RESEARCH ARTICLE



Accumulation and release of organic phosphorus (P) from legacy P-affected soils to adjacent drainage water

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Abstract

Legacy effects of P in agricultural soils have been highlighted in recent literature. However, co-accumulation and release of organic P (Po) have often been ignored in current agro-environmental assessments. The mineralizable Po fraction has a potential to increase the activity of phosphate in pore water, increasing fertility or degrading water quality. In this study, the effects of agricultural management practices (fertilizer applied corn-soybean rotation cropland and dairy manure applied pasture) on the Po/phosphate ratio were investigated in P-rich (290–1232 mg kg⁻¹) agricultural soils and adjacent ditchwater using experimental soil–water chemistry. The effect of agricultural management was significant on both Po and the Po/phosphate ratio in soil and adjacent ditchwater. The P_o content, dominated by orthophosphate monoesters, in the manure-amended pasture (average ~ 245 mg kg⁻¹) was significantly greater than that in the fertilizer-applied cropland (average 103 mg kg⁻¹). The Po/phosphate ratio was also significantly greater in the manure-amended pasture (0.54) than in the fertilizer-applied cropland (0.42). Similarly, water quality data also showed that ditchwater near the pasture had a significantly greater flux of dissolved non-reactive P and a greater Po/phosphate ratio compared to the water near the fertilizer-applied sites. Furthermore, a greater Po/phosphate ratio in ditchwater was often observed during wet periods, and the ratio was positively correlated to the discharge (r=0.42, p=0.003). The study showed the agricultural management-specific Po accumulation and release and – Po/phosphate ratio that might affect the fate of P in agroecosystems.

Keywords Organic phosphorus · P · Ratio · Soil · Ditchwater · Agricultural management

Introduction

The role of phosphorus (P) is irreplaceable to living organisms. Both inorganic P (Pi) and organic P (P_0) species are ubiquitous in the environment. For instance, in the biological system, Pi forms fundamental materials such as phospholipids for building cell membranes, genetic materials (e.g., DNA, RNA), coenzymes (e.g., esters of phosphoric or pyrophosphoric acid), and biochemical energy reservoirs (e.g., ATP, creatine phosphate, and phosphoenolpyruvate),

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to ensure the functionality of the cell structure (Follmi 1996; Smil 2002). Meanwhile, P_o , such as phytic acid (IHP), is the principal storage form of P in plant tissues (Oatway et al. 2001; Turner et al. 2002), which functions as intracellular regulation of surface receptors and ion channels (Sasakawa et al. 1995). In agriculture, rock phosphorus, synthetic fertilizer, and manure are widely used for crop production (Johnston et al. 2014).

In intensively managed agricultural soils, the continual input of P fertilizers along with the low availability of P for plants has resulted in the accumulation of P in agricultural soils and drainage ditch sediments (Withers et al. 2001). The excessive P in soils can lead to serious environmental issues such as land degradation and eutrophication in aquatic systems (Correll 1998; Daniel et al. 1998). Numerous studies have shown a high level of P in soils and sediments. For example, total P (TP) in surface soils of long-term manure- and fertilizer-amended agricultural soils is 900–4000 mg kg⁻¹ (Sui and Thompson 2000; Pizzeghello et al. 2011). Inorganic P generally accounts for 60–80%

of TP and mainly occurs in mineral forms, whereas P_o is about 30–50% of TP (Smil 2002; Sims and Pierzynski 2005). In agricultural ditch sediments, TP ranges from 250 to 2250 mg kg⁻¹, which is mainly dominated by Pi species (Xu et al. 2013; Soliman et al. 2017; Bastami et al. 2018).

The influence of agricultural management on soil TP concentration has been widely discussed (Sharpley et al. 1984; McDowell et al. 2005; Delgado et al. 2006). It is clear that the accumulation of TP in soils is influenced by the history of fertilizer application (Tiessen et al. 1984; Sharpley et al. 2001). However, no study has carefully documented the co-accumulation of Pi and P_0 (i.e., the ratio between P_0 and Pi) in different agricultural systems, including adjacent drainage water. Dissolved phosphate or dissolved reactive phosphate (DRP) has been of major interest in the scientific community to evaluate water quality. But Po species like IHP could competitively adsorb in soil minerals (Anderson et al. 1974; Guan et al. 2005; Berg and Joern 2006), desorbing phosphate to increase its solubility in porewater. Phosphorus mineralization/immobilization, which controls the amount of P_o in soil, is influenced by agricultural management and other environmental factors such as climate. Therefore, it is of interest to understand the ratio of P_o and Pi in agricultural soils. Organic P species are also an important mineralizable fraction in soil that has been ignored in the current nutrient assessment and management practices. Furthermore, it is unknown how, or if, the P_o/phosphate molar ratio changes as P is translocated from agricultural soils to drainage ditchwater. Understanding the environmentally relevant Po/phosphate ratio in agricultural soils and adjacent water bodies is important in understanding the P nutrient cycling in various agricultural systems. The objectives of the study were (1) to investigate the environmentally relevant P_o/phosphate ratio in different agricultural management systems (fertilizerapplied corn-soybean rotation cropland and dairy manureapplied pasture) and (2) to evaluate how the ratio changes as P translocated from agricultural soils to adjacent ditchwater. We tested the following hypotheses in this study:

- Total P_o concentration and the P_o/phosphate ratio in P-rich soils was influenced by agricultural management practices.
- The P_o/phosphate ratio and total P_o concentration in ditchwater were different from those in nearby agricultural lands that are dominated by corn and soybeans.

Materials and methods

Study sites and materials

included ditches adjacent to, and draining, different agricultural fields (Fig. S1). The B2 field and the west side of B3 have been used for corn and soybean rotation (corn in 2021, soybean in 2020, corn in 2019) and have received long-term fertilizer applications at ~45–56 kg of ammonium sulfate per hectare since the 1960s. The field received spring tillage in every odd-numbered year. The B1 and the east side of the B3 field received dairy manure at an annual application rate of ~25-35 tons per hectare. Total P in dairy manure was 0.8-1.2% by dry weight. The field has never been tilled. The east side of the B3 field is a pasture with teff hay. Soil sampling was conducted in the area of three white grids (B1-B3) (Fig. S1). B3 was divided into B3-F (F, fertilizer applied) and B3-P (P, pasture) (Fig. S1). According to the US Department of Agriculture (USDA) soil taxonomy and phaeozems by World Reference Base for Soil Resources, soils in all areas are poorly drained dark-colored mollisols. Soils were sampled from locations along the drainage ditches to investigate the contribution of P losses from soils to ditchwater.

Surface soil samples (0-20 cm) were collected in spring before planting. A grid sampling method was used. The number of grids at sites B1, B2, and B3 are 16, 8, and 8, respectively (Fig. S1). Soil samples were collected in duplicate at each location. The soil samples were air-dried, crushed, and passed through a 2-mm sieve, and some selected fresh samples were processed for NMR analysis (Text S1). Water samples were collected weekly at Sd1–Sd4 (Fig. S1) and collected biweekly during summer when water levels became low. Sd1 was not near agricultural fields, but Sd2-Sd4 were influenced by various agricultural practices nearby. At each location, approximately 0.5 L of water sample was collected using a grab sampler, and the flow rate, temperature, turbidity, pH, and NO₃⁻ concentration were measured (bi)weekly from March to August 2021. The flow rate and the temperature of ditchwater were measured by the Flowatch Flowmeter Station (RainmanWeather, Jacksonville, FL). Water pH and turbidity were measured by the pH meter (VWR sympHony, B40PCID Benchtop Multi-Parameter Meter, Radnor, PA) and the turbidity meter (Thermo Scientific, AQ4500 Turbidimeter, Waltham, MA), respectively. The concentration of nitrate was measured colorimetrically at 220 nm according to Patey et al. (2008). All reagents were prepared using ultrapure water (18.2 M Ω cm), and ACSgrade chemicals were used unless otherwise mentioned in the text.

Physicochemical characterization of soils

Soil samples were characterized for pH at a solid/water ratio of 1:2 in ultrapure water (McLean 1983; Thomas 1996) and % organic matter content using a loss-on-ignition method (Schulte and Hopkins 1996). Texture was characterized using a hydrometer method soil (Gee and Bauder 1986),

and cation exchange capacity (CEC) was characterized using an ammonium acetate (NH_4OAc) method at pH 7 (Ross and Ketterings 1995). Mehlich 3 and Bray-I agronomic P extractions were also conducted (Bray and Kurtz 1945; Mehlich 1984).

Total phosphorus (TP), total inorganic (TPi), and total organic P (TP_o) fractions in soil

Total inorganic and organic P contents in soil/sediment were measured by the concentrated sulfuric acid and dilute sodium hydroxide solutions (Kuo 1996). Duplicates were obtained for each sample. Briefly, a 1.0-g air-dried sample was mixed with 1.5 mL of concentrated H_2SO_4 in a 50-mL volumetric flask. After mixing with deionized water, samples were cool to room temperature. Filtrates through Whatman no. 2 filter paper were analyzed as an acid extract. The soil/sediment residue was extracted with 0.5 M NaOH solution, and the filtrate was saved as a base extract.

For total P (TP) determination (Arenberg and Arai 2021; Bowman 1989), both acid or base extracts were digested with $K_2S_2O_8$ and 5.5 M H_2SO_4 at 150 °C for 30 min. After cooling, *p*-nitrophenol was added. pH was adjusted with 1–10 M NaOH solutions until the color just changed to yellow. The P concentration was then determined using the molybdenum blue method (Murphy and Riley 1962). The concentration of TP was calculated by summing the P concentration in acid and base extracts.

For TPi determination, phosphate concertation was analyzed in undigested acid or base extracts using the modified ascorbic acid method (Miller and Arai 2016) to avoid the hydrolysis of P_0 species. The concentration of Pi in soil was calculated by summing the P concentration in the acid and base extracts. Accordingly, TP_0 fraction in the initial soil/sediment sample was calculated using the following equation (Kuo 1996):

 $TP_0 = TP - TPi$

To support TPi and TP_0 fractionation, solution P^{31} nuclear magnetic resonance (NMR) spectroscopy was used (Text S1).

Inorganic P fractionation in soils

Inorganic P in calcareous soils generally contains loosely bound soluble P, non-occluded phosphate, iron-occluded P, and calcium P, which were extracted in sequence by different solutions (Hedley et al. 1982; Kuo 1996). Initially, a 0.5-g soil sample and a 25-mL 1 M NH₄Cl solution were mixed and shaken for 30 min at 80 rpm on an orbital shaker to extract the pool of soluble P. Then, the paste samples were extracted with 25-mL 0.1 M NaOH and 1 M NaCl solution for 17 h for non-occluded phosphate. The iron-occluded phosphate fraction was extracted by the sodium citratebicarbonate-dithionite (CBD) method. Briefly, sample residue from the last step was mixed with 20 mL of 0.3 M Na₃C₆H₅O₇ and 2.5 mL of 1 M NaHCO3, and was placed in a water bath at 85 °C. Then, 1.0 g of Na₂S₂O₄ was added to each sample, and samples were kept in the 85 °C water bath for 15 min. Finally, the calcium P fraction was extracted by 25 mL of 0.5 M HCl for 1 h. During the extraction, all tubes were shaken on an end-over shaker at 30 rpm. After each extraction step, samples were centrifuged at 2862 g for 10 min, and the P analysis of supernatants was conducted by the modified molybdenum blue method (He et al. 1998). Phosphorus standards were prepared to contain the same volume of extracting solution as in the extracts. For each sample, two replicates were obtained.

Dissolved phosphorus (DP), particulate P (PP), and TP in water

The concentration of DP that includes dissolved reactive P (DRP) and dissolved non-reactive P (DNRP) was measured in water samples that were passed through 0.22-µm PVDF filters. For DRP analysis, 2 mL of the filtered water sample was transferred into a 50-mL volumetric flask. Five drops of *p*-nitrophenol were added. pH was adjusted to 6.5–7.2 with 1 mM NaOH or HCl solutions. The DRP concentration was determined by the modified ascorbic acid method (Miller and Arai 2016). For DP concentration, about 5-mL of the filtered water sample was digested with 0.2 g of $K_2S_2O_8$ and 2 mL of 5.5 M H₂SO₄ solution on a hot plate at 150 °C for 30 min. After cooling, five drops of *p*-nitrophenol were added. pH was adjusted with 10 M NaOH solution until the color changed to yellow. The DP concentration was measured by the modified molybdenum blue method (He et al. 1998) to minimize the effect of other ligands. The DNRP was calculated using the following equation:

DNRP = DP - DRP

Unfiltered water samples were digested by the same procedure described above, and TP was determined by colorimetry. The PP concentration was calculated using the following equation:

PP = TP - DP

Short-term water extraction of dissolved and particulate P from agricultural soils

Numerous researchers identified the high correlation between the water-extractable P from soils (with the soil to solution ratio ranges from 1:10 to 1:200) and the dissolved reactive P in surface runoff (Pote et al. 1996; Aase et al. 2001; Turner et al. 2004). The rapid release of P in surface runoff during a storm event was simulated by the short-term water extraction experiment for selected soil samples. A 25-g soil sample and 500-mL deionized water were mixed in a 500-mL bottle. All soil suspensions were continuously shaken on a shaker at 80 rpm for 5 min. Then, the samples were let stand for 44 s to settle sand particles. Then, the upper part solution was carefully siphoned and filtered through a 1.0-µm filter paper. After prefiltration, the solution was secondarily filtered through a 0.22-µm filter paper to separate the colloidal P from water. The residues were digested, and colloidal P was determined by colorimetry (He et al. 1998; Miller and Arai 2016). Filtered water was transferred to a new 500-mL bottle. Five grams of aluminum hydroxide was added to concentrated dissolved P, and the mixture was shaken at 80 rpm for 24 h. After that, the majority of the water was decanted, and the residues were centrifuged for 10 min at 4000 rpm to isolate the solids from the residue water. The residue was air-dried and used for 31-P NMR analysis (Text S1 and S2).

Statistical analyses

All statistical analyses were performed using the RStudio Desktop 1.4 (RStudio Team 2020). To investigate the distribution of soil P, spatial statistical analyses were conducted. The spatial autocorrelation for each soil variable was assessed using Moran's I test. If no spatial autocorrelation was found in the tested variables, this indicated that variables were independent. The effect of agricultural management and the distance to ditch on the soil P properties, including TP, TP_o, phosphate, water-extractable P, and P_o/phosphate ratio, were analyzed by the Student *t*-test. RandomForest was used to evaluate the importance of soil variables' effect on the soil P content. Based on the Random-Forest results, Pearson correlation was tested between the important soil variables and soil P parameters. The mixed model was also used with the sampling area as the grouping effect. If the significant effect found in Pearson correlation cannot be scaled down to each sampling area, it generally means that the found initially significant effect is probably due to the difference in sampling location. All p-values were reported for the significance analyses.

The differences in the P fluxes, including DRP, DNRP, PP, and the DNRP/DRP ratio between each two sampling sites, were tested by the Student *t*-test. The objective was to determine whether the P loss in different tributaries was significantly different from each other. One-way ANOVA analyses were conducted to test the effect of climate (i.e., precipitation and temperature) on water DRP, DP, PP, and TP content and the DNRP/DRP ratio in two tributaries (Sd1-2, Sd3) and the watershed outlet (Sd4). According to the National Oceanic and Atmospheric Administration website (NOAA 2021), daily average precipitation and daily average air temperature were reported. Pearson correlation was also used to test the linear correlation between weekly average precipitation/temperature and water properties or water P characteristics (DRP, DP, PP, and TP content and the DNRP/ DRP ratio) for every water sampling point, and significant correlations were reported.

Results and discussion

Physicochemical characterization of soil

Table 1 shows the physicochemical properties of all soil samples. According to the US Department of Agriculture Soil Survey report, the major soil series at the study area is Drummer silty clay loam (0 to 2% slopes, fine-silty, mixed, superactive, mesic Typic Endoaquolls) (USDA Soil Survey 2019). Soil organic matter content (%OM) was influenced by the source of fertilizer, either manure or synthetic fertilizer. The %OM ranged from 4.2 to 7.3% for the fertilizeramended fields (B2 and portion of B3) and from 7.7 to 12.2% for the manure-applied pasture (B1 and B3-P). The effects of fertilizer on %OM was also reported in previous studies (Vitosh et al. 1973; Hooda et al. 2001). The average soil pH was 6.3 in the fertilizer-amended field, and 6.85 in the manure-applied field due to the difference in the % base saturation (Amundson et al. 2003; Rogovska et al. 2007). The average % base saturation (BS) in the manure-applied field was 91.43%, whereas that in the fertilizer-applied field BS, it was 82.46%. Cation exchange capacity varied from ~ 17.6 to 35.9 cmol_c kg⁻¹, which was consistent with the soil survey report of mollisols (USDA Soil Survey 2019). No significant difference in CEC was found between the manure- and fertilizer-applied fields. The dominant soil texture mainly was silty clay loam and silt loam. Mehlich III P was 23–515 mg kg⁻¹ in all soil samples, which was much greater than the recommended soil P test value for corn and soybean growth in this region $(15-22.5 \text{ mg kg}^{-1})$ according to the Illinois Agronomy Handbook (2009) (Fernández and Hoeft 2009). The Bray I P content showed a similar trend. Both agronomic soil P tests showed a more significant P in the manure-applied field than in the fertilizer-applied field.

Total Pi and P_o fractionation and Pi fractionation in soils

The results of total P fractionation and the Pi fraction are summarized in Table 2. The content of TP ranged from ~ 280 to ~ 1230 mg kg⁻¹ in all soil samples. The TP in the manure-applied pasture (~460–1230 mg kg⁻¹) was higher than the TP in the fertilizer-applied sites (~ 280–430 mg kg⁻¹). A similar trend regarding

Sampling area	Distance to the ditch	Soil texture	CEC (cn	nol _e kg ⁻¹)	Hq		4M0%		% base s	aturation	Mehlich] (mg kg ⁻¹	III P	Bray I P kg ⁻¹)	(mg
B1 Manure applied pasture	Far	Silt loam	31	±2	6.60	± 0.00	12.16	± 0.09	88.99	± 0.01	93	+ 4	86	+3
	Far	Loam	31	+1 -1	7.30	± 0.00	10.2	± 0.4	94.23	± 0.02	88	±2	73.00	± 0.00
	Far	Silt loam	34.4	± 0.2	6.00	± 0.00	7.7	± 0.1	79.43	± 0.00	57	+1	43.5	±0.7
	Far	Clay loam	28	+3	6.35	± 0.07	7.8	± 0.1	85	+1	70	+ 8	56	1 6
	Near	Silt loam	30	+1 3	7.00	± 0.00	11.2	± 0.1	95.34	± 0.00	115	+ 8	87	+3
	Near	Silt loam	33	+ 3	6.8	± 0.2	11.0	± 0.4	93	+3	86	±5	65	+1 1
	Near	Silt loam	36	+1 1	6.85	± 0.07	10.6	± 0.4	93	+1 1	06	+1	62	9Ŧ
	Near	Silt loam	33.64	± 0.08	6.75	± 0.07	10.81	± 0.07	91	+1	06	+5	71.5	± 0.7
	Near	Silt loam	30	±2	6.80	± 0.00	11.9	± 0.4	92.14	± 0.02	54	±2	45	+ 1
	Near	Clay loam	26.1	±0.4	7.05	± 0.07	8.0	± 0.1	95.4	± 0.1	62	±2	49.00	± 0.00
	Near	Silt loam	30.9	±0.4	6.60	± 0.00	10.2	± 0.3	88.89	± 0.01	110.00	± 0.00	89	+ 4
	Near	Loam	27	+1	7.30	± 0.00	10.9	±0.2	95.66	± 0.01	93	+1	63.5	± 0.7
	Far	Loam	17.6	± 0.5	6.30	± 0.00	7.84	± 0.01	84.10	± 0.01	70	+1	57	+ 4
	Far	Silt loam	27.5	± 0.3	6.65	± 0.07	11.7	± 0.4	90	+1 1	55	9 +	43.5	± 0.7
	Far	Silt loam	27.2	± 0.7	6.70	± 0.00	10.9	± 0.5	90.50	± 0.01	167	±7	132	± 11
	Far	Silt loam	29.0	±0.9	6.60	± 0.00	9.5	± 0.4	88.99	± 0.01	50	±2	29	+1
B2: Fertilizer applied cropland	Far	Silty clay loam	32.1	± 0.7	6.20	± 0.00	5.63	± 0.06	82.58	± 0.00	31.5	± 0.7	18.5	± 0.7
	Near	Clay loam	27.1	± 0.2	6.20	± 0.00	4.2	± 0.1	82.61	± 0.01	39.5	± 0.7	25.5	± 0.7
	Far	Clay loam	28	±2	6.65	± 0.07	5.3	± 0.1	06	+1 1	43	+1 1	25.00	± 0.00
	Near	Clay loam	26.2	±0.6	6.10	± 0.00	4.88	± 0.03	81.11	± 0.03	54	+	39.5	± 0.7
	Far	Silty clay loam	35	+1	5.95	± 0.07	6.0	± 0.2	78	±2	23.00	± 0.00	7.5	± 0.7
	Near	Silty clay loam	30	±2	5.6	± 0.1	5.03	± 0.06	67	1 4	31.00	± 0.00	16	±2
	Far	Silty clay loam	35	1 3	5.80	± 0.00	6.27	± 0.08	73.05	± 0.02	41.5	± 0.7	21.5	± 0.7
	Near	Silty clay loam	33.0	± 0.1	5.70	± 0.00	5.56	± 0.04	69.82	± 0.03	35.5	± 0.7	16.5	±0.7
B3-F ^c Fertilizer applied cropland	Far	Silt loam	33.5	± 0.4	6.85	± 0.07	7.0	± 0.2	93	+1	54.5	± 0.7	31.5	±0.7
	Near	Clay loam	25	+1	7.3	± 0.1	6.4	± 0.2	95.6	± 0.2	75.5	± 0.7	52.5	±0.7
	Far	Silty clay loam	35	±2	6.60	± 0.00	7.3	± 0.2	88.77	± 0.00	110	±7	80	±16
	Near	Silty clay loam	31	±2	6.60	± 0.00	7.0	± 0.2	88.84	± 0.01	43.5	± 0.7	30	1 4
B3-P ^d Manure applied pasture	Near	Silty clay loam	27.2	± 0.6	7.25	± 0.07	8.96	± 0.08	95.66	± 0.06	169	±4	126	±5
	Far	Silt loam	27	±2	7.55	± 0.07	9.3	± 0.2	95.98	± 0.07	516	± 16	320	±2
	Near	Silt loam	20.87	± 0.01	7.20	± 0.00	9.2	± 0.2	95.57	± 0.02	166	±5	140	±11
	Far	Silt loam	29.5	± 0.6	7.25	± 0.07	9.7	± 0.4	95.61	± 0.05	395	+6	288	±27

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CEC cation exchange capacity, %OM organic matter content, F fertilizer applied, P pasture

Table 2 Total P, total Pi, total Po, and Pi fractionation of soil samples in various agricultural systems

Sampling area	ТР		TPi		TP _o		Solubl	e Pi	Non-oc Pi	cluded	Fe-occlu	ided Pi	Ca-Pi		TP _o /TPi
(mg P kg ⁻¹ soil)														
B1	866	±1	565	±2	301	±3	12.2	±0.2	192.0	±0.2	150	±6	116.5	±0.3	0.53
	645	± 18	417	± 4	228	±15	5.3	± 0.2	184.6	± 0.6	119	±6	106.1	± 0.5	0.55
	488	± 0.6	344	±3	144	±3	1.9	± 0.2	131.4	± 0.8	104	<u>+</u> 4	113	± 1	0.42
	581	± 22	349	±9	232	±13	2.2	± 0.5	161	± 2	125	± 2	83.2	± 0.2	0.67
	993	±5	712	±13	280	±17	13.63	± 0.00	320	± 1	270	± 2	125	±4	0.39
	676	± 1	417	± 15	258	± 17	11.6	± 0.4	169.2	± 0.2	146	±6	94.6	± 0.2	0.62
	755	±5	485	± 12	270	± 8	9.1	± 0.3	172	± 2	115	± 2	110	± 1	0.56
	811	±4	493	±11	318	±15	17.8	± 0.6	185	±1	152	<u>±</u> 4	111	±3	0.64
	761	±16	437	±16	323.8	± 0.2	8.0	± 0.4	175	±3	154	±6	82	± 2	0.74
	692	±0.9	445	± 10	247	±11	6.0	± 0.2	145	± 2	168	±6	112	± 1	0.56
	719	±15	431	±3	288	±18	16.7	± 0.2	196.7	± 0.8	132	± 4	68	±3	0.67
	602	±11	353	±3	248	± 8	14.1	± 0.2	147.5	± 0.3	100.81	± 0.06	87	±3	0.70
	437	±18	306	± 8	131	± 10	5.4	± 0.1	167	± 2	90	± 2	15.9	± 0.6	0.43
	570	± 2	351	± 8	219	± 10	4.5	± 0.2	139	± 4	75	±6	61	± 1	0.62
	633	±4	416	±9	217	±6	37.4	± 0.3	227	± 2	80.8	± 0.2	44	± 1	0.52
	463	±15	241	± 1	222	±14	2.4	± 0.2	92.4	± 0.2	56	<u>±</u> 6	56	± 2	0.92
B2	346	<u>±</u> 9	231	<u>+</u> 4	115	±5	0.6	± 0.1	61	± 2	59	±6	124.6	± 0.5	0.50
	311	±6	255	± 8	56	± 14	0.96	± 0.00	83	± 2	63	± 2	106	± 2	0.22
	373	±11	241	± 1	132	± 12	0.8	± 0.1	61	± 2	34	±4	123	±3	0.55
	407	± 10	296	± 2	111	± 8	1.2	± 0.2	113	± 2	82	± 2	115.95	± 0.04	0.37
	285	±14	182	± 2	103	± 12	0.8	± 0.1	41.9	± 0.9	40	± 2	96	± 1	0.57
	286	±19	197	±3	89	±16	0.4	± 0.1	62.6	± 0.4	53.78	± 0.06	66	± 1	0.45
	357	± 12	234.7	± 0.3	122	± 12	0.24	± 0.00	68.0	± 0.4	50.20	± 0.08	99	± 1	0.52
	352	±6	209	± 2	143	± 8	0.6	± 0.1	65.8	± 0.6	44	± 2	79.40	± 0.09	0.69
B3-F	392	±15	258	±5	134	± 20	0.4	± 0.1	68.21	± 0.06	36	±6	81	± 2	0.52
	394	±14	320	±15	74	±29	2.3	± 0.1	120	±1	86	±6	114	± 1	0.23
	400	± 1	364	±16	36	± 14	3.8	± 0.2	169	± 1	87	± 2	113	± 1	0.10
	426	±7	313	±6	113	±13	1.19	± 0.00	99	± 1	77	±4	113	± 2	0.36
B3-P	654	± 23	484	± 10	171	± 14	21.6	± 0.2	254	± 2	90	±6	84	±3	0.35
	963	± 22	849	±7	113	±15	63	± 2	465	<u>±</u> 4	192	±4	83	± 1	0.13
	678	±9	502	± 10	176	± 2	24.2	± 0.5	265	±4	128	±4	71.9	± 0.9	0.35
	1232	± 23	929	<u>±</u> 6	302	± 28	73	± 1	482	± 2	244	± 8	90	± 2	0.33

F fertilizer applied, P pasture

agricultural management effect was also found in the literature (Sharpley et al. 1984; Lehmann et al. 2005; McDowell et al. 2005; Delgado et al. 2006). Total Pi, especially phosphate, was the dominant fraction (~52–91% of TP) in soils. The effect of agricultural management can be seen both in TPi and TP_o. While the synthetic fertilizer-applied field had TPi ~ 180–360 mg kg⁻¹ and TP_o ~ 36–140 mg kg⁻¹, the manure-applied field had TPi ~ 240–930 mg kg⁻¹ and TP_o ~ 110–320 mg kg⁻¹. A high TP_o content in cattle manure-amended soils was also reported by other researchers (Hooda et al. 2001; Sharpley et al. 1984). This was attributed to the high P_o content in dairy manure ranging from ~ 700 to 3000 mg kg⁻¹ based

on dry mass (Barnett 1994; Sharpley and Moyer 2000; He and Honeycutt 2001; Ajiboye et al. 2004; He et al. 2004; Li et al. 2014) and because its high OC content (i.e., high C/P ratio) facilitated P immobilization. The P_o /phosphate ratio ranged from ~0.1 to 0.92 across the whole study site, which was consistent with findings by other researchers (Tarafdar and Jungk 1987; Hooda et al. 2001; Pizzeghello et al. 2011, 2014; Abdala et al. 2015, 2018; Tian et al. 2017). Generally, the average value of the P_o /phosphate ratio in the manure-applied field (0.54) was higher than that in the fertilizer-applied field (0.42). The higher P_o fraction in manure-applied soils was probably due to the higher OM content in the manured-applied pasture (i.e.,

C:P>300), where the P immobilization process dominated (Dalal 1977; Sanyal et al. 1991).

The results of the Pi fractionation suggested that the content of each Pi fraction was generally in the following order: soluble P < Fe occluded P ~ calcium-associated P<non-occluded P. For all soil samples, the non-occluded P fraction (e.g., amorphous Fe or Al associated P) was dominant, which was 23-61% of TPi. The dominance of the nonoccluded P fraction can be explained by the high P retention capacity of amorphous Fe and Al (oxyhydr)oxides (Peña and Torrent 1984; Arai and Sparks 2001; Agbenin 2003; Borggaard et al. 2004; Arai et al. 2005; Hartono et al. 2005; Bera et al. 2006; Li et al. 2007). The calcium-associated P fraction (Ca-P) was generally 6-56% of TPi. No significant difference was found in the Ca-P content between manureand fertilizer-applied fields. This was probably due to the similar Ca content from both sites. Mehlich III Ca concentration was 3750 mg kg⁻¹ in the manure-applied field and ~ 3800 mg kg⁻¹ in the fertilizer-applied field. The high Ca content in soils accounts for a major Pi fraction as Ca-P precipitates (i.e., hydroxyapatite) and adsorbed phosphate in calcite. The fractions of Fe occluded P (Fe-P) and soluble P were 10-39% and 0.11-20% of TPi, respectively. Both fractions were significantly higher in the manure-applied field. The higher Fe–P content in the manure-applied sites was attributed to the higher Mehlich III Fe concentration in the manure sites (~244 mg kg⁻¹) than in the fertilizer sites (~184 mg kg⁻¹). Previous studies found higher waterextractable P and available P concentration from manureapplied soil than from fertilizer-applied soil (Aase et al. 2001), suggesting that the application of dairy manure increased bioavailable P in soils. In our study, we observed a similar result that the manured applied pasture had generally greater non-occluded P (average 214 mg kg⁻¹) than in the fertilizer site (average 84 mg kg⁻¹).

Furthermore, the NMR analysis (Fig. 1, Text S1 and S2) indicated that common P_o species were orthophosphate monoesters, such as neo-inositol hexakisphosphate, adenosine-5 phosphate, myo-inositol hexakisphosphate, choline phosphate, scyllo-inositol hexakisphosphate, α -glycerophosphate, and β -glycerophosphate in most samples. The NMR analysis also showed that pyrophosphate was less than 3.1% of TPi, indicating that the value of TPi can be treated as the phosphate concentration in soils (Table S2).

Characterization of drainage water

Meteorological data (air temperature and precipitation) of the study area, stream discharge, and water temperature are summarized in Fig. 2. Turbidity, pH, and NO_3^- concentration are also shown in Fig. S2. High rainfall events generally correlated to stream discharges at all sampling sites (Fig. 2b–e) as discharge generally increased during high rainfall seasons and decreased in drier periods. An extremely high discharge was found on June 30, 2021, in Sd1, Sd2, and Sd4, which was associated to storm events during June 25–30, 2021. The lack of this discharge peak in Sd3 can be explained by the plant uptake from nearby agricultural croplands. Overall, stream discharges were greater from March to May and lower from June to August due to the increased evaporation and plant uptake. Daily average air temperature fluctuated over time, but the overall trend generally increased from March to August. Weekly water temperatures from Sd1 to Sd4 (Fig. 2b–e) generally followed the trend of air temperature, which also gradually increased throughout the study period.

Turbidity ranged from about 1 to 62 NTU (Fig. S2a). Water from Sd1 had the highest turbidity (~5 to 62 NTU), and Sd2 and Sd4 had 2.7 to 20 NTU. Sd3 had the lowest turbidity (~1 to 12 NTU). The high turbidity in Sd1 was probably due to the upstream lake, which has good mixing of the water and lake sediment, and the low turbidity in Sd3 can be explained by the high sand content in the Sd3 sediment. Over time, the high turbidity values in water were associated with the storm events due to the increased soil erosion (Ziadat and Taimeh 2013; Lee et al. 2016; Wu et al. 2021).

pH of the water was at near neutral during wet periods and increased to ~8 during the dry period at the sites (Fig. S2b). A similar pH trend was also identified in previous studies (Lynch et al. 1986; Laird 2006; Dinsmore et al. 2013). During the dry period, the flow was intercepted with subsoils where calcite and dolomite were dominant minerals in subsoils (Xu et al. 2020).

Figure 3 shows the results of weekly dissolved reactive P (DRP), dissolved non-reactive P (DNRP), and particulate P (PP) from four sampling locations. The DNRP refers to the dissolved form of P that was non-reactive to the molybde-num blue method. Therefore, it represents the concentration of dissolved P_0 and colloids (<0.2 µm).

At Sd1 (Fig. 3a), the dominant form of P was PP, which ranges from ~0.001 to 0.143 mg L^{-1} . The high concentration of PP was correlated with the high water turbidity found in Sd1 (Fig. S2b). The concentrations of DRP and DNRP in Sd1 were generally less than 0.014 mg L^{-1} since the sampling location was not near agricultural land. Water from Sd2 that was impacted by the manure-applied field (Fig. 3b) showed the same pattern as Sd1, in which PP was also the dominant form of P (0.001–0.068 mg L^{-1}). The DRP and DNRP concentrations in Sd2 were 0.005–0.030 mg L^{-1} and $0.002-0.042 \text{ mg L}^{-1}$, respectively. In most cases, the concentration of DNRP was greater than DRP in Sd2, which was potentially caused by the nearby the manure-applied sites in B1. The relatively high concentration of released DNRP in overland flow or tile water near manure-applied fields was also reported by others (Hooda et al. 1999; McDowell and Sharpley 2002; McDowell et al. 2005;



Fig. 1 ³¹P solution state NMR spectra of soil extracts from the **a** soil sample from the B2 field spiked with reference Po compounds, **b** B1 manure-applied pasture, **c** B2 and B3-F fertilizer-applied cropland, **d** B3-P manure-applied pasture. Soils were extracted with NaOH-EDTA. Peaks assigned as Ortho-P, orthophosphate; n, neo-IHP; m, myo-IHP; α , α -glycerophosphate; β , β -glycerophosphate; A,

Kleinman et al. 2009). At Sd3, which was impacted by the fertilizer applied field (Fig. 3c), the content of PP was generally low (0.001–0.046 mg L^{-1}). The dissolved P in Sd3 was 0.007-0.074 mg L⁻¹ and was generally dominated by DRP $(DRP 0.005-0.062 \text{ mg } \text{L}^{-1} \text{ and } DNRP 0.002-0.039 \text{ mg } \text{L}^{-1}).$ Although the concentration of DRP was greater in Sd3 than in Sd2, the flux of DRP and DP was consistently lower in Sd3 due to the difference in discharge. Total P in Sd4, which was impacted by both B3-F and B3-P, was dominated by PP $(0-0.123 \text{ mg L}^{-1})$ in most cases (Fig. 3d). The concentration of PP was also positively associated with water turbidity. In the dissolved form of P, the content of DNRP was consistently greater than DRP except for several dates in June and July. The dominance of DNRP in Sd4 can be ascribed to the nearby pasture in B3-P (Fig. 3d). During the dry period, DRP is released from soil to water through subsurface flow. However, the DNRP content was generally higher during wet periods. Generally, greater DNRP/DRP ratios were

adenosine 5' monophosphate; C, choline phosphate; S, scyllo-IHP; DNA, deoxyribonucleic acid; and Pyro-P, pyrophosphate. Unknown monoester peaks are denoted by 1, 2, 3, and 4, and unknown diester peak is denoted as d1. Orthophosphate monoesters are found in the region from ~5.8–3.0 ppm, and orthophosphate diesters are found in the ~0.15- to – 0.75-ppm region

found during wet seasons due to mobile P_o species in soils (Frossard et al. 1989; Simard et al. 1995; McDowell et al. 2005; Pizzeghello et al. 2016).

Dissolved and colloidal P in soil water extracts

The results of water-extractable colloidal P in the selected soil samples are summarized in Table 3. It is important to note that the soil colloids in this study are operationally defined as soil particles with a diameter between 0.22 and 2 μ m. The percent recovery rate of colloids was 0.43 to 0.86% in all sites. For P concentration, colloidal P was greater in the manure sites than the fertilizer-amended site. The concentration of TP in soil colloids was 964–1385 mg kg⁻¹ in the manure-applied field and 578–706 mg kg⁻¹ in the fertilizer-applied field. For both fields, Pi was dominant, which ranged from 54 to 85%, while P_o only made up ~ 15–46% of the total colloidal P.

Fig. 2 a Daily average precipitation and air temperature in the whole study site from March 21 to August 12, 2021. Precipitation is denoted by filled bars, and air temperature is denoted by the dotted line. **b–e** Weekly discharge and temperature of water from sampling sites Sd1, Sd2, Sd3, and Sd4. Discharge is denoted by filled bars, and water temperature is denoted by dotted lines



No significant difference was found in Pi and P_o fractions between manure- and fertilizer-applied sites. Compared to soil P data (Table 2), TP and Pi concentrations in soil colloids were greater. However, the concentrations of P_o in soils and colloids were not significantly different.

The dissolved P concentration during the water extraction is also presented in Table S3. The concentrations of soil dissolved Pi (DPi), dissolved P_o (DP_o), and dissolved total P (DTP) were well correlated with the TPi, TP_o, and TP concentrations in soils collected at adjacent fields (Table 2), respectively. High DPi, DP_o, and DTP (~24 to 170 mg kg⁻¹) were found in the manure-applied pasture. For all soil samples, DP_o was the dominant form of the soil dissolved P species, which ranged from ~ 60 to 81% of DTP. This result might be explained by the high mobility and solubility of P_o species (e.g., phytic acid) in soils (Frossard et al. 1989; Simard et al. 1995; McDowell et al. 2005; Pizzeghello et al. 2016). The DP_o/DPi ratio was also well correlated with the $TP_o/phosphate$ ratio. However, the DP_o/DPi ratio ranged from 1.5 to 4.3, while $TP_o/phos$ phate was generally less than 0.9. This suggested that soil released more P_o during storm events.



Fig. 3 a-d Flux of dissolved reactive P (DRP), dissolved non-reactive P (DNRP), and particulate P (PP) of water from sampling sites Sd1, Sd2, Sd3, and Sd4

Table 3 Water extractable soil colloidal P in selected soil samples under various agricultural management practices

Sampling area	% clay	of bulk	%recovery	CIP _C ^a		COP _C ^a		CTP _C ^a		CIP _S ^a		COP _S ^a		CTP _S ^a	
	soil		rate by wt	(mg P l	cg^{-1} coll	oids)				(µg P l	kg ⁻¹ soil	l)			
B1	24.7	±1.54	0.65	804	±1	160	±2	964	±4	5224	±6	1039	±13	6263	±26
	30.7	± 0.79	0.73	760	± 1	257	± 2	1018	±11	5550	±7	1879	±14	7429	± 51
	20.1	± 0.88	0.43	652	± 0.6	548	±3	1200	± 2	2802	±3	2358	± 12	5159	±9
B2	29.1	±1.29	0.86	494	± 2	84	±5	578	± 8	4245	±17	726	±43	4971	±69
	32.7	± 1.30	0.56	569	±4	136	<u>±</u> 1	706	±7	3188	±22	763	±6	3951	±39
	35.7	±0.16	0.68	345.9	± 0.8	256.0	± 0.8	602	±13	2352	±6	1741	±6	4093	± 88
B3-F	32.9	± 0.60	0.56	517	±3	141	± 1	657	±5	2895	±17	787	±6	3682	± 28
В3-Р	24.9	± 0.73	0.44	1099	± 2	286	± 2	1385	± 12	4834	±9	1259	±9	6093	±53

 CIP_C concentration of soil colloidal inorganic P in colloids, COP_C concentration of soil colloidal organic P in colloids, CTP_C concentration of soil colloidal inorganic P in soil, COP_S concentration of soil colloidal organic P in soil, COP_S concentration of soil colloidal organic P in soil, CTP_S concentration of soil colloidal total P in soil, F fertilizer applied, P pasture

Relationship between agricultural management and P in soil and ditchwater

Effects of agricultural management on soil P distribution

In this study, the effect of agricultural management on soil P contents and the P_o/phosphate ratio was found to be significant (Table S4). Soil TP, phosphate, Po, water-extractable P, and the P_o/phosphate ratio were all significantly higher in the manure-applied field than in the fertilizer-applied field. Dairy manure is often considered as organic fertilizer, and the P_0 content in manure is much greater than in inorganic fertilizer (e.g., diammonium phosphate). The reported $P_0/$ phosphate ratio in dairy manure was 0.15-0.48 (Barnett 1994; Sharpley and Moyer 2000; He and Honeycutt 2001; Ajiboye et al. 2004; He et al. 2004; Li et al. 2014). Therefore, the application of manure can increase the P_o content and the P_o/phosphate ratio in soils. As for the spatial distribution of P in soils, we expected the P concentration gradient would vary as a function of distance from the ditches. However, the effect of distance to the ditch was insignificant across all soil P parameters. The lack of spatial distribution in soil P can be explained by the fact that the pathway of P loss was probably dominated by subsurface base flow in this relatively flat landscape instead of surface runoff. Hence, the evidence of P transport might not be found in surface soil P content.

The result of the RandomForest model (Fig. S3) showed that several soil variables might contribute to soil P contents and ratios, including the OM content (%OM), %base saturation (%BS), pH, and elevation. It is important to note that the % variation explained in the RandomForest models was generally greater than 50% for soil P contents. The results of the Pearson correlation and the mixed model are summarized in Table S5 "Overall" and "Within-group," respectively. When considering the overall dataset, the soil P_o content was significantly influenced by %OM, %BS, and pH, and the elevation was important for the P₀/phosphate ratios. However, when scaled down to within each sampling area (B1, B2, B3-F, B3-P), %OM was still an important factor for the soil Po contents, while the effects of %BS and pH on P_o were largely reduced or even insignificant. This suggests that the significant effects of %BS and pH were probably due to the difference in sampling location. Therefore, %OM might be the significant driver of soil P_0 content. The %OM was, in turn, influenced by the agricultural management (Table 1). Thus, the effect of agricultural management on soil P_o was probably mainly due to the difference in %OM in soils. Table S5 also suggested that the P₀/phosphate ratio in water extracts was influenced by the %BS and elevation. The %BS was negatively correlated to this ratio, whereas elevation was positively correlated, but the Pearson coefficients indicated that neither correlation was strong. For the P_{o} /phosphate ratio in soil, although the significant effect of elevation and %OM exists in the overall dataset, it cannot be scaled down to individual groups. Therefore, the current study can only conclude that the P₀/phosphate ratio was influenced by agricultural management.

Agricultural management on P content in adjacent ditchwater

The release of P into aquatic systems has become a concern as the increased P concentration in rivers and lakes has been reported for the past few decades (Caraco et al. 1992; Sharpley et al. 1994; Gibson et al. 1995; Foy and Bailey-Watts 1998). Non-point sources, such as agricultural cropland and dairy farms, are considered to be the primary cause of P input to surface water bodies (Sharpley et al. 1994; Gibson et al. 1995; Jennings et al. 2003). For instance, Sharpley et al. (1994) found that 55% of the investigated river length and 57% of the investigated lake area in the USA were influenced by the diffuse P losses from agricultural sources. Hence, the content and flux of P in water can be affected by nearby soils. In the current study, the effect of agricultural management was also found to be significant on the water P flux (Table S6).

Water samples from different locations have a different flux of P. The flux of DRP and DNRP (i.e., dissolved P_o and colloidal $P < 0.22 \mu m$) in Sd1 were generally far less than those in the other three sampling sites. Water from Sd2, which was near the manure-amended site (B1), had significantly greater DRP and DNRP flux than water in Sd3 near the fertilizer-applied sites (B2). Samples in Sd4, which was adjacent to both the pasture (B3-P) and the fertilizer-applied sites (B3-F), also had significantly greater DRP and DNRP than water in Sd3. The flux of PP generally followed the following trend: $Sd4 \sim Sd2 > Sd1 > Sd3$. If we assume DNRP represents dissolved P_o in water, one can set the ratio of P_o /phosphate in water equal to the DNRP/DRP ratio. The DNRP/DRP ratio showed a significant difference between Sd1 and Sd2, Sd1 and Sd4, and Sd2 and Sd3. This suggested that water near the manure-amended sites generally had a greater DNRP/DRP ratio than water near the fertilizerapplied sites. The contribution of manure application to soil P loss in surface runoff has been well documented by other researchers (Sharpley 1997; Sharpley et al. 2001; Kleinman and Sharpley 2003; Daverede et al. 2004). Similar to our findings, Daverede et al. (2004) also found significantly greater DRP and TP in the simulated rainwater from manure sites than a triple superphosphate-applied field. Several earlier studies indicated that the interaction between organic and inorganic P species, such as competitive adsorption, could influence the retention and release of Po from soils (Anderson et al. 1974; Guan et al. 2005; Berg and Joern 2006). Therefore, the significantly greater DNRP/DRP ratio is found in ditchwater near manure sites.

The results of climate effect on P flux and ratio were summarized in Table S7. The wet/dry period had a significant impact on both the DNRP content and the DNRP/DRP ratio. The influence of the wet/dry period was also found in the flux of DRP, DNRP, and PP, which was mainly due to the more significant discharge of ditchwater during the wet periods. The temperature seemed to have a negligible influence on the soluble P characteristics.

Relationship of TP_o between soils and adjacent ditchwater

When the TP and P_o content in soils was compared with that in water, both generally followed a similar trend; manure amendments resulted in more TP and P_o in both soils and nearby ditchwater. The effect of agricultural management on both TPi and TP_o content in soils and DRP and DNRP flux in water (Table S4 and S6) was significant. The P flux in the ditchwater near the manure-amended sites was greater than that in the fertilizer-applied fields. Similarly, the P_o /phosphate ratios in soil and water samples near the manure-amended sites were greater than those measured near the fertilizer-applied sites, indicating that agricultural management was one of the major factors that influenced the P_o /phosphate in the environment.

The P concentration in the soil water extract also showed a similar trend (Table S4) The dissolved P in the water extract stimulates the release of P from soils after a sudden rainfall event. It was mentioned in the previous section that the P_0 /phosphate ratio (1.5–4.3) in the water extract was generally greater than the P_o/phosphate ratio (0.1-0.9) in soils. A similar trend was also found in the DNRP/DRP ratio in the ditch water samples (Table S7). This DNRP/DRP ratio ranged from 0.47 to 3.84 during the wet period and from 0.06 to 1.72 during the dry period. Furthermore, a positive correlation between the DNRP/DRP ratio and discharge (Fig. S4) indicated that the DNRP/DRP ratio increased with increasing discharge by precipitation. The release of DNRP or Po species in soil was facilitated after a storm event, resulting in an increase of the P_o/phosphate ratio in adjacent ditch water. The result of soil colloidal P in DNRP suggested that the concentration of soil colloidal P per soil mass (CTP_s) in B1 and B3-P was still greater than in B2 and B3-F (Table 3). Similarly, the flux of PP was also found to be significantly greater in Sd2 and Sd4 than in Sd3 (Table S6), indicating the possibility of soil PP loss into water.

Conclusions

Agricultural management significantly influenced the P_o contents and the P_o /phosphate ratios in both soil and water environments. Samples near the manure-applied pasture have significantly greater P_o content and P_o /phosphate ratio (0.54) than samples near the fertilizer-applied field.

Organic P such as P monoesters was dominant in the soil water extract (~60–81% of total dissolved P), while phosphate was the dominant fraction of soil TP (~52–91% of TP in soil). Water samples also showed significantly greater P_o concentration and the P_o /phosphate ratio during the wet period. These findings imply that during storm events, the loss of P_o from soil to water system can be substantial, especially for the manure-applied pasture. The transport of P_o and the change in the P_o /phosphate ratio from agricultural systems to water should be of concern as it may affect the P cycle in soils and the fate of P in the soil–water environments.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval The authors confirm that ethical standards were addressed.

Consent to participate The authors confirm the volunteer's declaration of consent.

Consent for publication The authors confirm the volunteer's consent for publication.

Competing interests The authors declare no competing interests.

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