Phosphorus Speciation and Sorption-Desorption Characteristics in Heavily Manured Soils

Zhengxia Dou*
C. F. Ramberg
J. D. Toth
Y. Wang
Univ. of Pennsylvania
382 West Street Rd.
Kennett Square, PA 19348

A. N. Sharpley
University of Arkansas
Dep. of Crop, Soil and Environ. Sci.
Fayetteville, AR 72701

S. E. Boyd
C. R. Chen
D. Williams
Z. H. Xu
Griffith University
Nathan Campus
Brisbane, Qld 4111
Australia

Managing heavily manured soils for decreased P loss to waters requires improved understanding of the chemical and sorption–desorption characteristics of P in these soils. We used soils from agricultural fields receiving ≥8 yr of dairy, poultry, swine manure or spent mushroom compost for the determination of P functional groups in NaOH-EDTA extracts by solution ^{31}P nuclear magnetic resonance (NMR) spectroscopy, degree of P saturation (DPS), and P sorption–desorption isotherms. The ^{31}P NMR results show that inorganic orthophosphate was the primary form of P in manure treated (79–93% of total extract P) and untreated soils (33–71%). Pyrophosphate and phosphate monoesters were identified in all soils, whereas phosphate diesters were present in small proportions (<3%) in only a few soils. Polyphosphate, a more condensed form of inorganic P, was present in seven out of nine manured soils (9–47 mg P kg^{-1}, <2%) but absent in untreated soils. Concentrations of inositol hexakisphosphate (IHP), mostly *myo*-IHP plus some *scyllo*-IHP, were similar in manured soils (52–116 mg P kg^{-1}, 2–8%) and untreated soils (43–137 mg P kg^{-1}, 6–22%), suggesting a lack of IHP accumulation despite long-term manure applications, including poultry manures that are typically rich in IHP. Most of the treated soils had DPS ≈ 80 to 90% compared with 11 to 33% for the untreated samples. Results from P sorption isotherms showed that potential P release was 3 to 30 times greater from treated than untreated soils. The lack of IHP accumulation in soils receiving long-term manure applications implies that manure-derived IHP may not be biologically and environmentally benign.

Abbreviations: DPS, degree of phosphorus saturation; ICP-AES, inductively coupled plasma–atomic emission spectroscopy; IHP, inositol hexakisphosphate; NMR, nuclear magnetic resonance.

Reducing P loss from agricultural land receiving long-term manure applications has been a research and management focus for many years. Heavily manured soils typically have P buildup to levels that far exceed the agronomic optimum required for satisfactory crop production. Such P-enriched soils are often associated with elevated P loss to waters, contributing to accelerated eutrophication of lakes, rivers, and estuaries (Sims et al., 2002; McDowell and Sharpley, 2001b). Information on the chemical composition and the sorption–desorption characteristics of P in these soils is essential to improving our understanding of P transformation and transport in the agroecosystem.

Comparing 20 agricultural soils that had received manure application for >10 yr with soils receiving no manure or fertilizer, Sharpley et al. (2004) found an overall change in P chemistry in manured soils. Sequential extraction results indicated considerable shifts from Al- and Fe- to Ca-P reaction products

in manured soils as compared with untreated soils, presumably a result of manure inputs, known to be high in P and Ca (Sharpley and Moyer, 2000). A substantial portion of P in animal manures may exist in various chemical forms other than inorganic orthophosphate, such as pyrophosphate, polyphosphate, phosphonate, monoesters including IHP (commonly known as phytate or phytic acid), and diesters (Turner, 2004). Of particular interest is IHP which can constitute a substantial proportion of total P in animal manures, especially poultry manures. Studies have shown that IHP composes 8% of total P in dairy feces (McDowell et al., 2008) and up to 80% in poultry manure samples (Turner, 2004; Maguire et al., 2004; Leytem et al., 2006; McGrath et al., 2005). The IHP molecules have high charge density and hence undergo strong interaction with clay minerals (Celi et al., 1999) or soil constituents such as (hydr)oxides of Al and Fe (Turner et al., 2002). This leads to a logical assumption that IHP would accumulate in soils receiving long-term manure applications because of their strong interaction with soil constituents. However, there has been a lack of experimental data showing that this is the case.

Another important aspect of soil P research is to study the relationship between potential P loss and risk-assessment indices based on soil P testing. Degree of P saturation has been widely validated on soils from different regions/nations and is generally accepted as a good indicator for the risk of P loss from agricultural soils (e.g., Mozaffari and Sims, 1994; Nair et al., 2004). The concept is based on the knowledge that P sorption in soil is associated with active sorption sites of Al and Fe and that P release from a soil is related to what proportion of these

sorption sites has been occupied by P (Breeuwsma et al., 1995). Operationally, DPS is determined by extracting the soil with an acid oxalate solution, then calculating DPS = $P_{ox} \div [\alpha (Al_{ox} + Fe_{ox})]$, where P_{ox}, Al_{ox} and Fe_{ox} are the elements in the extract on a molar basis and α is an empirical parameter with its value depending on soil physical and chemical properties (Nair and Graetz, 2002). A number of studies have demonstrated that once DPS reaches a threshold level, the concentration of P in leachate or runoff increases rapidly and can exceed critical to-tal P concentrations considered environmentally unacceptable for flowing waters (0.010–0.076 mg L^{-1}; U.S. Environmental Protection Agency, 2001). Threshold DPS has been established to be 25% for Netherlands soils (Breeuwsma et al., 1995), 20% for Florida sandy soils (Nair et al., 2004), and ~56% for typi-cal soils in the Mid-Atlantic region of the USA (McDowell and Sharpley, 2001a, 2001b; Maguire and Sims, 2002).

Determination of soil P sorption isotherms provides an-other useful means for studying soil P sorption–desorption dy-namics (Nair et al., 1984). By equilibrating soil with a series of solutions containing varying amounts of P (0–50 mg P L^{-1}, for example), the amount of P sorbed and/or desorbed is mea-sured, from which P sorption–desorption kinetics are modeled and several parameters such as P sorption maximum (S_{max}) and equilibrium P concentration at zero sorption (EPC_0) can be derived (Graetz and Nair, 2000). When comparing heavily manured soils with similar soils not receiving manure, deter-mination of soil P sorption isotherms may provide valuable information regarding the direction (desorption or sorption) and relative magnitudes of P release or retention.

The objectives of the present work were to: (i) identify P functional groups in heavily manured soils compared with similar soils not receiving manure using solution ^{31}P NMR techniques, and (ii) determine DPS and P sorption–desorption characteristics of these soils. The study included five paired (manure treated and untreated) and five unpaired (manure treated only) soils, all from agricultural fields. Types of animal manures applied included swine (*Sus scrofa*), dairy (*Bos taurus*),

chicken (*Gallus gallus*), and duck (*Anas platyrhynchos*) manure; one soil received spent mushroom compost[*Agaricus bisporus* (Lange) Imblach]. Duration of manure applications was esti-mated to be eight to >10 yr before soil sample collection.

MATERIALS AND METHODS
Sample Description

Soil samples were obtained from 10 farmers' fields in Pennsylvania. The treated soils received animal manure or spent mushroom com-post, whereas untreated soils were from fields adjacent to the treated ones but planted in native or introduced grasses and not receiving manure or fertilizer (Table 1). Information on manure application rates and duration was obtained by interviewing relevant landown-ers. Land use and nutrient management practices were typical for the region. All samples were from the 0- to 5-cm depth, air-dried and sieved (2 mm). Rather than a traditional soil sampling depth of plow layer, the 0- to 5-cm sampling depth was chosen to reflect the depth of soil interacting with runoff water and thus more important from an environmental point of view. The original sampling scheme included multiple subsamples from each field. However, in the present study, a composite sample made of equal gravimetric portions of three to six subsamples was used. Due to expensive NMR machine time, P specia-tion by solution ^{31}P NMR has typically been done using composite samples. To check the quality of laboratory analysis, selected samples were analyzed in duplicate or triplicate. Relative standard deviations averaged 7% of the means.

Chemical Analysis

Soil pH, organic C, and particle-size distribution were deter-mined using standard procedures as previously described (Sharpley et al., 2004). Total soil P was determined following microwave-assisted acid digestion (Walter et al., 1997), along with total Al, Fe, Ca, K, Mg, Cu, Mn, and Zn, by inductively coupled plasma–atomic emission spectroscopy (ICP–AES). Mehlich-3 extractable P (Mehlich, 1984) was determined by shaking 1 g soil in 10 mL of Mehlich-3 solution end-over-end for 5 min. Phosphorus concentration in filtered extracts

Table 1. Description of site management and selected soil properties. †

Soil Series‡	Management	Manure Application			pH		Clay		Organic C		Total P	
		Type	P Rate§	Duration§								
			kg ha^{-1} yr^{-1}	yr			%		g kg^{-1}		mg kg^{-1}	
					Tr¶	Unt¶	Tr	Unt	Tr	Unt	Tr	Unt
Bucks	Reduced tillage corn/barley	Poultry manure	120	10	7.4	6.6	15	13	17.3	7.1	2523	858
Chester	Pasture	Dairy manure	120	10	6.5	5.6	23	26	19.4	9.3	2788	866
Duffield 1	Reduced tillage corn/soybean	Dairy manure	120	10	6.9	6.2	18	21	16.2	7.7	2255	685
Glenelg	Reduced tillage/corn	SMC #	95	10	6.7	6.9	27	–	142.8	–	4866	604
Watson	No-till corn/soybean	Swine slurry	85	8	6.8	5.6	35	–	15.2	–	1137	639
Duffield 2	Reduced tillage, corn	Poultry manure	90	10	7.1	–	20	–	16.4	–	1974	–
Hagerstown	Reduced tillage corn/winter rye	Poultry manure	150	10	6.7	–	22	–	22.4	–	1478	–
Manor	Corn/alfalfa	Dairy manure	110	10	6.8	–	15	–	23.3	–	808	–
Berks/Weikert 1	Grass hay/corn/soybeans	Duck manure	29–69	> 10	6.1	–	15	–	16.5	–	3492	–
Berks/Weikert 2	Corn/soybeans/wheat	Poultry (layer)	110	> 10	7.1	–	25	–	35.2	–	3578	–

† Some of the data were taken from Sharpley et al. (2004). Reprinted by permission of American Society of Agronomy.

‡ Soil descriptions: Bucks, fine-loamy, mixed, active, mesic Typic Hapludults; Chester, fine-loamy, mixed, semiactive, mesic Typic Hapludults; Duffield 1, fine-loamy, mixed, active, mesic Ultic Hapludalfs; Glenelg, fine-loamy, mixed, semiactive, mesic Typic Hapludults; Watson, fine-loamy, mixed, active, mesic Typic Fragiudults; Hagerstown, fine, mixed, semiactive, mesic Typic Hapludalfs; Manor, coarse-loamy, micaceous, mesic Typic Dystrudepts; Berks, loamy-skeletal, mixed, active, mesic Typic Dystrudepts; and Weikert, loamy-skeletal, mixed, active, mesic Lithic Dystrudepts.

§ Estimated based on producer interviews.

¶ Treated and untreated soils, respectively.

Spent mushroom compost.

was determined by an adaptation of Murphy and Riley's colorimetric molybdenum-blue method (John, 1970).

Determination of DPS followed the protocol described by Schoumans (2000). Briefly, 2.5 g of soil was shaken in a 50-mL oxalate solution (0.11 M ammonium oxalate monohydrate + 0.13 M oxalic acid dihydrate, pH 3) for 2 h in the dark at ambient temperature. Filtrates were determined for P (P_{ox}), Al (Al_{ox}), and Fe (Fe_{ox}) with ICP–AES, and DPS was calculated as:

$$DPS = P_{ox}/[\ \alpha\ (Al_{ox} + Fe_{ox})]$$

The numerical value of α typically ranges from 0.4 to 0.6 for soils in our region (Sims et al., 2002). We assigned α = 0.5 in the present study because this value has been used in several studies for soils similar to ours (e.g., Sims et al., 2002; McDowell and Sharpley, 2001b; Maguire and Sims, 2002) and we wanted to relate our findings to those studies.

Determination of P sorption isotherms followed the procedure described by Graetz and Nair (2000), with 1 g of soil shaken in 25 mL of 0.01 M $CaCl_2$ solutions containing 0, 0.1, 0.5, 1.0, 5.0, 10.0, 20.0, or 50.0 mg P L^{-1} for 24 h at 25°C. Filtrates were analyzed for P by the colorimetric molybdenum-blue method. A linearized Langmuir adsorption equation was employed:

$$C/S = 1/(kS_{max}) + C/S_{max}$$

where C equals P concentration in solution after 24 h equilibration, mg L^{-1}, S = S' + S_0, the total amount of P retained, mg kg^{-1}, S_0 = P originally sorbed, mg kg^{-1}, S_0 = P_{ox} in the present study, S' = P sorbed by the solid phase during the 24-h equilibration, mg kg^{-1}, S_{max} = P sorption maximum, mg kg^{-1}, k = a constant related to binding energy, L mg^{-1} P.

In addition, EPC_0, the equilibrium P concentration at zero sorption, for each soil was determined graphically from isotherm plots of P sorbed vs. P in solution. Linearized isotherm equation parameters were determined using the PROC REG option in SAS (SAS Institute 1999).

Solution ^{31}P-NMR

Extraction for ^{31}P-NMR analysis followed the procedure described by Cade-Menun and Preston (1996). Briefly, 5 g of soil was shaken in 100 mL solution (0.25 M NaOH + 0.05 M EDTA) end-over-end for 16 h at ambient temperature. After centrifugation (2000 × g for 10 min) and filtration (Whatman 41, Whatman Chemical Separation, Inc. Clifton, NJ), an aliquot was analyzed for total P by ICP–AES. For NMR analysis, 70 mL of filtrate was lyophilized. The freeze-dried extract (~250 mg) was redissolved in 0.9 mL of 1 M NaOH and 0.1 mL of D_2O and allowed to stand for 10 min with occasional vortexing. Samples were then centrifuged at 1500 × g for 5 min and the supernatant transferred to 5-mm diam. NMR tubes. Solution ^{31}P NMR spectra were acquired at 162 MHz on a Varian Inova 400 spectrometer (Varian, Palo Alto, CA) using an acquisition time of 1.6 s, a recycle delay of 2.4 s and a pulse angle of 65°. A 1-Hz line broadening function was applied to the data before Fourier transformation. Chemical shifts were measured relative to an external standard H_3PO_4. Approximately 6000 scans were accumulated.

The general functional classes of P compounds were assigned according to Turner et al. (2003a): phosphonates around 19 ppm, inorganic orthophosphate approximately 6.1 ppm, orthophosphate monoesters at 3 to 6 ppm, orthophosphate diesters between –0.5 and 2.0 ppm, pyrophosphate around –4 ppm, and polyphosphate about

–20 ppm. Within the orthophosphate monoesters, *myo*-IHP was identified by the signature signals at 5.85, 4.92, 4.55, and 4.43 ppm in the ratio of 1:2:2:1 (Turner et al., 2003a). Integral areas for all major species were determined using the deconvolution routines implicit in the Varian 6.1C software package (Varian Inc., Palo Alto, CA). The identified P functional groups were expressed as percentages of total signal area, relative concentrations (mg P kg^{-1}) were calculated by multiplying the percentage peak area by extract total P.

RESULTS AND DISCUSSION
Selected Soil Parameters

Compared with untreated soils, long-term manure applications increased total P in the soils by three- to fourfold in most cases (Table 1), whereas the Glenelg soil (fine-loamy, mixed, semiactive, mesic Typic Hapludults) receiving spent mushroom compost had nearly eight times more P than the untreated soil. Treated soils also exhibited substantial increases in pH, ranging from 0.7 unit (Duffield [fine-loamy, mixed, active, mesic Ultic Hapludalfs] receiving dairy manure) to 1.2 units (Watson [fine-loamy, mixed, active, mesic Typic Fragiudults] with swine slurry) except the Glenelg which had similar pH in treated and untreated samples. There was also a twofold increase in soil organic C in treated soils compared with the untreated samples (Table 1). However, differences between treated and untreated soils in total Al, Fe, K, Mg, Cu, Mn, or Zn concentrations were generally small or negligible, although total Ca increased 2.5 to 11.3 times in all treated soils except the Duffield series receiving dairy manure (data not presented).

^{31}P NMR

Of total P in soils, NaOH-EDTA extracted 68 to 97% (Table 2). This recovery was comparable with that reported by Koopmans et al. (2003; 70–78%) for a surface (0–5 cm) acidic sandy soil receiving long-term manure or fertilizer applications, and by Cade-Menun and Preston (1996; 71–90%) for forest floor samples. Lower recoveries have been reported for semi-arid soils (12–45%; Turner et al., 2003b) or alkaline soils from an Idaho dairy farm receiving manures (18–45%; Hansen et al., 2004).

Our ^{31}P NMR spectra were of very good quality for most soils (see Fig. 1 for the Chester soils [fine-loamy, mixed, semi-active, mesic Typic Hapludults] as examples). On a percentage basis, orthophosphate accounted for the greatest portion of total extract P in most soils (Table 2), with manured soils (excluding the Glenelg soil receiving spent mushroom compost) having proportionally more orthophosphate (79–93%) than the untreated soils (33–71%). On a concentration basis (mg P kg^{-1} soil, values in parentheses in Table 2), differences between treated and untreated soils were even greater with treated soils having three to five times more orthophosphate than untreated soils. Orthophosphate as the primary form of extract P in manured soils has been reported previously, 78 to 80% in 0- to 10-cm alkaline soils receiving >10-yr dairy manure (Hansen et al., 2004) and 66 to 83% in acidic sandy soils after 11-yr applications of various manures (Koopmans et al., 2003).

Pyrophosphate was detected in all soils and accounted for as little as <1% or as great as 4.85% of extract P (Table 2). Pyrophosphate is believed to be a P storage form produced

Table 2. Distribution of P functional groups in NaOH-EDTA extracts of soils as percentage of total peak area of the ^{31}P nuclear magnetic resonance (NMR) spectra, or as concentration (mg P kg^{-1}soil; value in parenthesis). The latter was calculated by multiplying NaOH-EDTA extract P by the relevant percentage of peak area.

Soil Series†		Recovery	Inorganic			Organic			
			Orthophosphate	Pyrophosphate	Polyphosphate	Monoesters			Diesters
		%				*Myo*-IHP‡	*Scyllo*-IHP§	Other	
Bucks	Tr ¶	84.2	90.4 (1919)	0.54 (12)	1.25 (27)	2.84 (60)	0.40 (8)	4.60 (98)	0
	Unt ¶	73.7	70.7 (447)	1.09 (7)	0	4.93 (31)	1.90 (12)	21.40 (135)	0
Chester	Tr	79.1	88.7 (1958)	0.43 (9)	2.15 (47)	3.40 (75)	0.40 (9)	4.73 (104)	0.15 (3)
	Unt	84.4	60.7 (443)	1.35 (10)	0	15.14 (111)	3.20 (23)	18.11 (132)	1.53 (11)
Duffield 1	Tr	80.4	89.2 (1617)	0.44 (8)	0.48 (9)	3.76 (68)	0.69 (13)	5.47 (99)	0
	Unt	68.4	59.4 (278)	2.37 (11)	0	7.90 (37)	3.78 (18)	26.58 (124)	0
Glenelg	Tr	77.9	45.0 (1707)	4.75 (180)	0	n/a #	n/a	47.24 (1790)	2.88 (109)
	Unt	68.0	46.1 (189)	4.53 (19)	0	15.89 (65)	3.53 (15)	29.98 (123)	0
Watson	Tr	69.6	80.9 (640)	1.33 (11)	0	6.76 (53)	1.65 (13)	9.40 (74)	0
	Unt	97.4	34.3 (213)	4.85 (30)	0	16.85 (105)	5.10 (31)	36.35 (226)	2.55 (16)
Duffield 2	Tr	91.2	88.7 (1597)	0.08 (1)	0.71 (13)	4.06 (73)	0.72 (13)	5.70 (103)	0
Hagerstown	Tr	77.9	81.3 (1055)	0.69 (9)	0.71 (9)	5.91 (77)	1.39 (18)	9.66 (125)	0.36 (5)
Manor	Tr	72.9	79.4 (467)	1.25 (7)	n/a	n/a	n/a	19.40 (114)	n/a
Berks/Weikert 1	Tr	79.9	93.0 (2686)	0.35 (10)	1.51 (44)	1.57 (45)	0.24 (7)	5.1 (147)	0
Berks/Weikert 2	Tr	82.7	89.9 (2571)	0.36 (10)	1.24 (35)	3.69 (106)	0.38 (11)	4.43 (127)	0

† Soil descriptions: Bucks, fine-loamy, mixed, active, mesic Typic Hapludults; Chester, fine-loamy, mixed, semiactive, mesic Typic Hapludults; Duffield 1, fine-loamy, mixed, active, mesic Ultic Hapludalfs; Glenelg, fine-loamy, mixed, semiactive, mesic Typic Hapludults; Watson, fine-loamy, mixed, active, mesic Typic Fragiudults; Hagerstown, fine, mixed, semiactive, mesic Typic Hapludalfs; Manor, coarse-loamy, micaceous, mesic Typic Dystrudepts; Berks, loamy-skeletal, mixed, active, mesic Typic Dystrudepts; and Weikert, loamy-skeletal, mixed, active, mesic Lithic Dystrudepts.

‡*Myo*-inositol hexakisphosphate.

§A distinct peak at 3.8 ppm, which is mostly likely to be *scyllo*-IHP (Turner and Richardson, 2004).

¶ Treated and untreated soils, respectively.

#Not available due to poor resolution.

by microbial activity (Condron et al., 1985). Polyphosphate, a more condensed form of inorganic P, was not detected in any of the untreated soils but was present in seven of the nine manured soils (see Fig. 1 for the distinct peak near −20 ppm
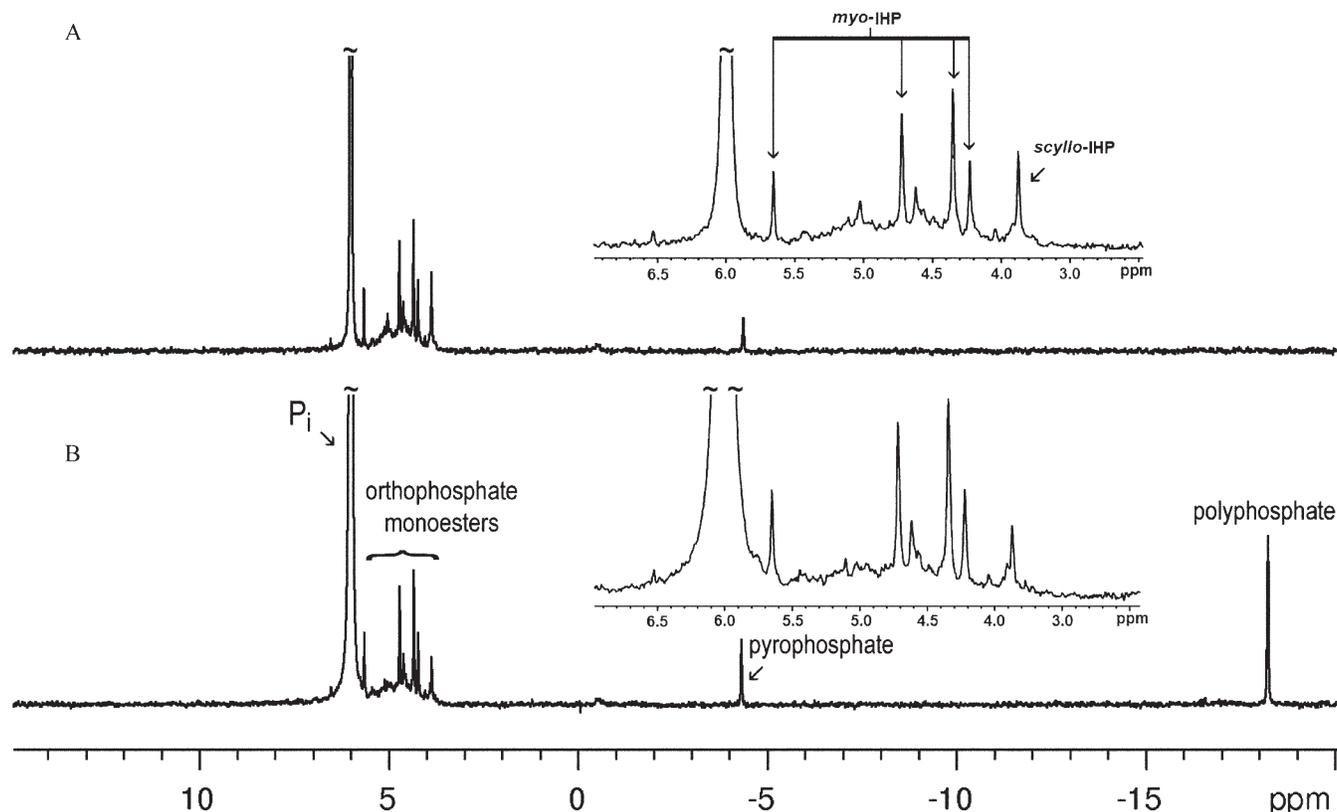

Fig. 1. Solution ^{31}P{^{1}H} nuclear magnetic resonance (NMR) spectra of NaOH-EDTA extracts of the Chester soils, untreated (A) or treated with dairy manure for 10 yr (B). The inset spectra were plotted using a line broadening of 1Hz to preserve the enhanced resolution.

for the Chester soil receiving dairy manure and the lack of it in the untreated sample). It is not clear what might have contributed to the formation of polyphosphate in these soils. Previous studies have not reported the detection of polyphosphate in a variety of semiarid arable soils of the Western USA (Turner et al., 2003b), wetland soils from Florida Everglades (Turner and Newman, 2005), grassland soils from New Zealand (Chen et al., 2004), or alkaline soils from an Idaho dairy farm (Hansen et al., 2004). Nearly all soils in these studies had total P < 1000 mg kg^{-1}. Notably, the seven soils with polyphosphate detected in the present study had total P ranging from 1530 to 3578 mg kg^{-1}, whereas all the soils with no detectable polyphosphate had total P < 1000 mg kg^{-1} (except the Glenelg soil receiving spent mushroom compost). Nevertheless, the presence of a high concentration of total P may not necessarily lead to polyphosphate formation, as a Netherlands study had acidic sandy soils high in total P (1134–2400 mg kg^{-1}) after long-term manure applications but no polyphosphate was reported (Koopmans et al., 2003).

It has been suggested that fungal biomass can be a rich source of polyphosphate when grown in P-rich media (Makaraov et al., 2002). We do not know if there was more fungal biomass in soils with polyphosphate detected than other soils in the present study. Reports on impact of manure applications on soil fungal biomass appear to be inconsistent. Bittman et al. (2005) reported a decrease in soil fungal biomass (although an increase in soil bacterial biomass) after multi-year applications of dairy manure slurry, which is in contradiction to the finding of Kabir et al. (1997). Previous studies have reported polyphosphate in cattle or swine manure (Turner, 2004; He et al., 2007), but not in poultry manure (Turner, 2004; He et al., 2007; Leytem et al., 2006). Also, Turner (2004) reported possible degradation of polyphosphate at greater pH during extraction of cattle or swine manure, as less polyphosphate was present in extracts of 0.25 M NaOH +50 mM EDTA than in extracts of 0.15 M NaOH +50 mM EDTA, while no polyphosphate was detected in extracts of 0.50 M NaOH +50 mM EDTA. For the present study, it is not clear why polyphosphate was present in seven of the soils receiving manure but not in the other two manured soils.

Organic P accounted for as little as 5.3% of extract P (the Berks [loamy-skeletal, mixed, active, mesic Typic Dystrudepts]/Weikert 1 [loamy-skeletal, mixed, active, mesic Lithic Dystrudepts] soil receiving duck manure) or as high as 60.9% (the untreated Watson soil). Of organic P forms, diesters were detected in only a few soils in relatively small proportions (<3% of extract P); these soils included both treated and untreated soils (Table 2). Phosphate diesters are present in considerable amounts in various animal manures, for example, 7 to 9% in fresh dairy feces or stored manure (Toor et al., 2005; He et al., 2007; McDowell et al., 2008), 3.4 to 4.2% in fresh layer manure (He et al., 2007), or up to 4.5% in poultry litter, 10.5% in cattle manure, and 1.2% in swine manure, depending on the pH of the extractant (Turner, 2004). However, studies of manured soils have found no detectable or only a small proportion (<1%) of phosphate diesters even after >10 yr applications of dairy manure (Hansen et al., 2004) or pig or poultry manure (Koopmans et al., 2003).

In the present study, the great majority of organic P was in the form of monoesters, with *myo*-IHP constituting 17 to 43% of the monoesters for the soils in which *myo*-IHP was present. There was also a distinct peak at 3.8 ppm (Fig. 1) in all soils where *myo*-IHP was detected, which is most likely *scyllo*-IHP (Turner and Richardson, 2004). More than half of the P monoesters were in the undistinguished "other" category (Table 2), which may include glucose phosphates, mononucleotides, phospholipid breakdown products, and other inositol phosphate compounds (P_1–P_5) (Turner et al., 2003a). It is interesting to note that in the Glenelg soil receiving spent mushroom compost, non-IHP monoesters were the largest component, constituting 47% of total extract P (Table 2). Total P in spent mushroom compost was reported to be 5.5 g kg^{-1} dry weight (Uzun, 2004). Although we found no information on chemical forms of P in spent mushroom compost, a substantial portion may be organic given that its major ingredients are composted straw, hay, peat, horse manure, poultry manure, and gypsum (Guo et al., 2001).

As for total IHP (*myo*-IHP plus *scyllo*-IHP), our data indicate little IHP accumulation in the manured soils, which is surprising in light of its chemical characteristics and expected fate. This can be viewed from three aspects. First, the amounts of IHP in all manure treated soils (52–116 mg P kg^{-1}) were similar to untreated soils (43–137 mg kg^{-1}) except the Manor soil (coarse-loamy, micaceous, mesic Typic Dystrudepts) receiving dairy manure that had no detectable IHP. Second, comparison of paired (treated/untreated) soils showed inconsistent patterns, with two treated soils having greater amounts of IHP (sum of *myo*- and *scyllo*-IHP) than the corresponding untreated soils (69 vs. 43, 81 vs. 55 mg P kg^{-1}), whereas the other three manured soils had less IHP than the untreated samples (84 vs. 134, 0 vs. 80, 67 vs. 137 mg P kg^{-1}). Third, considering soils receiving poultry manures, the concentrations of IHP in these soils (52, 69, 86, 95, and 116 mg P kg^{-1}) were not greater than the other soils (43, 55, 80, 134, and 137 mg P kg^{-1}). Typically, a large portion of total P in poultry manure is in the form of IHP, for example, 26 to 56% in broiler and turkey manures (Maguire et al., 2004), and 35 to 80% in layer manure (Leytem et al., 2006). Even after 440 d in storage, broiler litter still had IHP constituting 53 to 78% of total P when stored dry and 30 to 38% when stored wet (McGrath et al., 2005). A recent study by Leytem et al. (2007) reported IHP constituting only 3 to 12% of the P in poultry manure but their study was based on atypical barley-based diets. In the present study, we did not have samples of the manures that were applied during the 8 to 10 yr before soil sample collection, hence we do not know how much IHP could have been applied with poultry manures. However, it is most likely that those poultry manures were from birds fed normal rather than atypical diets and thus contained considerable amounts of IHP. Assuming an average of 30% total P as IHP (a conservative value considering the literature cited above), estimated amount of IHP applied would be in the magnitude of 300 kg P ha^{-1} (average application rate of 100 kg total P ha^{-1} yr^{-1} for 10 yr; Table 1). This would translate to approximately 450 mg IHP-P kg^{-1} for the top 5 cm of soils, or 150 mg kg^{-1} for the top 15 cm of soils (assuming a bulk density of 1.33 g soil cm^3). Clearly, the amounts of IHP detected in the poultry manured soils (52, 69, 86, 95,

Table 3. Results of Mehlich 3 soil test P (M-3), degree of P saturation (DPS), and P sorption isotherm parameters using the Langmuir adsorption equation.

Soil Series†		M-3 P	$Al_{ox} + Fe_{ox}$	P_{ox}	DPS‡	Langmuir Adsorption Isotherm Parameters§					
						C	S_0	S′	S_{max}	k	EPC_0
		mg kg^{-1}	——mmol kg^{-1}——		%	mg L^{-1}	———mg kg^{-1}———			L mg^{-1}	mg kg^{-1}
Bucks	Tr	626	89.1	39.7	89	1.17	1230	169	1404	3.16	1.96
	Unt	40	68.3	8.4	25	0.12	260	242	503	1.86	0.12
Chester	Tr	886	98.6	42.4	86	1.69	1314	101	1420	3.85	3.45
	Unt	20	90.9	14.9	33	0.17	462	162	625	3.62	0.20
Duffield 1	Tr	676	78.8	36.5	93	1.36	1129	117	1250	3.42	2.81
	Unt	31	88.9	7.7	17	0.09	239	207	444	1.08	0.08
Glenelg	Tr	1369	141.1	139.1	197	2.52	4309	203	4525	0.44	5.81
	Unt	21	81.6	4.7	11	0.08	145	355	505	1.41	0.08
Watson	Tr	346	90.7	18.1	40	0.37	561	179	741	2.93	0.45
	Unt	33	173.3	9.5	11	0.10	295	323	617	1.66	0.10
Duffield 1	Tr	691	80.7	30.6	76	0.84	949	175	1129	3.20	1.31
Hagerstown	Tr	336	86.0	24.4	57	0.52	757	428	1196	1.79	0.64
Manor	Tr	239	58.2	13.4	46	0.80	414	152	571	1.32	1.70
Berks/Weikert 1	Tr	1242	139.0	72.0	104	3.45	2230	94	2336	2.83	12.58
Berks/Weikert 2	Tr	1119	125.2	60.4	96	2.24	1869	239	2132	1.71	4.47

† Soil descriptions: Bucks, fine-loamy, mixed, active, mesic Typic Hapludults; Chester, fine-loamy, mixed, semiactive, mesic Typic Hapludults; Duffield 1, fine-loamy, mixed, active, mesic Ultic Hapludalfs; Glenelg, fine-loamy, mixed, semiactive, mesic Typic Hapludults; Watson, fine-loamy, mixed, active, mesic Typic Fragiudults; Hagerstown, fine, mixed, semiactive, mesic Typic Hapludalfs; Manor, coarse-loamy, micaceous, mesic Typic Dystrudepts; Berks, loamy-skeletal, mixed, active, mesic Typic Dystrudepts; and Weikert, loamy-skeletal, mixed, active, mesic Lithic Dystrudepts.

‡ Degree of P saturation determined from oxalate extraction, molar basis, $DPS_{ox} = P_{ox}/[0.5\,(Al_{ox} + Fe_{ox})]$.

§ Langmuir adsorption isotherm parameters: C, P concentration in 0.01 M $CaCl_2$ solution after 24-h equilibrium; only the 0 P solution results were presented. S_0, Previously adsorbed P ($S_0 = P_{ox}$; note that S_0 and P_{ox} are in different units.). S′: Amount of P adsorbed after a 24-h equilibration in 0.01 M $CaCl_2$ solution; only the 50 mg P L^{-1} solution results were presented. S_{max}, P sorption maximum. K, binding energy. EPC_0, Equilibrium P concentration at zero sorption.

116 mg P kg^{-1}) are considerably less than the range estimated, suggesting a lack of IHP accumulation in these soils.

The literature also lacks clear evidence indicating IHP accumulation in manured soils. Hansen et al. (2004) found IHP to be 71 or 63 mg P kg^{-1} in 0- to 10-cm soils from an Idaho dairy farm after >10 yr of solid or liquid dairy manure application; a subsoil layer (45–65 cm) contained much smaller amounts of IHP (6 and 8 mg P kg^{-1}). Unfortunately, there was no untreated control for comparison in their study, therefore one cannot be sure whether the IHP in the 0- to 10-cm surface soil originated from manure or merely reflected background levels. As demonstrated by the results of the present study as well as by Chen et al. (2004), natural soils without manures can contain IHP in a magnitude extending from tens to >100 mg P kg^{-1}. A Netherlands study of P speciation in soils receiving long-term manure applications did include untreated controls (Koopmans et al., 2003). However, their ^{31}P NMR spectra did not distinguish IHP from other monoesters. Their data showed an approximately 1.5-fold increase in monoesters in three manure-treated soils but no monoester increase for two other soils receiving manures.

The apparent lack of IHP accumulation in the heavily manured soils in the present study raises an important question: What could have happened to manure-borne IHP? One possibility is some downward movement into deeper soil layers. The report by Koopmans et al. (2007) showed an enrichment of phosphate monoesters (although IHP was not distinguished from other monoesters) in 0- to 5-cm and 5- to 10-cm layers of a sandy soil after 11-yr application of pig slurry or poultry manure, as compared with controls. The manure was surface applied onto grassland without mechanical incorporation, therefore the enrichment of phosphate monoesters in both 0- to 5- and 5- to 10-cm soil layers suggested downward

movement. A second possibility is potential IHP loss through overland flow or leaching. Although direct evidence of IHP loss in overland flow is hard to find, Toor et al. (2003) presented ^{31}P NMR spectra of a leachate sample showing that 59% of the total P was monoesters. Using enzymatic hydrolysis, the researchers found IHP to be 12 μg P L^{-1} in a control sample and 69 μg P L^{-1} in leachate from dairy effluent treatment (Toor et al., 2003). A third possibility is for IHP to undergo transformation into inorganic orphophosphate or other chemical forms. This is substantiated by the study of Leytem et al. (2006). Their ^{31}P NMR data showed the presence of IHP in soil extracts immediately following manure incorporation. However, after 12-wk incubation, IHP was no longer detectable while Olsen P in the soils increased (Leytem et al., 2006). Another support came from the study of Chen et al. (2004) through a 10-mo pot experiment growing radiate pine in New Zealand grassland soils. These researchers estimated that P release from IHP accounted for 18 to 100% of the total mineralization of phosphate monoesters contributing to P uptake by the tree seedlings. To provide interpretation of their findings, the authors cited Martin and Cartwright (1971) who suggested that P in IHP was more available for mineralization in weakly P-fixing soils than in high P-fixing soils. Then, it is likely that the manured soils in the present study featured weak P-fixation after large amounts of P had been applied, thus exposing manure-derived IHP to microbial degradation and preventing IHP from accumulating in the soils.

Degree of Phosphorus Saturation

Phosphorus sorption capacity was nearly exhausted in most of the treated soils, as DPS was generally in the range of 80 to 90% compared with 11 to 33% for the untreated soils (Table 3). Two treated soils, Glenelg receiving spent mush-
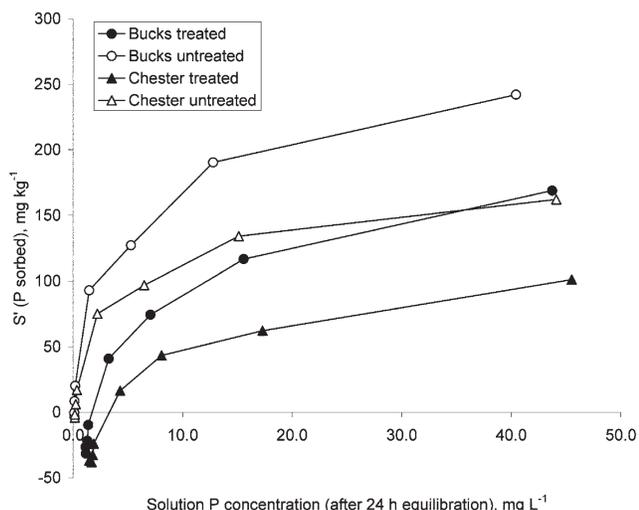
Fig. 2. Phosphorus sorption isotherm: The amount of P retained on the solid phase from a series of P solutions was less in heavily manured soils (Chester receiving dairy manure and Bucks with poultry manure) than the corresponding untreated soils. Differences also existed between different soils (Chester vs. Bucks).

Fig. 3. The amount of P retained by soil samples after 24-h equilibration in 0.01 M $CaCl_2$ solution containing 50 mg P L^{-1} (S' on left Y axis) and the amount of P released after 24 h equilibration in 0.01 M $CaCl_2$ solution containing 0 P (C on right Y axis) were a function of previously sorbed P (S_0) in the soils. Arrows point to an "outlier"- the Glenelg soil that received mushroom compost for 10 yr. Excluding this soil would increase R^2 values to 0.9142 for the linear function and 0.3133 for the power function, respectively.

room compost and Berks/Weikert 2 receiving duck manure, had DPS exceeding the theoretical maximum [100% of 0.5 (Al_{ox} + Fe_{ox})]. Koopmans et al. (2003) also found DPS exceeding 100% (ranging from 107–208%) in acidic sandy soils after 11 yr of manure applications. They suggested a buildup of Ca-P compounds in those manured soils. Similarly, Sharpley et al. (2004) found the application of dairy or poultry manure shifted P from Al- and Fe- to Ca-reaction products in a wide range of soils from New York, Oklahoma, and Pennsylvania, such that Ca-bound P in manured soils averaged 49% of total inorganic P as compared with 22% in unmanured soils. It is likely that for our treated soils, the near-saturation of P sorption capacity, combined with climate and soil conditions favoring vigorous microbial activity, prevented manure-borne IHP from accumulating in the soils. This implies that depending on climate and soil conditions, manure-borne IHP may not be biologically and environmentally benign but may be converted into other P forms that can be subject to potential loss in waters. Further investigation of this issue is needed.

Potential P loss can exceed critical environmental concentrations well before a soil is completely saturated with P (e.g., Breeuwsma et al., 1995; Maguire and Sims, 2002). Compared with the environmental threshold established for soils typical in our region (DPS ∼56%; Sims et al., 2002; McDowell and Sharpley, 2001a), 8 out of 10 treated soils in the present study had DPS exceeding the threshold, while none of the untreated soils was above the threshold (Table 3). In addition, Mehlich-3 P obtained through routine soil testing has also been used for environmental assessment purposes. For example, based on more than 450 soils from the Mid-Atlantic region, Sims et al. (2002) designated Mehlich-3 P of >100 mg kg^{-1} to be "above optimum" (soil P will not limit crop yields, no P application recommended) and >150 mg kg^{-1} to be in the "environmental" category (improved P management must be implemented to reduce potential for nonpoint source P pollution). Accordingly, our treated soils had Mehlich-3 P ranging from 239 to 1369 mg kg^{-1}, compared with 20–64 mg kg^{-1} for the

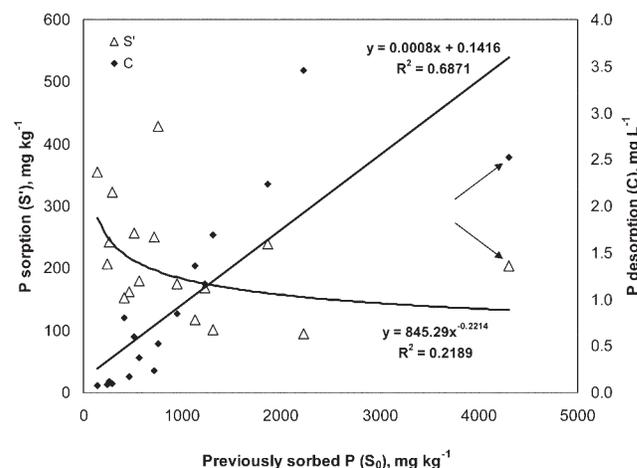untreated soils (Table 3). Clearly, these manure treated soils are at high risk regarding potential P loss to waters.

Phosphorus Sorption-Desorption Equilibrium

Phosphorus sorption isotherm parameters (Table 3) for treated soils exhibited trends typical of reports in the literature: (i) a net P release at lower P solutions because of desorption exceeding sorption, (ii) increased P sorption at higher P solutions but with a diminishing increment (Fig. 2). Comparing the paired (treated and untreated) soils, it is clear that less additional P was sorbed by treated soils (S' in Table 3; Fig. 2).

Potential P release (desorption) can be assessed by two parameters, the equilibrium P concentration in solution after the soil was shaken in 0.01 M $CaCl_2$ containing 0 P for 24 h (C in Table 3) and equilibrium P concentration at zero sorption (EPC_0 in Table 3). The value of C ranged from 0.37 to 3.45 mg L^{-1} in treated soils, which is 3- to 30-fold greater than for untreated soils and mostly >10 times the critical environmental concentration thresholds (0.010–0.076 mg L^{-1}). The value of EPC_0 had the same trend as C but a greater magnitude, ranging from 0.45 to 12.58 mg L^{-1} in treated soils but 0.08 to 0.20 mg L^{-1} in untreated soils. In other words, net P release from the treated soils occurred even when the solution contained as much as 0.45 to 12.58 mg P L^{-1}.

The amount of previously sorbed P (S_0) affected P release and P retention. As illustrated in Fig. 3, P release from soils after 24-h equilibration in 0.01 M $CaCl_2$ solution containing 0 P increased linearly with S_0 (R^2 = 0.6871). The Glenelg soil receiving mushroom compost appeared to be an outlier on the chart (Fig. 3), excluding it from the linear regression led to a higher R^2 (0.9142). On the other hand, the effect of S_0 on P retention was not as remarkable as for P release. The amount of P retained by the soils after 24-h equilibration in 0.01 M $CaCl_2$ solution containing 50 mg P L^{-1} decreased with increasing S_0. The relationship could be described by a power function but it had R^2 value of only 0.2189 (Fig. 3). Excluding the Glenelg

soil treated with mushroom compost improved the R^2 value to 0.3133. Apparently, P retention during the sorption–desorption equilibration was affected by other factors such as Ca, Al, and Fe in addition to the amount of previously sorbed P in these soils.

Traditionally, the Langmuir equation has been used to assess P sorption capacity (S_{max}) and P sorption affinity (k) of soils. For a given soil, greater amount of sorbed P would be associated with a lower k value, while S_{max} would remain unchanged. However, results from the present study did not fit to the conventional expectation. First, for paired soils, k values are greater instead of smaller for treated soils that contained large amounts of sorbed P after long-term manure applications as compared with respective untreated soils (Table 3). Furthermore, the magnitudes of S_{max} were vastly different for the paired soils (two to eight times greater for treated soils than the corresponding untreated soils), except for the Watson series soils whose treated and untreated samples differed only slightly (Table 3). A close examination revealed that for treated soils, S_{max} values resembled the magnitudes of previously sorbed P (S_0), whereas for untreated soils S_{max} was substantially greater than S_0 (Table 3).

The discrepancy between expectation and observation regarding S_{max} and k may be explained, at least in part, by earlier findings that precipitation of P reaction products influenced P retention in treated soils, in addition to traditional Al and Fe sorption mechanisms (Sharpley et al., 2004). The implication is that alternative models may be needed to better describe P retention dynamics for soils containing large amount of P such as the treated soil in the present study, although it is beyond our scope to do so here. Nevertheless, it is important to recognize that where P-retaining mechanisms other than Al- and Fe-governed sorption are at work, a straightforward comparison of k or S_{max} values between P-enriched (treated) and untreated soils may not be appropriate. Furthermore, S_{max} derived from the Langmuir equation should not be confused with P sorption saturation determined by oxalate extraction [α ($Al_{ox}+Fe_{ox}$)]. The latter did have similar magnitudes for treated and untreated soil pairs (Table 3).

CONCLUSIONS

Long-term application of animal manures or spent mushroom compost increased soil total P by 1.4- to 8-fold and Mehlich-3 P by 10- to 65-fold as compared with untreated soils. Most of the manured soils had DPS in the 80 to 90% range compared with 11 to 33% for the untreated soils. While the capacity for retaining additional P was markedly reduced in the treated soils, the potential for P release was dramatically increased in these soils. When in contact with water or soil solutions, net P release from the treated soils could be 10 times greater than critical environmental concentration thresholds for flowing waters and 3 to 30 times greater than the untreated soils. Solution ^{31}P NMR results showed that inorganic orthophosphate was the primary form of P in manured (79–93%) and untreated soils (33–71%). Our data showed little indication of IHP (phytate) accumulation in the manured soils, even those receiving poultry manures which typically contain large amounts of IHP. A plausible, but unproven explanation is that the active sorption sites in manured soils had been saturated, therefore exposing manure-borne IHP along with other organic P to potential transformation and/or transport.

REFERENCES

Bittman, S., T.A. Forge, and C.G. Kowalenko. 2005. Responses of the bacterial and fungal biomass in a grassland soil to multi-year applications of dairy manure slurry and fertilizer. Soil Biol. Biochem. 37:613–623.

Breeuwsma, A., J.G.A. Reijerink, and O.F. Schoumans. 1995. Impact of manure on accumulation and leaching of phosphate in areas of intensive livestock farming. p. 239–251. In K. Steele (ed.) Animal waste and the land-water interface. Lewis Publ., New York.

Cade-Menun, B.J., and C.M. Preston. 1996. A comparison of soil extraction procedures for ^{31}P NMR spectroscopy. Soil Sci. 161:770–785.

Celi, L., S. Lamacchia, F.A. Marsan, and E. Barberis. 1999. Interaction of inositol hexaphosphate on clays: Adsorption and charging phenomena. Soil Sci. 164:574–585.

Chen, C.R., L.M. Condron, B.L. Turner, N. Mahieu, M.R. Davis, Z.H. Xu, and R.R. Sherlock. 2004. Mineralization of soil orthophosphate monoesters under pine seedlings and ryegrass. Aust. J. Soil Res. 42:189–196.

Condron, L.M., K.M. Goh, and R.H. Newman. 1985. Nature and distribution of soil phosphorus as revealed by a sequential extraction method followed by ^{31}P nuclear magnetic resonance analysis. J. Soil Sci. 36:199–207.

Graetz, D.A., and V.D. Nair. 2000. Phosphorus sorption isotherm determination. p. 35–38. In G.M. Pierzynski (ed.) Methods of phosphorus analysis for soils, sediments, residuals, and waters. Southern Coop. Ser. Bull. 396. SERA-17 IEG. North Carolina State Univ., Raleigh, NC.

Guo, M., J. Chorover, R. Rosario, and R.H. Fox. 2001. Leachate chemistry of field-weathered spent mushroom substrate. J. Environ. Qual. 30:1699–1709.

Hansen, J.C., B.J. Cade-Menun, and D.G. Strawn. 2004. Phosphorus speciation in manure-amended alkaline soils. J. Environ. Qual. 33:1521–1527.

He, Z., B.J. Cade-Menun, G.S. Toor, A. Fortuna, C.W. Honeycutt, and J.T. Sims. 2007. Comparison of phosphorus forms in wet and dried animal manures by solution P-31 nuclear magnetic resonance spectroscopy and enzymatic hydrolysis. J. Environ. Qual. 36:1086–1095.

John, M.K. 1970. Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. Soil Sci. 100:214–220.

Kabir, Z., I.P. O'Halloran, P. Widden, and C. Hamel. 1997. Seasonal changes of arbuscular mycorrhiza fungi as affected by tillage practices and fertilization: Hyphal density and mycorrhiza root colonization. Plant Soil 192:285–293.

Koopmans, G.F., W.J. Chardon, J. Dolfing, O. Oenema, P. van der Meer, and W.H. van Riemsdijk. 2003. Wet chemical phosphorus-31 nuclear magnetic resonance analysis of phosphorus speciation in a sand soil receiving long-term fertilizer or animal manure applications. J. Environ. Qual. 32:287–295.

Koopmans, G.F., W.J. Chardon, and R.W. McDowell. 2007. Phosphorus movement and speciation in a sandy soil profile after long-term animal manure applications. J. Environ. Qual. 36:305–315.

Leytem, A.B., D.R. Smith, T.J. Applegate, and P.A. Thacker. 2006. The influence of manure phytic acid on phosphorus solubility in calcareous soils. Soil Sci. Soc. Am. J. 70:1629–1638.

Leytem, A.B., P.A. Thacker, and B.L. Turner. 2007. Phosphorus characterization in feces from broiler chicks fed low-phytate barley diets. J. Sci. Food Agric. 87:1495–1501.

Maguire, R.O., and J.T. Sims. 2002. Measuring agronomic and environmental soil phosphorus saturation and predicting phosphorus leaching with Mehlich 3. Soil Sci. Soc. Am. J. 66:2033–2039.

Maguire, R.O., J.T. Sims, W.W. Saylor, B.L. Turner, R. Angel, and T.J. Applegate. 2004. Influence of phytase addition to poultry diets on phosphorus forms and solubility in litters and amended soils. J. Environ. Qual. 33:2306–2316.

Makaraov, M., L. Haumaier, and W. Zech. 2002. The nature and origins of diester phosphates in soils: A ^{31}P-NMR study. Biol. Fertil. Soils 35:136–146.

Martin, J.K., and B. Cartwright. 1971. The comparative plant availability of ^{32}P myo-inositol hexaphosphate and $KH_2{}^{32}PO_4$ added to soils. Commun. Soil Sci. Plant Anal. 2:375–381.

McDowell, R.W., Z. Dou, J.D. Toth, B. Cade-Menun, P. Kleinman, K. Soder, and L. Saporito. 2008. Extractability and speciation of phosphorus in feeds and feces of different dairy herds. J. Environ. Qual. 37:741–752. 2008.

McDowell, R.W., and A.N. Sharpley. 2001a. Approximating phosphorus release from soils to surface runoff and subsurface drainage. J. Environ. Qual. 30:508–520.

McDowell, R.W., and A.N. Sharpley. 2001b. Phosphorus losses in subsurface flow before and after manure application to intensively farmed land. Sci. Total Environ. 278:113–125.

McGrath, J.M., J.T. Sims, R.O. Maguire, W.W. Saylor, C.R. Angel, and B.L. Turner. 2005. Broiler diet modification and litter storage: Impacts on phosphorus in litters, soils, and runoff. J. Environ. Qual. 34:1896–1909.

Mehlich, A. 1984. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun. Soil Sci. Plant Anal. 15:1409–1416.

Mozaffari, M., and J.T. Sims. 1994. Phosphorus availability and sorption in an Atlantic coastal plain watershed dominated by animal-based agriculture. Soil Sci. 157:97–106.

Nair, V.D., and D.A. Graetz. 2002. Phosphorus saturation in Spodosols impacted by manure. J. Environ. Qual. 31:1279–1285.

Nair, P.S., T.J. Logan, A.N. Sharpley, L.E. Sommers, M.A. Tabatabai, and T.L. Yuan. 1984. Interlaboratory comparison of a standardized phosphorus adsorption procedure. J. Environ. Qual. 13:591–595.

Nair, V.D., K.M. Portier, D.A. Graetz, and M.L. Walker. 2004. An environmental threshold for degree of phosphorus saturation in sandy soils. J. Environ. Qual. 33:107–113.

SAS Institute. 1999. SAS system for Windows, rel. 8.00. SAS Inst., Cary, NC.

Schoumans, O.F. 2000. Determination of the degree of phosphate saturation in non-calcareous soils. p. 31–34. In G.M. Pierzynski (ed.) Methods of phosphorus analysis for soils, sediments, residuals, and waters. Southern Coop. Ser. Bull. 396. SERA-17 IEG. North Carolina State Univ., Raleigh, NC.

Sharpley, A.N., and B. Moyer. 2000. Forms of phosphorus in manures and composts and their dissolution during rainfall. J. Environ. Qual. 29:1462–1469.

Sharpley, A.N., R.W. McDowell, and P.J.A. Kleinman. 2004. Amounts, forms, and solubility of phosphorus in soils receiving manure. Soil Sci. Soc. Am. J. 68:2048–2057.

Sims, J.T., R.O. Maguire, A.B. Leytem, K.L. Gartley, and M.C. Pautler. 2002. Evaluation of Mehlich 3 as an agri-environmental soil phosphorus test for the Mid-Atlantic United States of America. Soil Sci. Soc. Am. J. 66:2016–2032.

Toor, G.S., L.M. Condron, H.J. Di, K.C. Cameron, and B.J. Cade-Menun. 2003. Characterization of organic phosphorus in leachate from a grassland soil. Soil Biol. Biochem. 35:1317–1323.

Toor, G.S., B.J. Cade-Menun, and J.T. Sims. 2005. Establishing a linkage between phosphorus forms in dairy diets, feces, and manures. J. Environ. Qual. 34:1380–1391.

Turner, B.L. 2004. Optimizing phosphorus characterization in animal manures by solution phosphorus-31 nuclear magnetic resonance spectroscopy. J. Environ. Qual. 33:757–766.

Turner, B.L., N. Mahieu, and L.M. Condron. 2003a. Phosphorus-31 nuclear magnetic resonance spectral assignments of phosphorus compounds in soil NaOH-EDTA extracts. Soil Sci. Soc. Am. J. 67:497–510.

Turner, B.L., B.J. Cade-Menun, and D.T. Westermann. 2003b. Organic phosphorus composition and potential bioavailability in semi-arid arable soils of the Western United States. Soil Sci. Soc. Am. J. 67:1168–1179.

Turner, B.L., and S. Newman. 2005. Phosphorus cycling in wetland soils: The importance of phosphate diesters. J. Environ. Qual. 34:1921–1929.

Turner, B.L., M.J. Papházy, P.M. Haygarth, and I.D. McKelvie. 2002. Inositol phosphates in the environment. Philos. Trans. R. Soc. Lond. B Biol. Sci. 357:449–469.

Turner, B.L., and A.E. Richardson. 2004. Identification of scyllo-inositol phosphates in soil by solution phosphorus-31 nuclear magnetic resonance spectroscopy. Soil Sci. Soc. Am. J. 68:802–808.

U.S. Environmental Protection Agency. 2001. Ecoregional nutrient criteria for rivers and streams. EPA-822-F-01–010. USEPA, Office of Water (4304), U.S. Gov. Print. Office, Washington, DC. Available online at http://www.epa.gov/waterscience/criteria/nutrient/ecoregions/rivers/index.html (accessed 12 November 2008, verified 26 Nov. 2008).

Uzun, I. 2004. Use of spent mushroom compost in sustainable fruit production. Special ed. J. Fruit Ornam. Plant Res. 12:157–165.

Walter, P.J., S. Chalk, and H.M. Kingston. 1997. Overview of microwave-assisted sample preparation. p. 55–222. In H.M. Kingston and S.J. Haswell (ed.) Microwave enhanced chemistry: Fundamentals, sample preparation and applications. Am. Chem. Soc., Washington, DC.