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Surface runoff phosphorus (P) loss in relation to phosphatase activity and soil P fractions in Florida sandy soils under citrus production

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Abstract

Phosphorus losses by surface runoff from agricultural lands have been of public concern due to increasing P contamination to surface waters. Five representative commercial citrus groves (C1–C5) located in South Florida were studied to evaluate the relationships between P fractions in soils, surface runoff P, and soil phosphatase activity. A modified Hedley P sequential fractionation procedure was employed to fractionate soil P. Soil P consisted of mainly organically- and Ca/Mg-bound P fractions. The organically-bound P (biological P, sum of organic P in the water, $NaHCO_3$ and NaOH extracts) was dominant in the acidic sandy soils from the C2 and C3 sites (18% and 24% of total soil P), whereas the Ca/Mg-bound P (HCl-extractable P) accounted for 45–60% of soil total P in the neutral and alkaline soils (C1, C4 and C5 soils). Plant-available P (sum of water and $NaHCO_3$ extractable P fractions) ranged from 27 to 61 mg P kg^{-1} and decreased in the order of C3 > C4 > C1 > C2 > C5. The mean total *P* concentrations (TP) in surface runoff water samples ranged from 0.51 to 2.64 mg L^{-1}. Total P, total dissolved P (TDP), and PO_4^{3-}-P in surface runoff were significantly correlated with soil biological P and plant-available P forms ($p < 0.01$), suggesting that surface runoff P was directly derived from soil available P pools, including H_2O– and $NaHCO_3$– extractable inorganic P, water-soluble organic P, and $NaHCO_3$- and NaOH-extractable organic P fractions, which are readily mineralized by soil microorganisms and/or enzyme mediated processes. Soil neutral (55–190 mg phenol kg^{-1} 3 h^{-1}) and natural (measured at soil pH) phosphatase activities (77–295 mg phenol kg^{-1} 3 h^{-1}) were related to TP, TDP, and PO_4^{3-}-P in surface runoff, and plant-available P and biological P forms in soils. These results indicate that there is a potential relationship between soil P availability and phosphatase activities, relating to P loss by surface runoff. Therefore, the neutral and natural phosphatase activities, especially the natural phosphatase activity, may serve as an index of surface runoff P loss potential and soil P availability.

Keywords: Phosphatase activity; Phosphorus fractionation; Phosphorus loss; Surface runoff; Sandy soils

1. Introduction

Bioavailable P, including dissolved- and particulate- P in surface runoff from agricultural lands accelerates surface water eutrophication (Sharpley et al., 1995). In the USA, P has been the major nutrient affecting fresh water quality (US Environmental Protection Agency, 1996a). It was estimated that over 70% of total P discharging into rivers originated from agricultural land (Lowrance, 1991). Therefore, best management practices (BMPs) are required to optimize P fertilization to meet the demands of crop production with minimal environmental impacts. According to the Everglades Forever Act in Florida (EFAF, 1994), concentrations of total P in surface runoff that discharges into the Everglades are mandated to be reduced to a threshold level of 0.05 mg P L^{-1}. Sharpley et al. (1996) reported that runoff water is considered as degraded if total P exceeds a guideline concentration of 0.10 mg P L^{-1}. Mean concentrations of total P in surface runoff from the five commercial citrus groves we monitored in the last three years were 9.6–34.5 times greater than the EFAF threshold and, hence, 4.8–17.3 times greater than the Sharpley's guideline level (unpublished data). Results from Koch (1991) and Newman

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and Pietro (2001) from the Stormwater Treatment Areas, part of the Everglades restoration program, have arrived at similar conclusions.

The P load in surface runoff is mainly derived from desorption in surface soils and suspended solids or sediment (Douglas et al., 1998; Hooda et al., 2000; Ng Kee Kwong et al., 2002). Phosphorus concentration in surface runoff is often observed to relate to fertilization rate, to P cycling in the plant-soil system, and to the amount of labile P in soils. However, P loadings in surface runoff are also influenced by topography, precipitation (intensity and duration), temperature, land use, and other field management practices.

Phosphorus cycling in soils and P bioavailability are regulated by biological (short term) and geochemical (long term) processes (Cross and Schlesinger, 1995). The Hedley P sequential extraction procedure that has been broadly applied in soil P studies provides an operational and chemical fractionation of soil P (Hedley et al., 1982). The P fractions are categorized as plant-available P and refractory P forms, or biological P (all the extractable organic fractions) and geochemical P forms (the remaining fractions) based on P availability (Cross and Schlesinger, 1995). Using the Hedley P fractionation procedure in three southern alpine Spodosols, Beck and Elsenbeer (1999) found that 65% of total P in topsoil (0–30 cm) was in organic forms, and that bicarbonate extractable organic P reached a maximum of 80% of the total available soil P. Graetz and Nair (1995) estimated that nearly 80% of total soil P in the A horizon of Florida Spodosols was leachable.

Phosphatases play a key role in soil P cycling by catalyzing mineralization of organic P and potentially relate to soil P bioavailability. Acid and alkaline phosphatases (orthophosphoric monoester phosphohydrolase), so classified because they show optimal activities in acid and alkaline ranges (Tabatabai, 1994), have been extensively studied. In addition, PO_4^{3-} is a competitive inhibitor of acid and alkaline phosphatases in soils (Juma and Tabatabai, 1978). No information, however, is available regarding the relationships between phosphatase activity and soil labile P fractions, that are readily lost by surface runoff.

Citrus is an important Florida agricultural crop with a 200-year planting history. Florida has the largest citrus production area (about 294,455 ha) in the USA, and produced about 15 million tons of fruit in the 2001–02 season, representing 78% of the total USA citrus production (Florida Agricultural Statistics Service, 2003). Phosphorus in surface runoff from citrus groves is suspected to contribute to the eutrophication of surface water bodies, such as the Indian River Lagoon and Lake Okeechobee in South Florida. Data from five citrus groves in South Florida monitored in 2002, indicated that, an average about 0.5–2.1 kg P ha^{-1} was transported by surface runoff into ditches at concentrations ranged from 0.6–2.0 mg P L^{-1} (unpublished data). Most soils in Florida are sandy and have low P-retention capacity in their upper horizon (Graetz and Nair, 1995; He et al., 1999). Therefore, the potential of P loss by surface runoff from sandy soils under citrus production is considerable.

The objectives of this study were to evaluate the relationships among P loadings in surface runoff, soil P fractions, and soil phosphatase activities, and to understand the linkage of P losses by surface runoff with soil phosphatase activity. Findings from this study are expected to finetune BMPs, by incorporating phosphatase activity as an indicator of soil P availability, with an adequate balance of agro-economical and environmental concerns.

2. Material and methods

Topsoil samples (0–15 cm) were collected in February, 2003 from five commercial citrus groves (named C1, C2, C3, C4, and C5) in St Lucie and Martin counties, Florida, USA. Three field replicates were taken from each site and each sample was a composite of 10 cores. Taxonomically, the soils of C1, C2 and C3 were Riviera sand (loamy, siliceous, hyperthermic Arenic Glossaqualfs), Nettles sand (sandy, siliceous, hyperthermic, ortstein Alfic Arenic Alaquods), and Wabasso sand (sandy, siliceous, hyperthermic Alfic Alaquods), respectively, and C4 and C5 were Pineda sand (loamy, siliceous, hyperthermic Arenic Glossaqualfs) (US Department of Agriculture, 1980, 1981). Visible plant materials were removed from soil samples and samples were air-dried and ground to pass through a 2-mm sieve in preparation for measuring pH, electrical conductivity (EC). Soil phosphatase activities were measured in the air-dried soils because the field moisture of these sandy topsoils (0–15 cm) was very low (less than 3% of oven-dried weight) except on rainy days. It has been reported that by using air-dried soils, enzyme assays can effectively discriminate between different field management practices even though activities were reduced by drying (Babdick and Dick, 1999). In this study, air-dried soils was used to provides a common basis for comparing soil phosphatase activity among different sandy soils and with phosphorus fractions. Subsamples were ground to pass through a 0.25-mm sieve for further chemical analyses and phosphorus fractionation. Some basic physicochemical properties of the soils are shown in Table 1.

Soil pH was measured in a 1:1 soil-deionized water mixture using a pH/ion/conductivity meter (Fisher Scientific, Accumet model 50, Pittsburgh, PA, USA) after 30 min shaking. Electrical conductivity (EC) was measured in a 1:2 soil-deionized water mixture after 30 min shaking, using the pH/ion/conductivity meter. Soil particle distribution was determined by a micro-pipette method (Miller and Miller, 1987). Soil total organic carbon and nitrogen were determined using a CN-analyzer (Vario Max CN, Macro Elemental Analyzer System GmbH, Hanau, Germany). Total P, Ca, Mg, Al, and Fe were determined by digesting the soil samples following the procedure of EPA method 3050B (US Environmental Protection Agency, 1996b)

Table 1
Basic properties of the five sandy soils (C1–C5) under citrus production

Site	C1	C2	C3	C4	C5
Particle Composition (g kg^{-1})					
Sand	908	966	935	880	945
Silt	42.8	9.2	4.3	39.1	13.4
Clay	49.6	25.0	61.0	80.6	41.2
pH (H_2O)	7.7 a	5.9 c	5.2d	7.1 b	7.8 a
EC (μS cm^{-1})	336.0 b	114.5 c	446.0 a	316.0 b	299.7 b
Total N(g kg^{-1})	0.50 b	0.38 b	0.94 a	0.39 b	0.51 b
Total organic C(g kg^{-1})	8.5 a	4.0 b	9.2 a	3.6 b	8.1 a
C/N Ratio	17.1	10.5	9.8	9.1	15.9
Olsen P (mg P kg^{-1})	18.5 b	22. 6 b	27.4 a	20.4 b	11.2 c
Total element (g kg^{-1})					
P	0.20 a	0.07 b	0.13 b	0.12 b	0.11 b
Ca	8.69 a	0.26 b	0.49 b	0.73 b	7.35 a
Mg	0.25 b	0.11 c	0.23 b	0.12 c	0.41 a
Al	3.76 b	0.94 c	2.34 bc	1.26 c	6.08 a
Fe	1.95 a	1.10 b	1.14 b	0.88 b	2.32 a
Water soluble (mg kg^{-1})					
Ca	113.87 a	10.02 d	27.69 c	27.16 c	76.08 b
Mg	11.24 a	3.59 b	9.70 a	5.49 b	5.04 b
Al	6.20 a	0.90 b	1.41 b	0.06 b	1.23 b
Fe	3.16 a	1.26 bc	1.06 bc	0.67 c	1.72 b

Significant differences among the five sandy soils are indicated by different letters ($p < 0.05$) letters for a one-way ANOVA-pairwise *t*-test.

and measuring concentrations in the digested solutions using Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES, Ultima, JY Horiba Inc. Edison, NJ, USA) following EPA method 200.7 (U.S. Environmental Protection Agency, 1998). Water soluble Ca, Mg, Al and Fe were extracted with deionized water at 1:10 soil:water ratio by shaking for 30 min and the concentrations in the extracts were measured using the ICPAES. Olsen P was extracted with 0.5 mol $NaHCO_3$ L^{-1} at 1:20 soil:solution ratio with a 30 min shaking period. Concentrations of P in the extracts were measured by the ascorbic acid-molybdenum blue method (Kuo, 1996).

The activities of natural, acid, neutral and alkaline phosphatases were measured with the substrate, phenylphosphate, incubated in deionized water (natural soil pH), acetate buffer (pH 5.0), citrate buffer (pH 7.0) or borate buffer (pH 10.0), respectively, at 37° C for 3 h (Öhlinger, 1995).

Soil phosphorus was sequentially fractionated mainly following the Hedley fractionation procedure (Hedley et al., 1982). One gram soil (<0.25 mm) was successively extracted with 30mL deionized water/0.5 mol $NaHCO_3$ L^{-1} (pH8.5)/0.1 mol NaOH L^{-1}/1 mol HCl L^{-1} solutions each for 16 h. The inorganic P (Pi) concentration of each extract was determined and referred to as water-soluble (H_2O-), $NaHCO_3^-$, NaOH-, or HCl-extractable Pi fraction. Total P (Pt) concentration of each extract was also determined after acid persulfate digestion (autoclaving for 30 min at 121° C and 1.05 kg cm^{-1} pressure)(EPA 365.1). Organic P (Po) concentration of these extracts was calculated as the difference between the total and inorganic P, referred to as H_2O–, $NaHCO_3^-$, NaOH-, or HCl-extractable Po fraction. However, the difference between Pt and Pi of the HCl-extractable fraction, i.e. the HCl-extractable Po fraction, was negligible. The soil residue was digested with concentrated H_2SO_4–H_2O_2 and the total residual P was measured. In each case, Pi concentration was measured on neutralized extracts or digested solutions by the ascorbic acid-molybdenum blue method (Kuo, 1996).

Surface runoff water samples were collected from a catchment of two citrus beds at each rain event with an autosampler (SIGMA 900MAX portable sampler) at each site, from February 21 to June 30, 2003 after soil sampling. All the citrus groves have beds of similar width (15 m) except that the C4 had beds of 19-m width. The bed length differed among the groves. The citrus groves are generally flat, with a drainage furrow between the two neighboring beds. The autosampler was installed at the outlet end of the drainage furrow and programmed to collect surface runoff water samples. The catchment areas were 2772, 2834, 2788, 1788, and 5771 m^2, respectively, for the C1, C2, C3, C4, and C5 sites. The tree densities are 14.1, 13.1, 15.8, 19.0, and 18.4 trees per 100 m for the C1, C2, C3, C4, and C5 sites, respectively. For each surface runoff event, the autosampler was triggered to collect water sample when the rainfall reached 0.25 mm/30 min and the drainage flow level exceeded 0.76 mm simultaneously. The autosampler then distributed the surface runoff samples into the first 2-h flush samples (3 samples) and the 24-h composite samples (3 samples). Solid concentrations of the water samples were measured using a gravimetric method after oven drying. Total P in the unfiltered surface runoff samples was determined by the molybdenum-blue method after digestion with acidified ammonium persulfate (EPA 365.1). Subsamples of the surface runoff water were further filtered through a 0.45 μm membrane filter for measurement of PO_4^{3-}-P using an Ion Chromatograph (IC, DX 500; Dionex Corporation Sunnyvale, CA) following EPA method 300.0, and total dissolved P using the ICPAES following EPA method 200.7.

Means and Standard Error (SE) for each measured variable were calculated by site. A one-way ANOVA with the pairwise *t* test procedure, and Pearson correlation (CORR) were conducted to analyze all the data using the SAS System for Windows, Release 8.02 (SAS Institute Inc., 1999).

3. Results and discussion

3.1. Soils and phosphorus fractionation

Soils selected for this study were generally sandy in texture (880-970 g sand kg^{-1}), two were acidic (C2 and C3) and three were neutral to weakly alkaline (C1, C4, and C5) (Table 1). The C1 and C5 soils had higher total Ca content (8.69 and 7.35 g Ca kg^{-1}) than the other soils (C2, 0.26 g Ca kg^{-1}; C3, 0.49 g Ca kg^{-1}; and C4, 0.73 g Ca kg^{-1}). Total and water-soluble Ca contents in the five soils were significantly and positively correlated with soil pH ($r=0.790$, $p<0.001$; $r=0.721$, $p<0.01$, respectively). Total organic carbon content in these soils was less than 10 mg C kg^{-1} and decreased in the order of C3 > C1 > C5 > C2 > C4. Olsen-P, extracted by 0.5 mol $NaHCO_3$ L^{-1}, decreased in the order of C3 > C2 > C4 > C1 > C5, and was significantly negatively correlated with soil pH ($r=-0.841$, $p<0.001$) and with the total Ca ($r=-0.709$, $p<0.01$), Mg ($r=-0.546$, $p<0.05$), Al ($r=-0.640$, $p<0.01$), and Fe ($r=-0.607$, $p<0.05$). These results suggest that Olsen-P in the soils is likely controlled by soil pH and the contents of Ca, Mg, Al, and Fe.

Phosphorus fractionation in the five soils determined by the Hedley's procedure showed that for soils C2 and C3, the size of different P fractions decreased in the order of residual P > $NaHCO_3$-P > NaOH-P > H_2O-P > HCl-P, for soils C1 and C4 the order was HCl-P > residual P > $NaHCO_3$-P > NaOH-P > H_2O-P, and for the C5 soil, the order was residual P > HCl-P > NaOH-P > $NaHCO_3$-P > H_2O-P (Table 2). In the water soluble P fraction, inorganic P was the dominant form, with organic P accounting for only 7.4–21.2% of the total P in the extracts or <1% of total soil P (Table 2, Fig. 1), which was negligible (Hedley et al., 1982). The $NaHCO_3$-P fraction was dominated by inorganic P, but the organic P was also considerable. However, the NaOH-P fraction was dominated by organic P, accounting for 55.0–86.6% of total P in extracts and had a significant correlation with soil total organic carbon content ($r=0.560$, $p<0.05$).

Organically bound P (the sum of organic P concentrations in the H_2O-, $NaHCO_3$- and NaOH-extracts), defined as the biological P by Cross and Schlesinger (1995), was markedly higher in the acidic soils (C2, 18.0% and C3, 23.8% of soil total P) than in the neutral and alkaline soils (4.8–9.6% of soil total P) (Table 3). These results disagree with earlier findings reported for the southern alpine Spodosols, which had up to 65% of soil P in the topsoil as organically bound P fractions (Beck and Elsenbeer, 1999). Perhaps this was because the alpine Spodosols had much higher organic carbon (about 100 mg C kg^{-1}) than the sandy soils (<10 g C kg^{-1}) in this study.

The geochemical P, consisting of inorganic P fractions extracted by H_2O-, $NaHCO_{3-}$, NaOH-, and HCl, and the residual P fractions, was the dominant P component of these sandy soils. It ranged from 76.3–95.2% of the soil total P (Table 3). The three neutral or alkaline sandy soils (C1, C4, and C5) had higher geochemical P fractions than the two acid sandy soils (C2 and C3), especially, the HCl-P fraction, defined as Ca/Mg-bound P fraction (Cross and Schlesinger, 1995), was evidently higher in the C1, C4 and C5 soils than in

Table 2
Phosphorus (P) fraction distribution in topsoil (0–15 cm) of the five citrus groves (C1–C5) in Florida, USA using the Hedley's fractionation framework

P fraction		C1	C2	C3	C4	C5
Water Soluble P	Inorganic P (Pi)	12.54 bc	9.39 bc	19.19 a	12.96 b	8.53 c
	Organic P (Po)	1.86 ab	1.25 c	1.54 bc	1.70 bc	2.30 a
	Total P (TP)	14.41 b	10.64 b	20.73 a	14.66 b	10.83 b
0.5 M NaHCO3-extractable P	Inorganic P (Pi)	28.61 a	21.39 ab	25.81 a	29.51 a	13.56 b
	Organic P (Po)	1.92 a	7.74 b	14.54 b	5.91 c	2.38 c
	Total P (TP)	30.53 ab	29.13 b	40.35 a	35.43 ab	15.94 c
0.1 M NaOH-extractable P	Inorganic P (Pi)	7.30 ab	5.37 bc	3.97 bc	9.85 a	3.40 c
	Organic P (Po)	9.93 b	11.64 b	25.54 a	12.02 b	14.77 b
	Total P (TP)	17.23 b	17.01 b	29.51 a	21.87 ab	18.16 b
1 M HCl-extractable P	Inorganic P (Pi)	107.6 a	4.16 d	13.26 c	59.52 b	60.98 b
Residual P	Total P (TP)	103.9 b	42.22 c	55.06 c	54.15 c	141.8 a

Significant differences among the five sandy soils are indicated by different letters ($p<0.05$) letters for a one-way ANOVA-pairwise *t*-test.

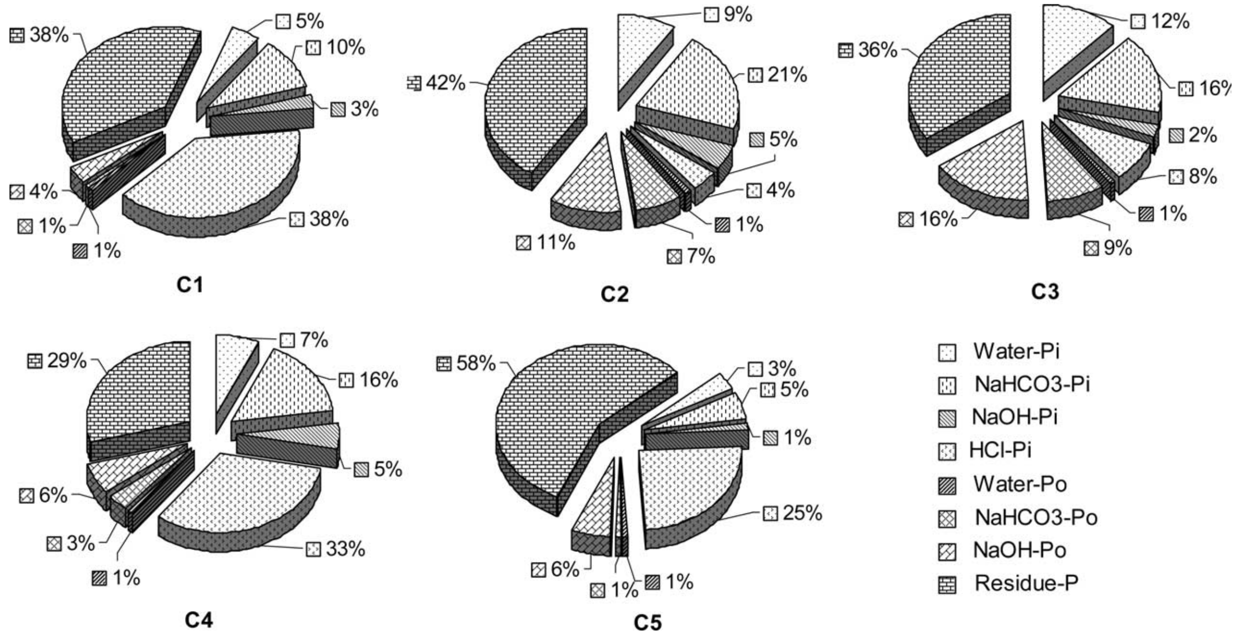

Fig. 1. Percentages of P fractions in the five sandy soils (C1–C5) under citrus production using the Hedley's fractionation framework.

the C2 and C3 soils. The Ca/Mg-bound P fraction dominated all the soil P fractions except for the residual P (44.8–59.9% of extractable P fractions) in the C1, C4 and C5 soils that contained high Ca contents; whereas in the C2 and C3 soils it was less than 10% of extractable P fractions (Fig. 1). The Ca/Mg-bound P fraction correlated positively with soil total Ca ($r = 0.776$, $p < 0.001$) and Mg ($r = 0.507$, $p = 0.05$), and water-soluble Ca ($r = 0.830$, $p < 0.001$) contents.

The amount of plant available P (PAP) significantly decreased in the order C3 > C4 > C1 > C2 > C5, but the percentage of plant available P with respect to total soil P decreased in the sequence C2 > C3 > C4 > C1 > C5. Consequently, the percentage of refractory P fractions changed in the reverse order (Table 3). The plant available P fraction had a significant correlation with Olsen P ($r = 0.840$, $p < 0.001$) in these sandy soils. Both were also significantly correlated to P fractions of water- (PAP, $r = 0.896$, $p < 0.001$; Olsen P, $r = 0.680$, $p < 0.01$), NaHCO$_3$- (PAP, $r = 0.980$, $p < 0.001$; Olsen P, $r = 0.855$, $p < 0.001$), and NaOH-extractable P (PAP, $r = 0.783$, $p < 0.01$; Olsen P, $r = 0.640$, $p < 0.01$). The water soluble P and NaHCO$_3$ extractable P have been considered as the easily lost P fractions through surface runoff or leaching (Graetz and Nair, 1995). For the five sandy soils under citrus production, there were about 10.2–35.5% of total soil P or 26.8–61.1 mg P kg^{-1} that are potentially subjected to surface runoff or leaching loss. Given the bulk density of the five sandy soils at about 1.4 g cm^{-3}, approximately 56–128 kg P ha^{-1} stored in the topsoil (0–15 cm) may be subjected to loss by surface runoff or leaching if not taken up by plants. Moreover, the sandy soils with low organic matter content usually have a low

Table 3
Definition of soil phosphorus fractions as biological, geochemical, plant-available, and refractory forms using the Hedley's fractionation framework in the five sandy soils (C1–C5) under citrus production in Florida, USA

Site		Biological P	Geochemical P	Plant-available P	Refractory P	Total P
C1	mg P kg^{-1}	13.71 b	269.9 a	44.93 b	238.7 a	283.6 a
	%	4.84	95.16	15.84	84.16	100.0
C2	mg P kg^{-1}	20.62 b	94.17 c	39.77 bc	75.02 c	114.8 d
	%	17.97	82.03	34.65	65.35	100.0
C3	mg P kg^{-1}	46.41 a	149.7 b	67.72 a	128.4 b	196.1 c
	%	23.67	76.33	34.54	65.46	100.0
C4	mg P kg^{-1}	19.64 b	185.6 b	54.81 ab	150.4 b	205.2 bc
	%	9.57	90.44	24.41	73.29	100.0
C5	mg P kg^{-1}	19.45 b	243.1 a	26.77 c	235.8 a	262.5 ab
	%	7.41	92.59	10.20	89.80	100.00

Data present in concentration (mg P kg^{-1}, mean (SE)) and percentage. Significant differences among five sandy soils are indicated by different letters ($p < 0.05$) letters for a one-way ANOVA-pairwise t-test.

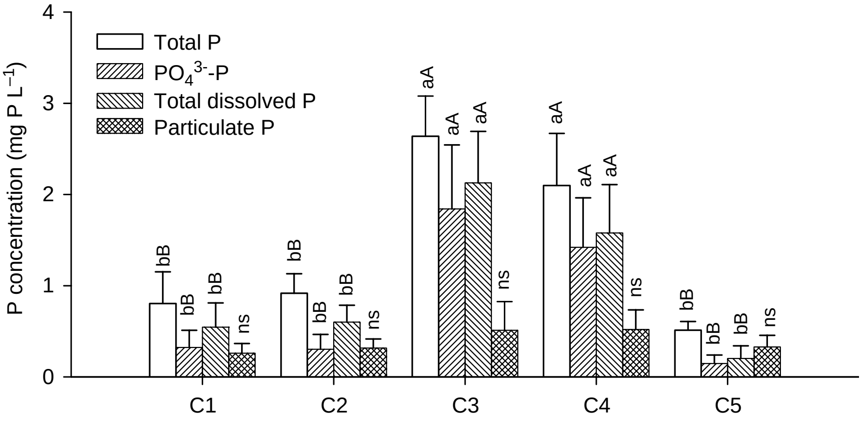

Fig. 2. P fractions in the first flush samples of surface runoff (collected in the first 2 h of each rain event) after soil sampling from February 21 to June 30, 2003 in the five sandy soils (C1–C5) under citrus production. Data as shown on the figure are the mean P concentrations from 11, 18, 8, 7, and 13 rain events of the C1, C2, C3, C4, and C5 sites, respectively. Letters above bars indicate the multi-comparison result of ANOVA-pairwise *t*-test by site, and the significance of small letters is at $p<0.05$, and the capital letters at $p<0.01$. 'ns' means not significant. Error bars around means are standard errors.

retention capacity for P, compared with other soils with high clay or organic matter content (Graetz and Nair, 1995; Zhang et al., 2002). Although the Ca- (for the neutral and alkaline soils) or organically-bound P (for the acid soils) dominated soil P fractions in these sandy soils, the potential for P losses by surface runoff or leaching is still considerable.

3.2. Phosphorus fractions in surface runoff

From February to June 2003, there were 11, 18, 8, 7, and 13 rain events for the C1, C2, C3, C4, and C5 sites, respectively. Mean concentrations of P fractions in the first 2-hour flush samples, including total P (TP, 0.51–2.64 mg P L^{-1}), PO$_4^{3-}$-P (0.15–1.84 mg P L^{-1}), and total dissolved P (TDP, 0.20–2.13 mg P L^{-1}), were significantly higher in the C3 and C4 sites than in the C1, C2, and C5 sites, whereas there was no statistical difference in the particulate-P among the different sites (Fig. 2). This trend was similar to the plant-available P fractions, the sum of water- and NaHCO$_3$-extractable P fractions (Table 3, $p<0.01$). All P fractions (TP, PO$_4^{3-}$-P, and TDP), except for the particulate-P, in the surface runoff were significantly and positively correlated with the total P of H$_2$O-, NaHCO$_3$-, or NaOH-extractable fraction ($p<0.01$, Table 4). A positive relationship was observed between soil organically-bound P

Table 4
Correlation coefficients (*r*) of between P fractions in surface runoff and soil P fractions, soil P forms (biological, geochemical, plant-available, and refractory forms), and soil basic properties

			P fraction in surface runoff			
			Total P (TP)	PO$_4^{3-}$—P	Total dissolved P (TDP)	Particulate P
Soil P fractionation	H$_2$O-extractable fraction	Inorganic	0.678*	0.694*	0.744*	0.080
		Organic	−0.347	−0.237	−0.320	−0.239
		Total	0.644*	0.671*	0.712*	0.057
	NaHCO$_3$-extractable fraction	Inorganic	0.403	0.483	0.516**	−0.212
		Organic	0.794*	0.731*	0.798*	0.35
		Total	0.698*	0.727*	0.785*	0.013
	NaOH-extractable fraction	Inorganic	0.092	0.161	0.143	−0.151
		Organic	0.604***	0.675*	0.664*	0.088
		Total	0.655*	0.758*	0.739*	0.022
	HCl-extractable fraction		−0.319	−0.163	−0.214	−0.495
	Residual fraction		−0.572***	−0.460	−0.544***	−0.333
	Total P		−0.400	−0.260	−0.318	−0.446
Soil P fraction grouping	Biological P (Organically-bound P)		0.716*	0.736*	0.757*	0.201
	Geochemical P		−0.490	−0.358	−0.417	−0.458
	Plant available P		0.714*	0.744*	0.799*	0.028
	Refractory P		−0.519***	−0.389	−0.455	−0.436
Soil basic property	Soil pH		−0.577***	−0.462	−0.553***	−0.337
	Olsen P		0.617***	0.557***	0.641*	0.177
	Total Ca		−0.613***	−0.520***	−0.587*	−0.354
	Total Fe		−0.603***	−0.483	−0.570***	−0.362

*, **, and *** represent significance levels at $p<0.001$, $p<0.01$, and $p<0.05$, respectively.

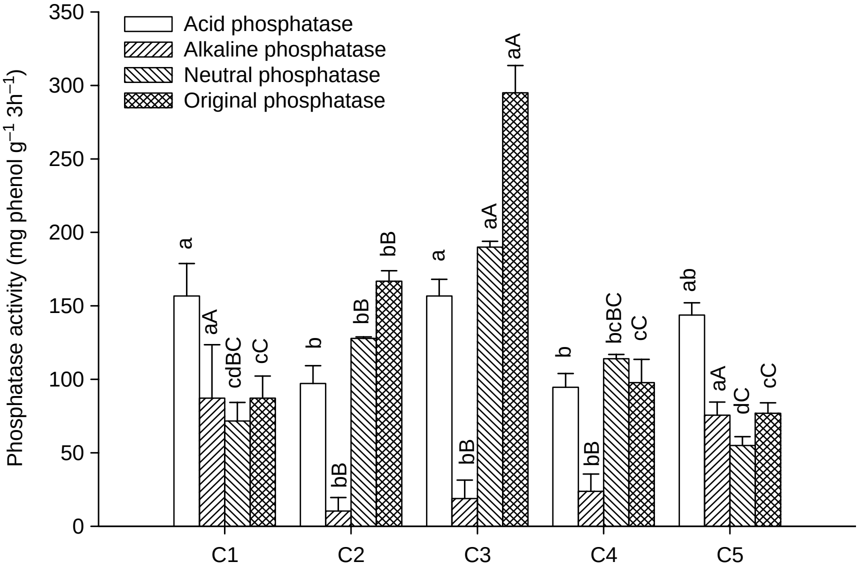

Fig. 3. Phosphatase activities in the five sandy soils (C1–C5) under citrus production. Acid, alkaline, neutral, and orignial phosphatase activities were determined in acetate buffer (pH 5.0), borate buffer (pH 10.0), citrate buffer (pH 7.0), and deionized water (field pH), and the disodium phenylphosphate was the substrate with a 3 h incubation period at 37 °C. Letters above bars indicate the multi-comparison result of ANOVA-pairwise *t*-test by site, and the significance of small letters is at $p<0.05$, and the capital letters at $p<0.01$. Error bars around means are standard errors.

fractions (sum of H_2O-, $NaHCO_3$-, and NaOH- extractable organic P) and P fractions (TP, PO_4^{3-}-P, and TDP) in surface runoff ($p<0.01$, Table 4). Phosphorus fractions (TP, PO_4^{3-}-P, and TDP) in surface runoff were correlated with soil water-extractable inorganic P and Olsen P (Table 4). These findings suggest that P fractions in surface runoff are not only directly derived from water- and $NaHCO_3$- (Olsen) extractable inorganic P, and water-soluble organic P in soils, but are also potentially linked to $NaHCO_3$- and NaOH-extractable Po fractions, which can be mineralized into inorganic P through microorganisms and/or enzymes mediated processes.

Basic soil properties influence P concentrations in surface runoff from these citrus groves. Soil pH and total Ca and Fe contents were negatively correlated with the concentration of TP and TDP in surface runoff ($p<0.05$, Table 4). These results indicate that soil pH and the contents of Ca and Fe may control soil P solubility since Ca and Fe can retain inorganic P in the soil matrix through precipitation and/or adsorption processes (Cross and Schlesinger, 1995). Zhang et al. (2002) reported that soil clay and total Fe contents had significant correlations with adsorption maxima of PO_4^{3-}-P in 80 Florida sandy soils.

3.3. Phosphatase activity

The acid phosphatase activity in the five sandy soils ranged from 94.6 to 156.7 mg phenol kg^{-1} 3h^{-1} and was significantly higher in the C1 and C3 soils than in the C2 and C4 soils ($p<0.05$), whereas the C5 soil had a higher phosphatase activity than the C2 and C4 soils but lower phosphatase activity than the C1 and C3 soils, though not significant (Fig. 3). The alkaline phosphatase activity (10.4–87.2 mg phenol kg^{-1} 3 h^{-1}) was obviously lower than the acid phosphatase activity. The C1 and C5 soils had higher alkaline phosphatase activity than the C2, C3, and C4 soils ($p<0.01$), which was positively influenced by soil pH ($p<0.001$) (Table 5). The neutral phosphatase activity, ranging from 55.0 to189.9 mg phenol kg^{-1} 3 h^{-1}, decreased in the order of C3 $>>$ C2$>$C4$>$C1$>$C5 ('$>>$' means the difference at $p<0.05$), and the natural phosphatase activity, ranging from 76.9 to 295.0 mg phenol kg^{-1} 3 h^{-1}, was in the order of C3$>>$C2$>>$C4$>$C1$>$ C5 (Fig. 3). Both were negatively correlated with soil pH ($p<0.001$) (Table 5). Trends in level of neutral and natural phosphatase activities in different soils were similar.

The activity of acid phosphatase was significantly correlated to total organic C ($p<0.001$) and total P ($p<0.01$), and the alkaline phosphatase activity was negatively correlated with Olsen P ($p<0.05$), and positively correlated with total P ($p<0.001$), total organic C ($p<0.05$), total Ca, Mg, Fe, Al ($p<0.01$), and water soluble Ca, Fe, Al ($p<0.01$) (Table 5). The neutral and natural phosphatase activities also had positive significant correlations with Olsen P ($p<0.001$), and negatively correlated with total and water soluble Ca ($p<0.05$), while the neutral phosphatase activity was negatively correlated to the total Fe and Al ($p<0.05$) (Table 5). The relationships between basic soil properties and phosphatase activities were similar to those between soil P fractions and P fractions in surface runoff, suggesting that the soil phosphatase activity may have potential relationships with soil P fractions and their runoff losses.

Pearson correlation analyses between phosphatase activities and soil P fractions confirmed the above results (Table 5). Both the neutral and natural phosphatase activities were significantly correlated with H_2O-inorganic

Table 5
Coefficients of Pearson Correlation Analysis between phosphatase activities and soil basic properties, soil P fractions, soil P forms (biological, geochemical, plant-available, and refractory forms), and P fractions in surface runoff

			Soil phosphatase			
			Neutral phosphatase	Acid phosphatase	Alkaline phosphatase	Natural phosphatase
Soil basic property		Soil pH	−0.872*	0.060	0.778*	−0.892*
		Total carbon	0.050	0.774*	0.545***	0.306
		Olsen P	0.915*	0.105	−0.619***	0.803*
		Total Ca	−0.770*	0.398	0.922*	−0.601***
		Total Mg	−0.369	0.66*	0.734*	−0.178
		Total Al	−0.529***	0.572*	0.801*	−0.362
		Total Fe	−0.597***	0.466	0.788*	−0.429
		Water soluble Ca	−0.687**	0.419	0.902*	−0.544*
		Water soluble Fe	−0.429	0.483	0.744*	−0.352
		Water soluble Al	-0.236	0.587*	0.703*	−0.197
Soil P fractionation	H_2O-extractable fraction	Inorganic	0.782*	0.447	−0.223	0.746*
		Organic	−0.478	0.417	0.700**	−0.454
		Total	0.735*	0.486	−0.155	0.701***
	$NaHCO_3$-extractable fraction	Inorganic	0.438	0.141	−0.107	0.225
		Organic	0.927*	0.041	−0.712***	0.942*
		Total	0.790*	0.127	−0.433	0.636**
	NaOH-extractable fraction	Inorganic	0.005	−0.161	−0.024	−0.280
		Organic	0.691***	0.414	−0.256	0.769*
		Total	0.705***	0.349	−0.271	0.656***
	HCl-extractable fraction	HClP	−0.446	0.570***	0.873*	−0.436
	Residual fraction	Residual P	−0.688**	0.466	0.880*	−0.535***
	Total P		−0.454	0.667***	0.911*	−0.386
Soil P fraction grouping	Biological P (Organically-bound P)		0.825*	0.292	−0.449	0.881*
	Geochemical P		−0.558***	0.597***	0.941*	−0.501
	Plant available P		0.810*	0.25	−0.364	0.688***
	Refractory P		−0.589***	0.597***	0.947*	−0.501
P fraction in surface runoff	Total P (TP)		0.704***	−0.090	−0.563*	0.638***
	PO_4^{3-}—P		0.650***	0.020	−0.457	0.603**
	Total dissolved P (TDP)		0.743***	0.009	−0.514**	0.680***
	Particulate P		0.184	−0.362	−0.408	0.154

*, **, and *** represent significance levels at $p<0.01$, $p<0.001$, and $p<0.05$ respectively.

P ($p<0.001$) and total P ($p<0.01$), $NaHCO_3$-organic P ($p<0.001$) and total P ($p<0.01$), NaOH-organic P and total P ($p<0.01$), total organically-bound P (biological P) ($p<0.001$), and plant available P ($p<0.01$), and negatively correlated with geochemical P, refractory P, and residual P ($p<0.05$). The acid and alkaline phosphatase activities had good relationships with inorganic P fractions, such as HCl-extractable P, residual P, geochemical P, and refractory P ($p<0.05$). Some of these relationships agreed with Chen's findings (Chen, 2003) that, in soils under an 18-year-old Chinese fir planation, both acid and neutral phosphatase activities were positively related to total, inorganic, organic and occluded P as well as to dilute acid extractable P, Al-P and Fe-P, whereas they were negatively related to Ca-P at a significance of 0.001. In this study, the neutral and natural phosphatase activities were positively correlated to the concentrations of TP, TDP, and PO_4^{3-}-P in the surface runoff water ($p<0.05$). The neutral phosphatase activities had positive correlations with P contents in surface runoff, probably because pHs of surface runoff water were neutral or slightly alkaline (Table 6). The acid phosphatase had no significant relationship with the P fractions in the surface runoff water, but the alkaline phosphatase had negative correlations with the concentrations of TP and TDP in the surface runoff water (Table 5).

In brief, this study revealed the relationships among the P loss by surface runoff, the soil P fractions, and the phosphatase mediated P mineralization. Phosphorus loaded in surface runoff is mainly derived from plant-available P fractions, which are transformed from relatively stable inorganic and organic P fractions in the soils. Because of their positive significant relationships with soil P fractions and P loss by surface runoff, the natural and neutral phosphatase activity may serve as an index of potential P loss in surface runoff and soil P availability to plants. The P release from organic P fractions by enzymes or microorganisms mediated

Table 6
Mean values and ranges of pH of surface runoff water from the five sandy soils (C1–C5) under citrus production in Florida, USA

Sites	No. of rain events	PH of surface runoff water	
		Range	Mean(SD)
C1	11	6.3–8.5	8.0 (0.4)
C2	18	6.6–7.8	7.2 (0.3)
C3	8	6.9–7.9	7.4 (0.2)
C4	7	6.4–7.5	7.0 (0.3)
C5	13	7.1–8.3	7.9 (0.3)

mineralization processes is primarily related to soil pH (Harrison, 1982) and the P solubility in soils is also of pH-dependence (Rodríguez et al., 1996).The natural phosphatase activity was related to in situ soil P fractions because it was measured at soil pHs corresponding to 'intact' P status in soils. It, therefore, had the positive correlation with the P fractions in the surface runoff. As a common recommendation, acid phosphatase activity is measured in acidic soils and alkaline phosphatase activity is measured in alkali soils. The acid and alkaline phosphatase activities are measured in artificial pH buffering systems, representing the potentials of phosphatase activity in these soils but not in situ soil phosphatase activity. Hence, natural phosphatase activity may be more adequate for comparing phosphatase status in soils with different pH. In addition, whether the relationship between phosphatase activity and soil P availability is intrinsic or not remains unclear and more elaborate work, such as isotope tracing study, will be needed to quantify the relationship between soil phosphatase activity and P loss by surface runoff.

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