relatively longer time. It was determined that the fractal distribution of these fractions varied according to the land use pattern and soil properties such as texture, organic matter, carbonates, and iron and manganese oxide minerals.

Keywords: Soil phosphorus, sequential extraction, soil properties, fruit orchards

1. INTRODUCTION

Phosphorus (P) is one of the plant nutrients limiting crop yield, especially in calcareous soils of arid regions. Therefore crop- or growth season-based continuous application of P fertilizers is commonly practiced (Zheng et al., 2004; Miller & Fox, 2011; Herrera et al., 2016). It is known that, the fruit orchards have continuously received higher P fertilizers to maintain sustainable yield and quality. However, most of the farmers in the region do not practice regular soil analysis for optimal fertilization. This environmentally fatal mistake has resulted in elevated levels of available P (Olsen P) in the region's soils (Durgun et al., 2017; Akgül et al., 2001). This practice can result in P enrichment in the surface horizons resulting its availability and partition among different geochemical fractions to change significantly (Uygur et al., 2017). Surface accumulation of P can have negative environmental impacts through increased leaching of the soluble P and runoff of P-rich particulates toward water resources (Skoulikidis & Amaxidis, 2009; Sharma et al., 2017; Paltineanu et al., 2021; Paltineanu et al., 2022; Sedratı et al., 2022).

During the soil formation, thermodynamically stable P minerals such as apatite (Lindsay, 2001) have been transformed into organic-P, non-occluded P, and occluded P as suggested in the conceptual model of Walker & Syers (1976). In contrast, soluble P forms in fertilizers are converted into more stable P compounds by adsorbing on oxide mineral surfaces

through inner-sphere complexes (Rodrigues et al., 2016) or reacting with Ca and Fe (Uygur et al., 2017).

In the short term, iron oxides were reported to be responsible for the removal of 70-85% of fertilizer P as moderately labile and non-labile forms in Oxisols (Rodrigues et al., 2016), whereas the ultimate chemical fraction of P in calcareous soils, especially those of low-P containing were Ca bound-P (Delgado & Torrent, 2000; Uygur & Karabatak, 2009). This fraction was related to soil carbonates and readily available Ca concentration of soil solution (Akinremi & Cho, 1991a, b; Tunesi et al., 1999). Either oxide-rich weathered soils or calcareous soils suffer from very low efficiency (mainly 15-25%) of fertilizer P in the application year. Continuous application of high amounts of fertilizer P in such soils set-up new equilibrium among the geochemical P fractions (Zheng et al., 2004; Koopmans et al., 2007; Miller & Fox, 2011; Amaizah et al., 2012; Herrera et al., 2016) even in a relatively short time (Uygur & Karabacak, 2009). Therefore, the objective of this study was: i) to evaluate the effect of cover crops, mainly fruits, on P fractions determined by a sequential extraction procedure that is a combination of Kuo (1996) and Hedley et al., (1982) methods and ii) to relate P pools with the soil properties.

2. MATERIAL AND METHODS

2.1. Site description

Atabey Plain that is the study area of this work, is the most significant agricultural area in the Isparta province of Türkiye. It is well-known for various fruit production, especially sweet cherry, apple, peach, and other fruits. The region's climate is specific for apple and sweet cherry; therefore, the best quality fruits are produced in this province. The regular application of commercial and organic fertilizers has relatively preserved the productivity of the orchards.

2.2. Descriptive soil properties

Soil sampling was taken from the 0-20 cm range where the fertilizer has been given, homogenised by regular soil tillage practices and phosphorus is virtually immobile (Kaçar 2013). 29 surface soil samples were taken from fruit orchards (5 apple, 6 peach, 13 sweet cherry, 3 pine, and 2 walnut-orchard soils). Soil samples under pine cover was taken as the control representing non fertilization soil. The samples were air-dried and sieved through a 2 mm screen for descriptive soil analyses, and sequential fractionation of P. Soil physical and chemical properties were determined by commonly used methods for calcareous soils (Sparks et al., 1996). The fraction of clay, silt, and sand-sized particles was determined by a hydrometer method after physical and chemical dispersion procedures. EC and pH of the soils were determined in the saturation paste by EC probe and combined pH electrode, respectively. The organic matter content of soils was determined by the dichromate oxidation method. The available P was extracted by 0.5 M $NaHCO_3$ at pH 8.5 using a solution-to-soil ratio of 20:1. Ammonium acetate extractable (1 M, pH 7) cations (Ca, Mg, Na, and K) and DTPA extractable (0.005 M DTPA + 0.01 M $CaCl_2$ + TEA, triethanolamine, at pH 7.3) micronutrients (Fe, Cu, Mn, and Zn) were determined by ICP-OES (Perkin-Elmer, Optima 2100). Cation exchange capacity was determined by Na saturations of exchange sites, followed by NH_4 replacement for Na. Iron and manganese (Mn) oxides were also chemically fractioned as amorphous and total, as suggested by Shuman (1985). Free Mn oxides were also solubilized by hydroxilaminhydrochloride (Chao, 1972). The soils were digested with aqua-regia (3:1 V/V, HNO_3: HCl) then Fe, Mn, and Zn concentrations of the digests were determined by the ICP-OES. The descriptive statistics for measured soil characteristics are given in Table 1.

2.3. Sequential extraction of phosphorus

Soils were subjected to sequential P fractionation procedures using a modified version of Hedley et al., (1992) and Kuo (1996). One gram of soil samples in triplicates was weighed into 50 mL centrifuge tubes, and the fractions from most soluble to least soluble were sequentially solubilized. The outline of the procedures and related geochemical phases are given below.

1. Labile or plant available P ($NaHCO_3$-P) soil extracted with 0.5 M $NaHCO_3$ solution for 30 min on a reciprocal shaker. This fraction was imparted to organic and inorganic P by determining the readily molybdate reactive P, which was regarded as inorganic P and total P after acid digestion. The difference between the molybdate reactive and total was considered as organic P.

2. Moderately labile P (NaOH-P), the residues from the previous fraction were treated with 25 mL of 0.1 M NaOH at a 1:25 soil solution ratio for 16 h on the shaker. As in the previous stage, this fraction was also chemically divided into organic and

Table 1. Descriptive soil properties

Parameters	Range	Min	Max	Mean	Std. Deviation	Variance	Skewness Raw data	Skewness Transformed data	Kurtosis Raw data	Kurtosis Transformed data*
T-Zn (mg kg^{-1})	42.9	47.6	90.5	63.6	8.49	72.1	1.272	0.130*	2.85	1.054*
OM (%)	2.60	0.65	3.25	1.99	0.51	0.26	-0.36	-0.357	2.15	2.149
CCE (%)	34.9	0.66	35.6	8.42	8.12	65.9	1.471	-0.173*	2.96	-1.407*
CEC (cmol kg^{-1})	34.9	17.1	52.0	33.8	9.83	96.5	0.041	0.041	-0.65	-0.648
pH	1.25	6.78	8.03	7.68	0.31	0.10	-1.67	-1.672	2.27	2.270
EC	368	105	473	267	104.1	10825	0.441	0.441	-0.58	-0.585
Ca (cmol kg^{-1})	29.8	6.90	36.7	23.8	8.36	69.85	-0.51	-0.509	-0.49	-0.498
K (cmol kg^{-1})	2.58	0.48	3.06	1.56	0.72	0.52	0.713	0.713	-0.62	-0.622
Mg (cmol kg^{-1})	13.1	2.71	15.8	6.20	3.05	9.30	1.610	0.561*	2.77	-0.012*
Na (cmol kg^{-1})	2.07	0.08	2.15	0.61	0.36	0.13	2.764	-0.718*	12.5	1.966*
P (mg kg^{-1})	214	2.50	216	41.9	41.1	1691	2.920	-0.194*	11.3	0.540*
Sand (g kg^{-1})	641	54.1	695	399	163	26565	0.022	0.022	-0.18	-0.187
Silt (g kg^{-1})	287	118	405	236	76.6	5872	0.350	0.350	-0.79	-0.798
Clay (g kg^{-1})	490	167	657	364	124	15468	0.197	0.197	-0.52	-0.516
MnOx(mg kg^{-1})**	162	77.0	239	154	37.7	1424	0.039	0.039	-0.18	-0.180
AMnOx(mg kg^{-1})	3268	49.0	3317	212	598	357833	5.358	-0.161*	28.8	-0.855*
TMnOx(mg kg^{-1})	3376	276	3652	584	622	386902	4.668	-0.525*	23.0	-0.212*
AFeOx (mg kg^{-1})	7424	1017	8441	2259	1395	1945142	3.361	0.287*	14.0	-0.166*
TFeOx (mg kg^{-1})	13051	11725	24776	18049	3457	11953116	0.274	0.274*	-0.62	-0.622*

* Data sets were log-transformed. Standard errors of skewness and kurtosis are 0.434 and 0.845 for N=29, respectively. CCE: calcium carbonate equivalent, OM: organic matter, CEC: cation exchange capacity, T: total, A: amorphous, ** Free Mn oxides.

3. inorganic. This fraction extracts P adsorbed on the Fe and Al (hydr)oxide surfaces.
4. Reducible P (CBD-P) residues from the second fractions were reacted with a citrate-bicarbonate-buffer system as described by Kuo (1996). This fraction solubilizes the P occluded with mainly Fe oxides.
5. Ca-bound P (Ca-P), the residues from the preceding extraction, were extracted with 1 M HCl for 16 h on a reciprocal shaker. This fraction dissolves sparingly soluble calcium phosphate precipitates.
6. Residual P (Res-P) the residues from the forgoing fractionation were acid digested with concentrated HNO_3-$HClO_4$ mixture (3:1, V/V).

After the extraction procedure of stages 1-4, the supernatants were separated by centrifugation. Then residues were rinsed with saturated NaCl solution and combined with the main extracts to minimise fractional contamination. The P concentrations of the supernatants were colourimetrically determined using the ascorbic acid method (Murphy & Riley, 1962). Therefore, before adding colour reagent into the supernatants of fraction-3, 1.5 mL 5% ammonium molybdate solution was pipetted to air-oxidised aliquots for 25 mL volumetric flasks (Weaver, 1974) to improve colour development.

2.4. Statistical analysis

The data were initially subjected to descriptive statistics to test the distribution of the parameters (Table 1). Any parameters showing high skewness (skewness $\geq 2 \times$ standard error) (Berkman & Reise, 2012) were log-transformed to maintain or approximate typical normal distribution because of very high positive skewness. The data were then subjected to further statistical analysis such as principal component analysis (PCA). The PCA was utilized to reveal soil parameters' effect on P partitioning among geochemical fractions. All statistical studies were carried out using R 4.2.1. package program (Team, 2013).

3. RESULTS AND DISCUSSION

Descriptive statistics for phosphorus fractions in different fruit orchards' surface soils are given in Table 2. Most of the fractions, especially labile and moderately labile, showed typical positively skewed distribution with skewness and/or kurtosis value $\geq 2 \times$ their respective standard errors. In contrast, CBD-P and Ca-P fractions had a typical normal distribution. The higher skewness values observed, the more mobile fractions indicate that the fruit orchards receive the more excessive P fertilizer. The inorganic fractions had a very high portion of the mobile and moderately labile

P fractions (Table 2). Fruit orchards had higher concentrations of labile or moderately labile inorganic P. NaHCO$_3$-Pi, and NaOH-Pi concentrations in the fruit orchards were 0.87-2.42 and 1.28-2.33 folds.

Interestingly the CBD-P and Ca-P fractions' data sets were normally distributed. These fractions can be the result of the continuous conversion of fertilizer P under excessive fertilization practice in the studied soils. Most examined soils may attain P saturation maxima when mobile fractions increase. (Table 3). The total fraction also indicated that fruit orchards have 1.81-2.15 fold higher P, which is also considered as supporting data of excessive P fertilization in the region. Typical total P in slightly weathered soils such as Entisols (580 mg P kg^{-1}) and Inceptisols (492 mg P kg^{-1}) are highly similar to the juniper soils (587 mg kg^{-1}), which was classified as Inceptisol (Akgül et al., 2001). The added fertilizer P, therefore, tended to accumulate Ca-P and CBD-P. As similar amounts of fertilizer, addition is practiced under the conditions governing the occurrence of either Ca-P or CBD-P; thus, only slightly skewed data can be obtained. Ca-P is considered the stable pool for P added by agricultural practices (Lindsay, 2001) in calcareous soils; even for moderately acid (Zheng & MacLeod, 2005) and peat-derived calcareous soils (Schlichting et al., 2002). The experimental soils have formed on calcareous parent material (Akgül et al., 2001) and have a pH range of 6.78-8.03 (Table 1), which is typically the abovementioned similar literature. CBD-P becomes the thermodynamically stable fraction upon weathering (Lindsay, 2001; Yang & Post, 2011), which may be related to the lower ratio (Fruit orchards) observed for CBD-P concentrations than the ones obtained for Ca-P.

Principal component (PC) analysis reveals the relationships between commonly observed soil characteristics and phosphorus fractions.

The PC analysis using a correlation matrix with a varimax rotation routine identified 7 components with an Eigenvalue above 1.00 (Table 4). These rotating components (PC1-7) explain individually 0.24, 0.21, 0.11, 10.44, 0.08, 0.07, 0.06, and 0.04 of the total variances, respectively, summing up 80% of the total variance in the soils. Five of the 7 routinely extracted components correspond closely to clear conceptual groupings of the P fractions and some soil properties affecting the respective fractions (Table 5). PC1 explains the effect of soil's physical properties. PC2 had very high positive loadings with Ca-P and T-P, NaHCO$_3$-Pt, and, NaHCO$_3$-P. The extractable P concentration and clay, MnOx, AFeOx, TFeOx, and TMnOx had positive relation, with sand negative relation. pH had relations with the PC4 that showed negatively loadings for Ca-P and Res-P forms. PC3 describes the relationship between general soil properties and phosphorus fractions. Organic matter, EC, K, P, TMnOx and AMnOx negatively affect NaHCO$_3$-Pi, NaHCO$_3$-Po, NaOH-Pt, and NaOH-Pi. The PCs may extract some degree of information about possible inter-fraction mobility of P in soils as well as the effect of soil parameters.

Table 2. Descriptive statistics for geochemical phosphorus (P) fractions

P fractions (mg kg^{-1})	Range	Min	Max	Mean		Std. Deviation	Variance	Skewness		Kurtosis	
				Statistic	Std. error			Raw data	Trans. data	Raw data	Trans. data
NaHCO$_3$-Pt	92.4	8.54	101	18.8	3.15	17.0	287.4	4.356**	0.755*	21.1**	0.298*
NaHCO$_3$-Pi	89.0	4.38	93.4	16.7	2.97	16.0	255.9	4.212**	-0.054*	20.1**	0.870*
NaHCO$_3$-Po	7.29	0.27	7.56	2.07	0.41	2.194	4.814	1.518**	0.491	1.06**	-0.785
NaOH-Pt	178	32.3	210	72.3	7.12	38.4	1472	2.027**	0.596*	4.94**	-0.401*
NaOH-Pi	181	26.0	207	64.4	6.79	36.5	1335	2.437**	0.507*	7.59**	-0.217*
NaOH-Po	29.4	0.55	30.0	7.92	1.58	8.493	72.13	1.344**	0.091	0.608**	-0.965
CBD-P	147	56.5	204	125	7.77	41.9	1753	0.221	0.221	-0.792	-0.792
Ca-P	828	200	1029	545	39.4	212	44922	0.250	0.250	0.092	0.092
Res-P	394	55.3	449	205	19.6	105	11089	0.908	-0.062	-0.184	-0.164
Tot-P	1249	560	1809	1056	60.5	326	106310	0.617	0.617	0.252	0.252

*a flier was excluded for obtaining normal distribution, ** log transformation was applied to the data set. Standard errors of skewness and kurtosis are 0.434 and 0.845 for N=29, respectively. Pt total, Pi inorganic, and Po organic fractions; CDB-P citrate-bicarbonate-dithionite extractable P, Ca-P HCl extractable P, Tot-P total of individual fractions.

Table 3. Distribution of geochemical fractions (mg kg^{-1}) in different fruit orchards

	NaHCO$_3$-P			NaOH-P			CBD – P	Ca – P	Res – P	Tot- P
	Pt	Pi	Po	Pt	Pi	Po				
Apple	14.3	12.6	1.7	71.9	64.9	7.0	134.1	522.8	232	1061
Peach	32.1	28.8	3.3	91.2	88.0	3.2	127.2	627.4	184	1201
Pine	13.27	11.94	1.33	45.7	37.7	8.00	94.3	203.7	170.5	587
Sweet cherry	17.7	15.6	2.1	76.2	65.2	11.0	123.4	569.4	196	1075
Walnut	11.8	10.4	1.4	53.6	48.2	5.4	148.9	679.4	294	1261

Table 4. The explained variances by the extracted principal component (PC)

PCs	Standard Deviation	Proportion of Explained Variance	Cumulative Proportion
1	2.58	0.24	0.24
2	2.41	0.21	0.45
3	1.75	0.11	0.55
4	1.51	0.08	0.64
5	1.35	0.07	0.70
6	1.25	0.06	0.76
7	1.12	0.04	0.80

Table 5. Loadings between the extracted components

Soil Parameters	Components						
	1	2	3	4	5	6	7
$NaHCO_3$-Pt		0.372	-0.828				
$NaHCO_3$-Pi		0.387	-0.835				
$NaHCO_3$-Po			-0.510				
NaOH-Pt			-0.652				
NaOH-Pi			-0.699				
NaOH-Po							
CBD-P					0.323		
Ca - P		0.554		-0.748			
Res-P				-0.674	0.317		
T-P		0.557					
T-Zn							
OM			-0.499				
CCE					0.470		
CEC				-0.328	0.636		
pH				-0.419			
EC			-0.382	-0.378	0.506		0.634
Ca					0.717		
K			-0.303	-0.337	0.430		
Mg				-0.713	0.362		
Na							0.564
P		0.516	-0.668				
Sand	-0.348				-0.880		
Silt					0.759		
Clay	0.549	-0.361			0.685		
MnOx	0.426				0.489		
AMnOx		0.415	-0.897				
TMnOx		0.351	-0.912				
AFeOx	0.324	0.933					
TFeOx	0.999						

*Loadings below 0.3 were suppressed. T: total, A: Amorphous, Pi: inorganic phosphorus (P), Po: organic P, Pt: total P, Ca-P: carbonate bound P, CBD-P: citrate-bicarbonate-dithionite extractable P, Res-P: residual P.

The scatter plot of PC 1 versus PC2 was given in Figure 1, which visualises the effects of soil properties (PC1) and Ca-P, T-P, $NaHCO_3$-Pt, and $NaHCO_3$-Pi (PC2). Pe4 soil sample stands out from the other soils mainly due to the parameter having negative loading (sand -0.348) with the PC1. The soils on the negative side of PC1 generally have high sand content, which is suggesting that this textural size consists of significant amounts of carbonates and NH_4-Ac extractable Na concentration. The soil parameters negatively loading with PC2 separated Ap1, Sc1, Sc2, and Sc9 soils according to clay. The soils on the negative side of the PC1 had comparatively higher amounts of Ca-P, T-P and $NaHCO_3$-Pi despite their very high sand content. Sc11, Sc13, Sc7, Ap5, Pe3, and Pe7 have been separately grouped. PC1 and PC2 positively grouped area where clay, MnOx, AFeOx, and TFeOx parameters having positive loadings with PC2 and positive loadings with PC1. Pe6 and SC12 fell apart depending on the Ca-P, T-P, and $NaHCO_3$-Pi. Since both PCs were related to NH_4-Ac extractable Na, the solubilising effect of Na on Ca-carbonates (Coto et al., 2012) may have a critical role in forming Ca-P fraction in the soils. On the other hand, the soils forming on calcareous parent materials such as calcareous alluvium, calcareous colluvium, travertine,

and calcareous and gypsum base were that the experimental soils formed on Mesozoic calcareous parent materials (Akgül et al., 2001). On the other hand, the presence of carbonates and/or Na-induced increased solubility of Ca drives thermodynamically stable sparingly soluble P fractions in soils (Lindsay, 2001; Uygur & Karabatak, 2009).
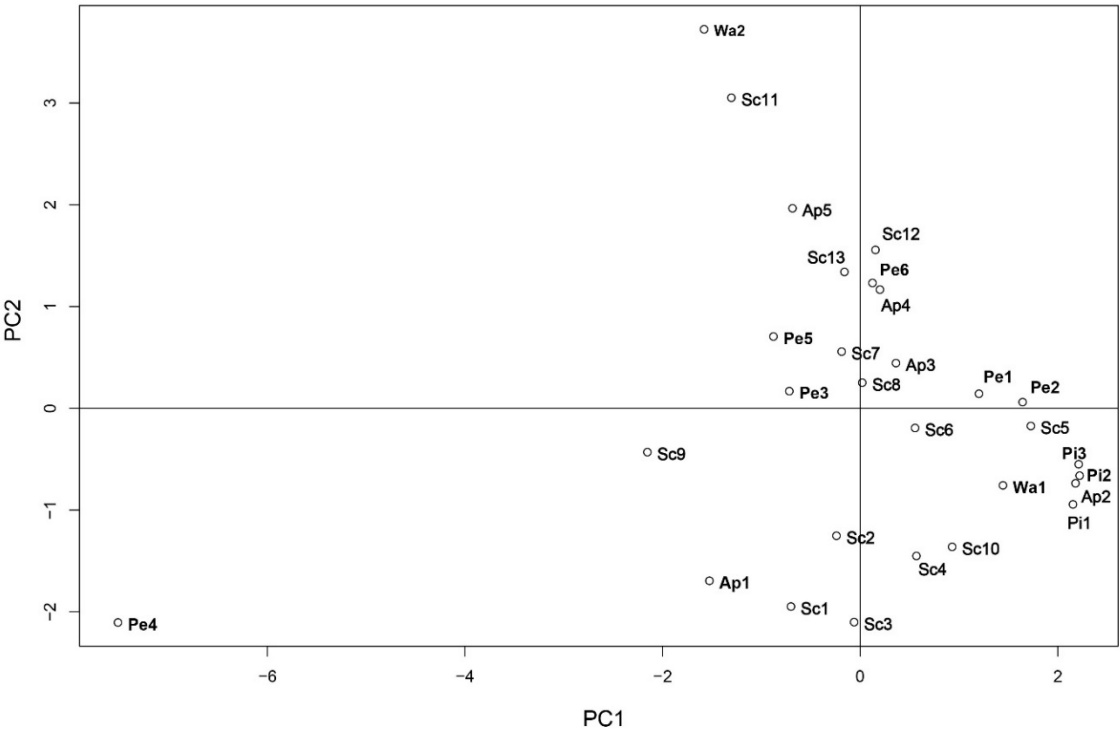

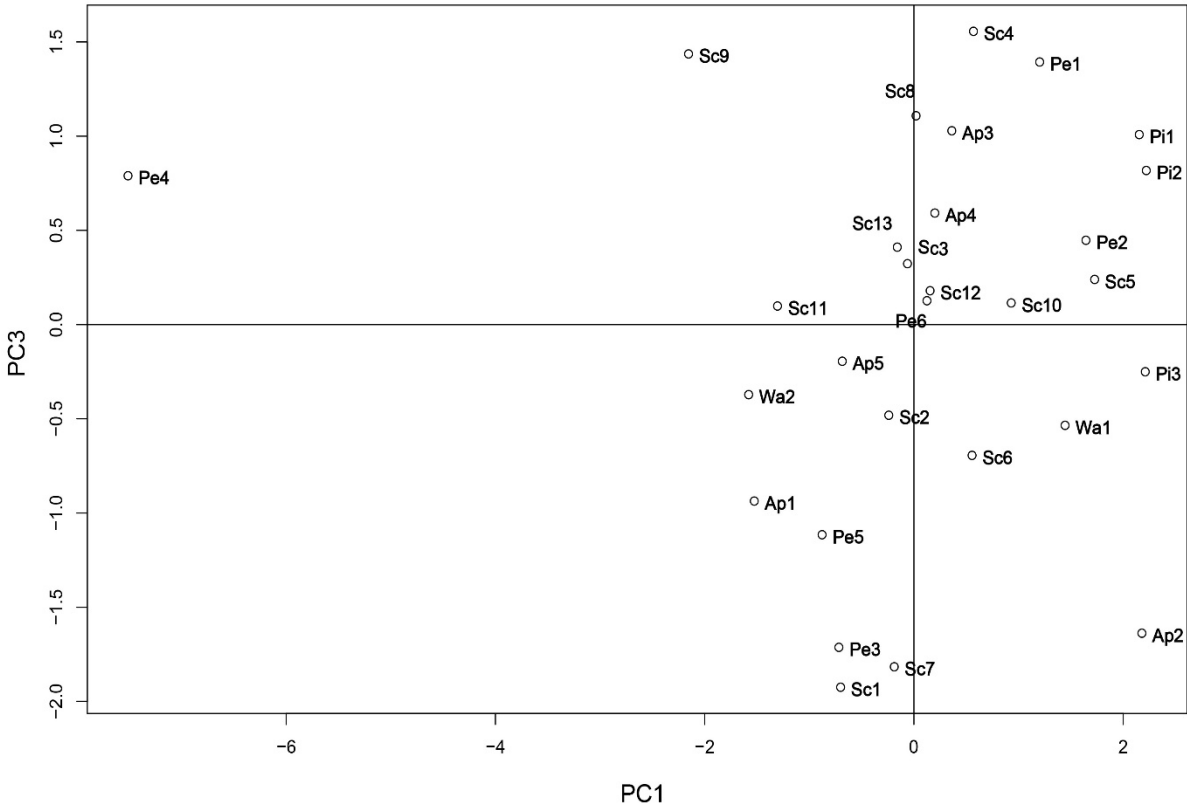Figure 1. Scatter diagram for PC 1 and PC 2. Ap Apple, Pe peach, Sc sweet cherry, Wa walnut, and Pi Pine.

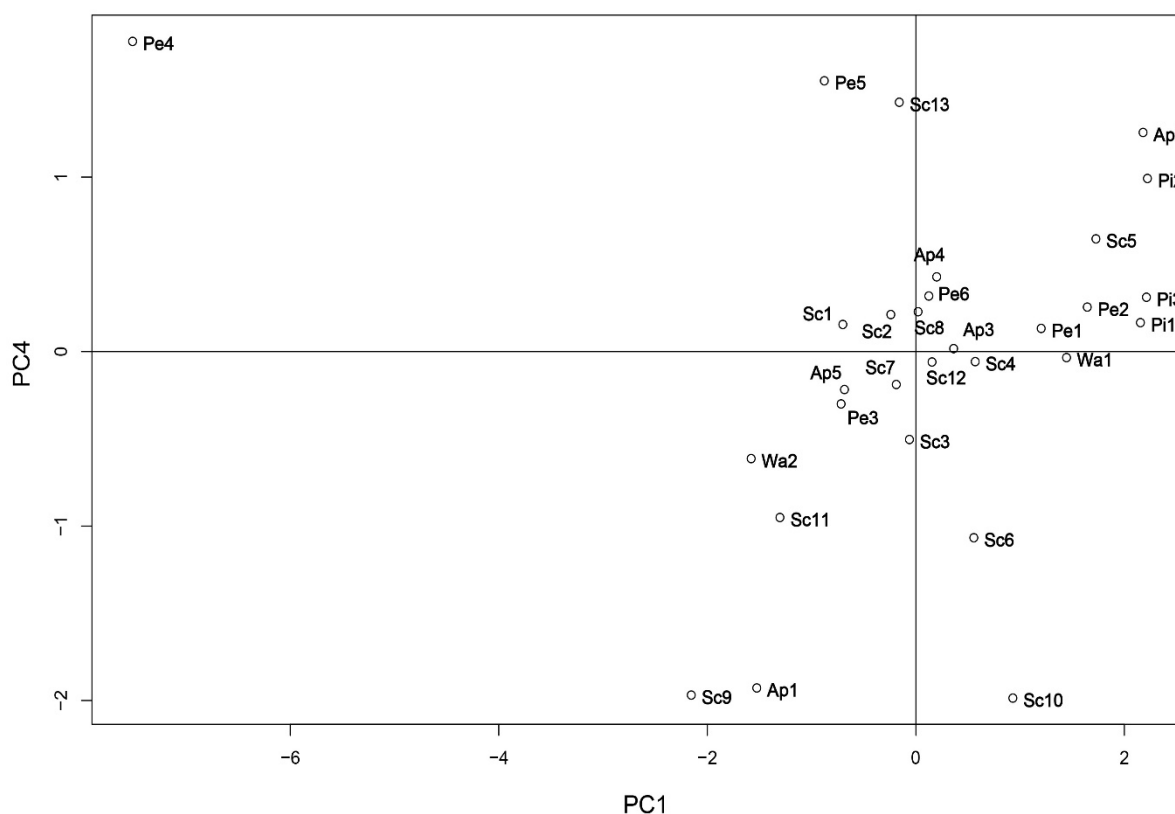Figure 2. Scatter diagram for PC 1 and PC 3. Ap Apple, Pe peach, Sc sweet cherry, Wa walnut, and Pi Pine.

Figure 3. Scatter diagram for PC 1 and PC 4. Ap Apple, Pe peach, Sc sweet cherry, Wa walnut, and Pi Pine.

The PC1 vs. PC3 plot given in Figure 2 serves to help visualise the $NaHCO_3$-Pt, $NaHCO_3$-Pi, $NaHCO_3$-Po, NaOH-Pt, and NaOH-Pi patterns of the soils. Sc1, Pe3, Sc7, Pe5, Ap1, Wa2, Sc2, and Ap5 soils stand out from the other soils depending on the soil property with negative loadings (sand for PC1) for both PCs. PC3, however, did not show any considerable positive loadings (above 0.3) for any soil properties. These soils and the others on the negative side of the PC3 had $NaHCO_3$-Pi, $NaHCO_3$-Po, NaOH-Pt, and NaOH-Pi fractions. Ap2 soil with the lowest NaOH extractable P fractions (32.7 mg kg^{-1}) was different from the others because of clay, MnOx, AFeOx, and TFeOx concentrations.

This suggests that excessive Ca concentration and fine texture induce Ca and clay minerals-related-adsorption reactions (Akinremi & Cho, 1991a, b; Uygur, 2009). In this case, the added fertilizer P is hindered from reacting with the oxide surfaces. Sc9 and Pe4 drew apart due to the negatively loading parameters for PC1 and the positively loading parameters for PC3. The coarse texture of these soils was likely to drive the added fertilizer P to be adsorbed on the oxide surfaces where an iron oxide coating increases the efficiency of iron oxides to fix P (Hamad et al., 1992).

Figure 3 shows the scatter diagram of PC1 and PC 4, which explains the distribution of soil samples by considering the Ca-P, and Res-P fractions and soil properties with considerable loadings for PC1. This suggested that clay minerals and very high extractable Ca scavenge the fertilizer P. This soil probably reached a P maximum sorption; therefore, the added P increases the available P concentration. Ince (2016) found that many soil samples in the region have already reached P saturation, and negative fertilizer requirements were determined for those soils.

4. CONCLUSION

It can be concluded that the partition of fertilizer P among geochemical fractions is mainly driven by soil texture, extractable Ca, calcium carbonate equivalent, and soil organic matter content. The fertiliser P is converted to CBD-P in coarse textured soils; whereas Ca-P was main sink for fine textured soils. Especially coarse textured soils pose a risk for phosphorus leaching.

The magnitude of amorphous oxides, mainly Fe and Mn oxides which is an indication of the redox status of soil, can play a critical role in the partition of fertilizer–P between the mobile and moderately mobile geochemical fractions.

Conflicts of Interest

There is no conflict of interest between the authors

of the article. HH, and VU designed the study and its trials were established, HH conducted the study, the data were analysed by FG, and the article was written by HH, FG, and VU. This study was financially supported by the Scientific Research Foundation of Süleyman Demirel University with project number 4486-YL1-17.

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