**The short-term transport and transformation of phosphorus species in a saturated soil
following poultry manure amendment and leaching**

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Abstract

The application of manures for fertilization will influence the composition of phosphorus (P) species in the soil profile and the extent of dissolved P leaching to surface waters. Spatial and temporal changes in the P species composition of poultry-manure-amended soil were assessed at two soil column depths (0-5, 10-15 cm) during 10 weeks of leaching. Leachate P was primarily composed of dissolved fractions (soluble reactive P; dissolved unreactive P) and reached a maximum in the fourth week of leaching, concurrent with maxima in the degree of P saturation (DPS; 80%) and water extractable P (WEP, 9 mg kg⁻¹) in the soil profile. In week 4, orthophosphate (orthoP) represented a greater proportion of total P in the 0-5 cm depth (80% or 11 mg/kg NaOH-EDTA P) than the 10-15 cm depth (72%, 571 mg/kg). In contrast, monoester- (17%, 150 mg kg⁻¹) and diester-P classes (10%, 60 mg kg⁻¹) were most abundant in the 10-15 cm depth. In week 4, the ³¹P NMR spectrum of the 0-5 cm depth soil was most similar to that of the poultry manure, which may have contributed to the mobility of P species, including orthoP, *myo*-inositol hexakisphosphate (phytate or *myo*-IHP), and nucleic acids. The appearance of *neo*- and *D-chiro*-IHP, as well as phospholipid signals during the leaching period indicate possible short-term (< 10 week) contributions of organic P to the generation and leaching of orthoP, specifically under P-saturated conditions. This work reaffirms current best management practices to consider soil DPS prior to manure amendment in order to minimize the generation of excess inorganic P and loss of dissolved P species to receiving waters.

Keywords: Phytate, organic phosphorus, degree of phosphorus saturation, soil, leachate, poultry manure

Abbreviations: diester-P, orthophosphate diesters; DPS, degree of P saturation; DUP, dissolved molybdate-unreactive P; IHP, inositol hexakisphosphate; monoester-P, orthophosphate monoesters; P_i , inorganic P; P_o , organic P; SRP, soluble molybdate-reactive P; TDP, total dissolved P; TP_L , total P in unfiltered leachate; TPP, total particulate P; TP_s , soil total P; WEP, water-extractable P.

1. Introduction

The application of phosphorus (P) fertilizers in excess of crop requirements represents a significant source of non-point nutrient pollution and water quality deterioration in agricultural watersheds such as the Chesapeake Bay (Sharpley, 2000; Sharpley et al., 2003) and Lake Champlain (Gaddis and Voinov, 2010; Kominami and Lovell, 2012; Meals and Budd, 1998; Michaud et al., 2009; Troy et al., 2007). The application of manure to soils nearing saturation with inorganic P (P_i) is of particular concern, as saturated soils have a limited capacity to retain P, leading to losses via leaching and subsurface transport (Sharpley et al., 1994). Manures rich in organic P (P_o ; e.g., poultry) may contribute to the loss of dissolved P species either directly through leaching and displacement of loosely-bound P_i (Sharpley and Moyer, 2000) or following biologically-mediated transformations in the soil profile (Crouse et al., 2002). The time period immediately following manure amendment may be the most critical for understanding the behavior of manure P_o in P-saturated soils under leaching conditions, as well as the fate and transport of excess P to surface waters (Kleinman et al., 2005a; Lehmann et al., 2005).

The loss of P_i and P_o from manure-amended soils has been assessed in both field and column leaching studies (Chardon et al., 2007; Lehmann et al., 2005; McDowell and Sharpley, 2001b). The leaching of manure P will depend on the chemical (e.g., degree of P saturation

[DPS], water-extractable P [WEP], pH, organic matter and mineral content) and physical (e.g., pore structure, bulk density) characteristics of the soil, which influence P species solubility and transport (Godlinski et al., 2004; McDowell and Sharpley, 2001a; McDowell and Sharpley, 2001b; Schelde et al., 2006). For example, P losses from manured soils are highly correlated with manure WEP, either as dissolved P_i or P_o (Chardon et al., 1997; Koopmans et al., 2007; Sharpley and Moyer, 2000). Leaching of WEP is dependent on the DPS in the soil, which can change soon after manure amendment (Koopmans et al., 2003; Koopmans et al., 2007; Nair et al., 2004). In a study of 429 global soils, high DPS (> 80%) was correlated with low soil P retention due to the presence of saturated surface binding sites, resulting in higher WEP concentrations and leaching (Pothig et al., 2010). Organic P species that interact more strongly with the soil solid phase (e.g., *myo*-inositol hexakisphosphate [phytate or *myo*-IHP]; Leytem et al., 2002) typically display slower biological turnover (Condrón et al., 1990); however, soils that are saturated with P will have a lower capacity to retain additional P_o , leading to higher equilibrium concentrations of dissolved P_o in soil pore water, and a greater propensity for leaching and transformation of P.

The preferential movement of P_o over P_i has been frequently reported in soil leaching studies (Anderson and Magdoff, 2005; Kang et al., 2011; Toor et al., 2005a; Toor et al., 2003). This in part is due to the transport of particulate P and dissolved P_o through preferential pathways or via dissolved P leaching under matrix flow conditions (Fuentes et al., 2012; Makris et al., 2006). For example, grassland lysimeters treated with dairy effluent contained a higher proportion of P_o (88%) than the original fertilizer input (14%), indicating preferential leaching of manure P_o (Toor et al., 2003). Similarly, packed soil columns amended with poultry manures leached > 50% more P than treatments amended with inorganic fertilizer (Kang et al., 2011).

Anderson and Magdoff (2005) demonstrated greater leaching of diester-P through packed soil columns compared to inorganic or monoester-P sources, indicating the importance of P species and class characteristics in the mobility of P_o amendments through the soil profile.

The short-term effects of precipitation on the leaching of P in amended soils has been demonstrated in several studies, ranging from simple leaching experiments (Bol et al., 2003; Soinne et al., 2008; Wood et al., 2005) to hydrologic models of nutrient dynamics in soils (Runyan and D'Odorico, 2012); however, the fate of P within the soil profile is typically not assessed in parallel to leaching metrics. In few examples, the movement of P_o to lower depths (70-80 cm) or accumulation in the upper layer (e.g., monoester-P; 0-30 cm) of manure-amended soils has been confirmed in weeks to months-long studies at the end of a leaching period by destructively sampling soils at various depths (Chardon et al., 1997). The behavior of manure P_o may be extrapolated through the analysis of P species composition and position at the end of a leaching experiment (Chardon et al., 1997; Godlinski et al., 2004; Kleinman et al., 2005a; Zhao et al., 2009); however, this approach does not capture changes in soil P composition between leaching events and over short time periods, which may be more critical for understanding fertilization-related nutrient losses. To overcome these limitations, a poultry manure-amended column system was designed so that soil and leachate composition could be monitored with minimal disturbance between leaching events. The objectives of the study were to (1) assess changes in the P species composition of a poultry manure-amended soil at two soil depths (0-5, 10-15 cm) during ten weeks of leaching; (2) quantify the associated dissolved and particulate leachate P losses; and (3) utilize multivariate methods to assess the influence of manure P_o on the composition of soil P as a function of depth and leaching event.

2. Materials and methods

2.1. Soil and manure collection

Fresh poultry manure from layer houses and pasture soil (0- 30 cm depth) were collected in Salisbury, VT in August 2008. The field had received poultry litter since the 1930s and was last cultivated with corn in 2003. The soil was a Berkshire and Marlow stony loam (3-12% slope; 11% clay, 54% silt, and 35% sand), mixed, frigid Typic Haplorthods (USDA, 1971). The pasture vegetation was composed of alfalfa (*Medicago sativa*), timothy grass (*Phleum pratense*), and brome grass (*Bromus willdenowii*).

2.2. Column experiment

Columns were wet-packed with sieved soil (6.4 mm) in distilled water. The three 25.4-cm diameter clear PVC columns were capped at the base with a plastic mesh-filter (1.6 mm) and 1.3 cm spigots for leachate collection and incubated indoors at ambient temperature (20-22°C). Columns with total soil masses of 10.8-20.2 kg ($1.1-1.9 \text{ g cm}^{-3}$) were fitted with off-set ports (2.5 cm diam.) to collect soil samples from 0-5 and 10-15 cm below the surface. The total soil mass removed due to sampling was less than 2% of the initial soil mass. Columns were inspected regularly for the formation of macropores near the soil sampling ports and along the inner wall of clear PVC and none were found. The water used to pack the columns was collected to determine column pore volume as well as week 0 leachate measurements. Fresh poultry manure was surface-applied (0.35 kg ; $6.8 \pm 0.3 \text{ g P}$; $> 1342.0 \pm 59.2 \text{ kg P ha}^{-1}$) to each column prior to the first leaching event (week 1). Columns were leached weekly with one pore volume of distilled water (2.3 – 2.5 L; 4.5 – 4.9 cm rain; pH 5) for 10 weeks. The chosen leachate volume equated to a weekly precipitation rate that was 40-50% greater than regional averages for May through September (NOAA, 2008). Composite leachate samples were collected within 24 h of each

irrigation event and stored at 4°C for subsequent analysis (< 48 h). Every two weeks, approximately 12 g of soil was removed from each port for analysis following complete column drainage.

2.3. Leachate analysis

Total P in unfiltered leachate (TP_L) was determined by perchloric acid oxidation (Robinson, 1941) followed by inductively-coupled-plasma-optical-emission-spectroscopy (ICP-OES; JY-Horiba Ultima 2C) for P and metals (iron, Fe; aluminum, Al; magnesium, Mg; calcium, Ca; manganese, Mn). Leachate was filtered (0.45 µm) and analyzed for total dissolved P (TDP) and metals by ICP-OES. Soluble reactive P (SRP) was determined by molybdate colorimetry as described by Watanabe and Olsen (1965). The difference between TDP and SRP was defined as dissolved unreactive P (DUP) (Koopmans et al., 2007). The difference between TP_L and TDP was defined as total particulate P (TPP). These definitions were analogously extended to dissolved and particulate metals fractions (Fig. S1).

2.4. Soil analysis

Soil moisture content was determined following oven drying (48 h, 60°C). Total P (TP_S) was determined on dry soil by digestion (Parkinson and Allen, 1975) followed by ICP-OES. Water extractable P (WEP) was determined on field-moist soil, which was extracted in distilled water (1:20 w/v) and analyzed for P by molybdate colorimetry (Watanabe and Olsen, 1965). Degree of P saturation (DPS) was determined by ammonium oxalate extraction of dried soils (pH 3, 1:20 m/v) and calculated based on P, Al, and Fe extract concentrations determined by ICP-OES (Schoumans, 2000). Organic P in initial soil and manure was determined by ignition at 550°C as described by Saunders and Williams (1955) and modified by Walker and Adams (1958). Soil pH was determined in distilled water (1:1 w/v) as described by Schofield and Taylor (1955). Total

carbon (C) and nitrogen (N) for selected samples were determined by elemental analyzer (CE Elantech Inc.; Fig. S1).

2.5. Phosphorus-31 NMR analysis

Soils collected from a single column were prepared for solution ^{31}P nuclear magnetic resonance spectroscopy (^{31}P NMR) as described by O'Halloran and Cade-Menun (2008). Briefly, 2 g of moist soil was extracted in 30 mL of 0.25 M NaOH + 0.05 M EDTA for 12 h on a rotary shaker (100 rpm), and then centrifuged (10,000 g, 10 min). A 1-mL aliquot of supernatant was diluted to 10 mL and analyzed by ICP-OES for total NaOH-EDTA-P; the remaining supernatant was lyophilized (Fig. S1). Lyophilized extracts were dissolved in 0.8 mL of D_2O , 0.3 mL of 10 M NaOH, and 1.2 mL of the NaOH-EDTA extractant solution. Solution ^{31}P NMR analysis was conducted using a Bruker AVANCE 500 MHz spectrometer equipped with a 10-mm broadband probe (parameters: 90° pulse, 0.68 s acquisition time, 4.32 s pulse delay, 20°C , ~2500 scans [4 h] for all but samples from week 10 [12 h]; no proton decoupling). One sample was spiked with phytate to confirm peak identifications. Chemical shifts of signals were referenced in parts per million (ppm) relative to an external orthophosphoric acid standard (85%), and the orthophosphate peak was standardized to 6 ppm for each sample during processing after analysis. Signals were assigned to P compounds based on the literature (Cade-Menun, 2005; He et al., 2011; Turner et al., 2012; Turner et al., 2003). Peak areas were calculated by integration on spectra processed with 1 Hz and 7 Hz line broadening, using NMR Utility Transform Software (NUTS, Acorn NMR, Livermore CA, 2000 edition). Spectra were processed with 7-Hz line-broadening for the overall spectrum, and with 1-Hz line-broadening for peak identification in the orthophosphate monoester region. The concentration of individual P

species was calculated by multiplying the ^{31}P NMR proportions by the total NaOH-EDTA-P concentration measured by ICP-OES.

Based on chemical shift ranges, peaks were grouped into specific compounds if they could be identified, or general compound classes. Inorganic P compounds included: orthophosphate (orthoP; 6 ppm), pyrophosphate (pyroP; -4.0 ppm), and polyphosphates from -3.5 to -27.5 ppm. Phosphonates (phos) were found between 28.5 and 7.7 ppm. Organic P compounds included orthophosphate monoesters from 6.8 to 4.0 ppm and 3.9 to 2.7 ppm, and orthophosphate diesters from 2.5 to -3.6 ppm. Orthophosphate monoesters were: *myo*-inositol hexakisphosphate (*myo*-IHP or phytate) at 5.5, 4.5, 4.1, and 4.0 ppm; *scyllo*-IHP at 3.7 ppm (Turner and Richardson, 2004); α -glycerophosphate (α -glyc) at 4.9 ppm and β -glycerophosphate (β -glyc) at 4.7 ppm (Doolette et al., 2009); glucose 6-phosphate (G6P) at 5.1 ppm; mononucleotides including AMP (Nucl) from 4.2 to 4.5 ppm; choline phosphates at 3.9 ppm. The remaining orthophosphate monoester region was subdivided into three subregions (Hill and Cade-Menun, 2009). The region from 6 to 7 ppm was called Mono1, and includes peaks tentatively identified as *neo*- (6.67 ppm) and *D-chiro*-IHP (6.48 ppm), based on Turner et al. (2012). Unidentified orthophosphate monoesters in the region from 5.8 to 3.7 ppm were classified as Mono2, while those from 3.6 to 2.5 ppm were classified as Mono3. Orthophosphate diesters were: DNA at -0.7 ppm with a shoulder at -0.9 ppm; other unidentified diester peaks from 2.5 to -0.6 ppm (OD1) and from -1.0 to -3.6 ppm (OD2).

Degradation of diester compounds (e.g., phospholipids) during ^{31}P NMR sample preparation can lead to the generation of glycerophosphates and mononucleotides (Doolette et al., 2009). Some of the unidentified orthophosphate monoester compounds occurring in the Mono2 range may also result from degradation; we assumed these could be as much as half of

the unidentified peaks in the Mono2 region. Therefore, α - and β -glyc, Nucl, and half of Mono2 were classified as originating from orthophosphate diesters in the original soil sample. Using this approach, we present two estimates of degradation: no degradation (the original, uncorrected total monoesters and diesters), and a high estimation of degradation (including the known degradation compounds plus half of the Mono2 region). We make the conservative assumption that the maximum degradation of diester compounds has occurred, and therefore, only discuss the corrected proportions of total monoester and diester-P.

2.6. Statistical analysis

All statistical analyses were conducted using JMP 9.0.0 software (2010 SAS Institute, Inc.). Analysis of variance (ANOVA) was used to compare average soil and leachate properties across weeks ($n = 3$, $\alpha = 0.05$). Comparison of average soil properties between depths was made using a two-sample t-test assuming equal variances ($n = 3$, $\alpha = 0.05$). The solution ^{31}P NMR species distribution of each sample was tested for normality (Shapiro and Wilk, 1965) on log (n+1)- and non-transformed data. A principal components analysis (PCA) of log (n+1)-transformed proportions was used to illustrate variability in ^{31}P NMR species ($n = 17$) composition among initial soil, poultry manure, and column soils collected following manure amendment and leaching; loading plots were generated to determine the contribution of individual P species to this variability.

3. Results and discussion

3.1. Initial soil and manure properties

Poultry manure and initial soil pH, moisture content, C:N, and P composition (Table 1) were within previously reported ranges (Kleinman et al., 2005b; Sharpley and Moyer, 2000;

Turner and Leytem, 2004). The poultry manure contained 19,434 mg kg⁻¹ total P, of which 6,345 mg kg⁻¹ was NaOH-EDTA-P and 7,381 mg kg⁻¹ total P_o (Table 1). The initial soil contained 1,360 mg kg⁻¹ total P, 387 mg kg⁻¹ NaOH-EDTA-P, and 243 mg kg⁻¹ total P_o (Table 1). The proportions of orthoP (58%), monoester-P (30%), and diester-P (10%) determined by ³¹P NMR were consistent with previous reports for poultry manure (He et al., 2008; Hill and Cade-Menun, 2009; Turner and Leytem, 2004). The solution ³¹P NMR spectra for the initial soil and manure are provided in Fig. S2. In the initial soil, DPS (42%) was below the phosphate saturation threshold (70%) described by Pothig et al. (2010), indicating the capacity of soil to retain P at the outset of the study (Table 1). Manure was substantially higher in C (34%), N (12%), total P, and WEP (234 mg kg⁻¹) than the initial soil (Table 1).

3.2. Leachate phosphorus analysis of poultry-manure-amended soil columns

An average of 3.7 ± 0.4 mg of total leachate P (TP_L; Table 2) was removed from column soils in week 0 during the packing procedure, primarily as total particulate P (TPP, 2.9 ± 0.2 mg; Table 2). The cumulative loss of leachate P fractions was linear over the 10 weeks of sampling (Table 2). Total leachate P was recovered at a rate of 5.4 mg P wk⁻¹ and represented 56 mg P (< 1%) lost from the columns after 10 weeks. Seventy percent of the cumulative TP_L loss was transported as TDP (39 mg), of which 24 mg was SRP (63% TDP; Table 2).

The greatest P losses due to leaching occurred in weeks 4 (6.3 ± 0.5 mg) and 10 (8.8 ± 1.8 mg), representing a 28% and 78% increase in P export relative to the weekly average (4.9 ± 0.4 mg TP_L), respectively. Total dissolved P was the major leachate fraction, contributing an average of 86% and 53% TP_L in weeks 4 and 10, respectively. In weeks 4 and 10, SRP accounted for 62-65% of the TDP fraction (Table 2). In addition to dissolved P being the dominant leached fraction from soils, previous studies have reported a rapid leaching of this

fraction soon after manure amendment (≤ 5 leaching events; Kleinman et al., 2005a; McDowell, 2008; Siemens et al., 2008). Dissolved unreactive P also reached a maximum in week 4 (1.9 mg; Table 2) but was not the major dissolved form ($< 40\%$ TDP). Smaller contributions of dissolved P_o to leachate P have been reported for soils with a history of long-term manure amendment (Lehmann et al., 2005).

The loss of TPP (4.4 mg, of TP_L) was greatest in week 10 and accounted for nearly half of TP_L in that week ($42 \pm 20\%$; Table 2). Cross-column differences in soil bulk density may have contributed to the large variation in TPP losses from individual columns throughout the study, possibly due to the formation of macropores in low-bulk density columns (Kleinman et al., 2005a). The leaching of TPP may have accounted for the significant drop in total soil P in week 10 (TP_S ; Table 3); however, this cannot be confirmed, as changes to the structural characteristics of the soil were not assessed in the current study. Rather, we have focused our analysis on the soil chemical properties associated with the leaching of dissolved P (e.g., WEP, DPS). As indicated by the leachate analysis, the greatest flush of dissolved P_i (SRP) and P_o (DUP) from the study soils occurred in week 4. It was therefore expected that the maximum loss of dissolved P from the poultry manure amended soil columns would be reflected in concurrent soil P analysis.

3.3. Changes in total and extractable phosphorus in poultry manure-amended soil columns during leaching

Soil WEP concentrations ranged from $0.8 - 9.2 \text{ mg kg}^{-1}$ (0-5 cm) and $1.2 - 9.3 \text{ mg kg}^{-1}$ (10-15 cm) during the study, with a maximum WEP concentration occurring in week 4 for both depths (Table 3). In week 4, the WEP concentration in the 0-5 cm depth was significantly higher than neighboring weeks ($P < 0.001$). In contrast, the WEP concentration at the 10-15 cm depth

was not significantly different in week 4 than the preceding weeks (9.3 mg kg^{-1} ; $\alpha = 0.05$), though it decreased steadily for the remainder of the leaching period (Table 3).

The week 4 maxima in WEP concentrations (Table 3) and dissolved leachate P (Table 2) corresponded to the greatest DPS measured throughout the study (80%, both depths; Table 3). Soils with $>70\%$ DPS corresponded to 4.7 mg kg^{-1} WEP, with these parameters being highly correlated ($R^2 = 0.94$). Pothig et al. (2010) used a WEP-based model to predict DPS, and found that soils saturated with P ($\text{DPS} > 70\%$) had, on average, more than 5 mg kg^{-1} WEP and displayed a greater propensity for P leaching. Similarly, when DPS was greater than 60% in the current study (corresponding to WEP concentrations above 2 mg kg^{-1}), leachate TDP displayed a step-increase that was 5.6-fold higher than the average loss in week 0 ($0.79 \pm 0.07 \text{ mg TDP}$; Fig. 1). Previous soil leaching studies have reported the leaching of soluble P from poultry manures with soil $\text{DPS} < 100\%$ (e.g., Holford et al., 1997). Rhue et al. (2006) developed a model to predict P leaching from manured soils and found that when DPS was less than 50%, pre-manure P leaching was at its lowest ($< 0.1 \text{ mg L}^{-1}$). Soil DPS prior to manure amendment in the current study (week 0, 41-49%) was similarly associated with the lowest amount of TDP measured in leachate (0.8 mg). Soils sampled after manure-amendment (week 2) had 20% higher DPS and at least 2-fold higher WEP and TDP concentrations compared to the unamended soil (week 0; Tables 2 and 3).

Soluble P in poultry manure likely contributed to the observed increases in soil P saturation ($\text{DPS} > 60\%$), and subsequent increases in soil WEP concentrations and TDP leaching from soils. The greatest changes in soil P properties following manure amendment occurred in the 0-5 cm depth interval. Solution ^{31}P NMR spectroscopy was employed to determine the contribution of individual manure and soil P species to the mobile P pool.

3.4. Solution ^{31}P NMR analysis of a poultry-manure-amended soil profile during leaching

Solution ^{31}P NMR spectra for soils collected from two depths in weeks 2 through 10 are provided in Fig. 2. Similar to WEP trends, significant differences in NaOH-EDTA-extractable P were observed with respect to time, but not depth ($\alpha = 0.05$). On average, NaOH-EDTA-extractable P concentrations were highest in week 4 (685 mg kg^{-1}) for both depths, then decreased in week 6 and remained at $\sim 330 \text{ mg kg}^{-1}$ for the remainder of the study (week 6-10; Table 3). The NaOH-EDTA extraction efficiency remained below 60% (Table 2), in contrast to multiple reports of near-100% recovery in similar poultry-manured soils (e.g., Turner, 2004; Hill and Cade-Menun, 2009). Changes in soil properties, such as total metals concentration, may have influenced NaOH-EDTA extract efficiency. Peak extraction efficiency (week 10) coincided with the lowest soil metals concentrations and the greatest cumulative metals loss due to leaching (Table S1, Fig. S3). In general, high recoveries of soil P by NaOH-EDTA derive from the dissolution of P-containing humic substances at alkaline pH, and the chelation of bridging metals via EDTA (Cade-Menun and Preston, 1996; Turner et al., 2005). In the current study, soils in weeks with the lowest extraction efficiency (week 6; Table 2) contained total measured metals concentrations that were sufficient to saturate $97 \pm 15\%$ of the $0.05 \text{ M Na}_2\text{EDTA}$ in the extract solution, with little remaining to facilitate the chelation of bridging cations. It is therefore likely that soil metals concentrations were in excess of extractant chelation capacity, leading to low P recoveries in the current study.

The greatest orthoP concentration (711 mg kg^{-1}) and proportion (80%) occurred in week 4, 0-5 cm depth soils (Table 4), which was consistent with soil WEP and dissolved leachate trends (Tables 2 and 3). Shifts were also observed in the concentrations and proportions of P_o species during the leaching study, particularly in week 4. A principal components analysis (PCA)

of the log (n+1)-transformed ^{31}P NMR proportions was used to identify differences in the P species composition of the initial poultry manure and soil as well as the leached soils. The first and second principal components accounted for 33.8% and 29.4% of the variance among samples, respectively (Fig. S4). Loading plots illustrate the contributions of IHP (25%) and Nucl (7%) to the P species composition of the poultry manure (Table 4, Fig. S4). The initial soil contained larger proportions of choline (2%), phosphonates (1%), Mono2 (4%), and orthoP (68%), which differentiated its P species composition from that of the poultry manure (Table 4, Fig. S4). The majority of leached soils were similar to the initial soil, with the exception of soils collected at 0-5 cm in week 4 (Fig. S4). Week 4 (0-5 cm) soils contained the largest concentration of total P and NaOH-EDTA-P. Poultry manure contained 14-fold more total P and 16-fold more NaOH-EDTA-P than the initial soil (Table 1). Similarly, *myo*-IHP (1612 mg kg^{-1}) was nearly 50 times higher in the poultry manure than in the initial soil (Table 4). We therefore conclude that the quantity of poultry manure applied to the study columns was sufficient to observe changes in the P composition of soils as a result of manure application and subsequent leaching.

The composition of manure P species was not entirely conserved in the week 4 soil (0-5 cm), which may be explained by the transformation of P species in the initial manure and subsequent transport of the transformed products into the column profile. For example, loading plots illustrate the influence of pyroP on the P species composition of the manure and week 4 (0-5 cm) soil, despite pyroP representing a small proportion of NaOH-EDTA-P in this week (1%, Table 4). OrthoP (80%), Mono1 (3%), and OD2 (1%) proportions were highest in week 4 relative to other weeks (OrthoP, 66-76%; Mono1, 1-2%; OD2, 0%), and contributed to the loading vector separation of the week 4 soil from poultry manure and soils collected in other

weeks. All other P species proportions remained the same or decreased in the 0-5 cm soil in week 4 (Table 4). In contrast, OD2 and Mono1 proportions were absent in the initial poultry manure, and may therefore represent P species that were generated during the leaching experiment (Fig. S4). Consistent with the PCA, OD2 concentrations at the 0-5 cm depth reached a maximum in week 4 (6 mg kg^{-1}), as did Mono1 (28 mg kg^{-1} ; Table 4). The identity of P species in the OD2 chemical shift range (-1.0 to -3.6 ppm) are not reported in the literature and are not defined in this study. Turner et al. (2012) identified *neo*- and *D-chiro*-IHP resonances in the Mono1 chemical shift range. Retroactive analysis of ^{31}P NMR spectra (Turner et al. 2012) and previous measurements of *neo*- (1-4%) and *D-chiro*- (3-10%) stereoisomers in soil (McKercher and Anderson, 1968; Young et al., 2013) were within the range measured for Mono1 in the current study (1-3 %; Table 4). In soils, *D-chiro*-IHP is formed through the epimerization of *myo*-IHP (L'Annunziata and Gonzalez, 1977), whereas *neo*-IHP has been found to originate in aerobic sewage sludge (Cosgrove, 1973) or parasitic amoebae (Martin et al., 2000). In a recent study of riparian soils from Vermont, the concentration of *neo*-IHP was significantly higher in poorly-drained than well-drained soils (Young et al., 2003). The transformation of manure P_o during the leaching period is the most likely explanation for the large proportion of orthoP, and the occurrence of Mono1 and OD2 species in the week 4 (0-5 cm) soil.

The composition of P species in the 10-15 cm soil differed from that measured at 0-5 cm in week 4. Total monoester-P (135 mg kg^{-1} , 17%) and diester-P ($\sim 78 \text{ mg kg}^{-1}$, 10%) concentrations were greater at 10-15 cm than 0-5 cm (Table 3). Specifically, *myo*-IHP (8%; 65 mg kg^{-1}), OD1 (1%, 6 mg kg^{-1}), DNA (1%, 5 mg kg^{-1}), and polyP (1%, 6 mg kg^{-1}) were greatest in the 10-15 cm depth (Table 3). The increase in *myo*-IHP at the 10-15 cm depth suggests that it had been transported to the lower depth within four weeks of manure application, as biogenesis

of this isomer has not been reported in soils, and few studies have demonstrated the production of forms such as *scyllo*-IHP (e.g., Cosgrove, 1964; Cade-Menun 2014; unpublished results). Unlike the 0-5 cm soil, the 10-15 cm soil did not contain a high proportion of orthoP (72%) relative to neighboring weeks. The 10-15 cm soil also lacked measureable proportions of OD2 and PyroP and had a lower proportion of Mono1 (2%), which contributed to the differentiation of the 0-5 cm soil in week 4 based on PCA (Table 4, Fig. S4). Phosphorus species previously identified within the OD1 chemical shift range (2.5 – -0.6 ppm) included phosphatidyl choline, phosphatidyl serine, and phosphatidyl ethanolamine (1.75 – 0.78 ppm; Turner et al. 2003a). These fatty acids are typically associated with plant and microbial sources, which can be transferred to poultry manure as sloughed-off intestinal cells, plant feed cell-walls, or gut microflora (Sharpley and Moyer, 2000; Toor et al., 2005b) . Accumulation of diester-P (i.e., OD1, DNA) in the 10-15 cm depth in week 4 may therefore be a result of manure-P transport, or alternatively, the generation of microbe-derived phospholipids and nucleic acids *in situ*. DNA and polyP are produced as a result of microbial activity and P storage (Achbergerova and Nahalka, 2011); the appearance of these compounds in excess of the initial soil and manure proportions is therefore indicative of microbial generation and degradation of soil P_o during the leaching period. Unlike the 0-5 cm soil, there was nothing to suggest that Mono1 and OD2 species had been produced at 10-15 cm in week 4; however, it is possible that these species were generated, and subsequently leached with orthoP prior to collection.

3.5. Transformation and leaching of manure organic P in a P-saturated soil

Higher concentrations of P_o species in the lower soil depth in week 4 were indicative of preferential P_o leaching, as has been described in previous column and lysimeter studies (Anderson and Magdoff, 2005; Chardon et al., 1997; McDowell, 2008; Toor et al., 2003). The

apparent mobility of *myo*-IHP is of interest, as its migration is typically low in soils due to precipitation or sorption reactions (Celi and Barberis, 2007). Dou et al. (2009) reported negligible accumulation of *myo*-IHP in poultry-manure amended soils with high P saturation ($\text{DPS} \geq 80\%$), and suggest that IHP leaching was more likely under these conditions. In week 4, the proportion of *myo*-IHP was greater at 10-15 cm than 0-5 cm with respect to monoester-P (48%), total P proportion (8%), and absolute concentration (65 mg kg^{-1} ; Table 4). Furthermore, P-saturated soils in this week (80% DPS; Table 3) would have a limited capacity to retain P from poultry manure, leading to the downward movement of *myo*-IHP within the column and an increase in leached dissolved P_o to its maximum value for the study (1.9 mg ; Table 2).

In previous studies, the preferential leaching of P_o has been demonstrated by comparing the proportion of dissolved P_o in leachate (%DUP) to that of the initial manure (% P_o). Leachate %DUP in excess of the initial manure composition suggests that P_o has become enriched in the leachate solution, as opposed to the leachate solution retaining the same proportion of P_o found in the original soil or manure (Toor et al., 2003; Toor et al., 2005a). Through a similar comparison, Toor et al. (2003) concluded that P_o increased in leachates from soils amended with dairy effluents due to the retention of orthophosphate in the soil profile. Other studies have reported the retention of monoester- and diester-P at shallower depths ($<40 \text{ cm}$) of manured soil columns and proportionally less monoester-P than orthoP in leachate (Fuentes et al., 2012; Koopmans et al., 2003; Koopmans et al., 2007). Although leachate P species were not determined in the current study, dissolved unreactive P proportions in week 1 leachates were, on average, 20% higher than the P_o composition of the initial manure (40%), indicating preferential loss of dissolved P_o soon after manure amendment.

Evidence for the movement of poultry manure-P through the column system is indicated by preferential losses of dissolved P_o (i.e., DUP) and the differential accumulation of P_i and P_o species at the two soil depths. Above-threshold DPS (80%) likely promoted P_o transport due to a lack of free sorption sites in the column matrix, leading to high monoester- and diester-P species concentrations at the 10-15 cm depth in week 4. The mobility of P_o observed in columns was also evident in leachate DUP, which reached a maximum in week 4 (1.9 mg) and remained high for the remainder of the study (1 – 1.7 mg; Table 2). This is consistent with previous characterizations of DUP leachate fractions, which were primarily composed of monoester-P (including IHP) and diester-P classes (Toor et al., 2003). However, cumulative losses of DUP were a minor fraction of TDP (35%), indicating a sustained and higher proportion of SRP leached from columns during weeks 4 through 10. The large proportion of SRP measured in the dissolved leachate fraction during weeks 4 through 10 may be due to the leaching of orthophosphate from the manure source, or orthophosphate generated from P_o via *in situ* enzymatic hydrolysis processes.

Koopmans et al. (2007) suggested that in addition to transport and loss, intra-column degradation of P_o and subsequent orthophosphate generation may lead to increases in the proportion of P_i lost during leaching. We observed a steady increase in the proportion of leachate SRP from 40% of TDP in week 1 to 60% in week 10 (Table 2). Previous studies have reported the conversion of monoester-P (e.g., IHP) to orthoP as linked to phosphatase activities in batch experiments (Crouse et al., 2002) or leaching studies of poultry manure-amended soils (Toor et al., 2003). The potential for native soil microorganisms to mediate these degradation processes has also been reported in multiple instances (Hill et al., 2007; Jorquera et al., 2008; Richardson and Hadobas, 1997).

4. Conclusions

We assessed short-term changes in the P species composition of a soil profile that had been amended with poultry manure and leached weekly for ten weeks. Maxima in soil P concentrations (e.g., total P, WEP) and ^{31}P NMR species (e.g., orthoP, IHP) coincided with soil P saturation ($\text{DPS} > 80\%$) and peak losses of dissolved leachate P in week 4. Poultry manure represented a concentrated source of orthoP and P_o species, particularly IHP, which were transported to lower depths within the first four weeks of leaching. Preferential leaching of dissolved P_o (i.e., DUP) was also apparent in the first week following manure application, further supporting the apparent downward movement of P_o in the column system. We provide evidence for the microbial cycling of manure and soil P within this time period, through the appearance of pyroP, DNA, and orthoP in excess of initial soil or manure proportions. The degradation of higher monoesters (e.g., IHP) and diesters (e.g. DNA) may have also contributed to the generation and leaching of orthoP. It was not possible to determine – on an individual P species basis – whether decreases in soil concentrations of P_o forms were due to leaching, transformation, or both; however, analysis of the soil column during the leaching period confirm the relatively short time-period over which soil P saturation and organic P transformations will become apparent within the soil and leachate record. A detailed analysis of leachate P species in future studies of intact soil columns could be used to parse apart these dynamic transport and transformation processes. Soils with a history of manure-amendment and high DPS display a greater potential for manure P_o turnover and loss over short time periods (< 10 weeks). Particularly in well-drained soils, efforts to study organic P transformations and mitigate the leaching of dissolved P should occur within the first several precipitation or leaching events following the application of manure. An understanding of these processes, and their timing, will

be necessary to minimize the loss of valuable plant-available P and improve the efficiency of nutrient use in agricultural systems.

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Captions for Tables and Figures

Table 1. Initial soil and poultry manure physical and chemical properties.

Table 2. Weekly and cumulative losses of leachate P fractions (mg, %TP) and P loss rates (mg P wk⁻¹) for 10 week leaching of poultry-manure-amended soil columns (n = 3).

Table 3. Composition of soil P, degree of phosphate saturation (DPS) and NaOH-EDTA extraction efficiency (EE) at two depths during ten-week leaching of poultry-manure-amended soil columns (n = 3).

Table 4. Concentrations and relative percentages of P compounds determined by solution ³¹P NMR spectroscopy of NaOH-EDTA.

Fig. 1. Relationship between soil water extractable P (WEP) and total dissolved P (TDP) losses in poultry manure-amended soil columns leached for ten weeks. Black symbols represent soils with degree of P saturation >60% or TDP loss >3 mg.

Fig. 2. Phosphorus-31 nuclear magnetic resonance spectra of poultry-manure-amended column soils sampled at two depths (0-5, 10-15 cm) during 10 weeks of leaching.

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Table 1

Initial soil and poultry manure physical and chemical properties.

Property	Soil ^a	Manure
pH	6.26	5.30
Moisture Content, %	9 (2)	41 (5)
Bulk density, g cm ⁻³	1.6 (0.2)	-
Total C, %	2.07	33.74
Total N, %	0.19	11.93
Degree of P Saturation, %	42 (5)	-
Total P ^b	1,360 (298)	19,434 (757)
Organic P ^c	243 (36)	7,381 (1,690)
NaOH-EDTA-P	387 (97)	6,345 (383)
Organic P ^d	134	2,690
Water Extractable P	0.43 (0.12)	234 (7)

^a Values shown are means (SD), n = 3. Properties with no SD, n = 1.

^b Total P, determined by digestion; All P measures: mg P kg⁻¹ dry weight, unless indicated otherwise.

^c Organic P determined by ignition or ^{d,31}P NMR.

Table 2
Weekly and cumulative losses of leachate P fractions (mg, % TP) and P loss rates (mg P wk⁻¹) for 10 week leaching of poultry-manure-amended soil columns (n = 3).

Leachate ^a	Week of Leaching											Cumulative P Loss ^b		Loss Rate ^c	
	0	1	2	3	4	5	6	7	8	9	10	--mg P--	% TP _L	mg P wk ⁻¹	R ²
	-----mg P-----														
TP _L ^a	3.7	2.3 [*]	4.0 [*]	5.0 [*]	6.3 [*]	4.6 [*]	4.9 [*]	4.8 [*]	5.0 [*]	4.9 [*]	8.8 ^{**}	55.6± 2.1	n.a.	5.4	0.97
TDP	0.8	2.1 [*]	3.5 [*]	4.0 [*]	5.4 ^{**}	2.5 [*]	3.5 [*]	3.6 [*]	3.9 [*]	4.6 ^{**}	4.7 ^{**}	38.7± 5.4	69.7± 3.8	3.7	0.83
SRP	1.0 [*]	1.0	2.2 [*]	2.3 [*]	3.5 ^{**}	1.5 [*]	2.2 [*]	2.5 [*]	2.6 [*]	3.2 ^{**}	2.9 ^{**}	24.4± 3.8	43.9± 1.7	2.3	0.77
TPP	2.9 [*]	0.9	1.0	1.0	0.9	2.1	2.1	1.1	1.1	0.3	4.1 ^{**}	16.8± 4.7	30.3± 1.4	1.6	0.54
DUP	0.5 [*]	1.1	1.3	1.7	1.9 ^{**}	1.0	1.3	1.2	1.3	1.4	1.7	14.4± 1.6	25.8± 0.4	1.4	0.86

^a Total leachate P (TP_L), Total Dissolved P (TDP), Total Particulate P (TPP), Soluble Reactive P (SRP), Dissolved Unreactive P (DUP).

^b Cumulative leachate P losses represented as percentage of TP_L leached; Not applicable (n.a.).

^c Loss rate determined by linear regression of cumulative leachate P (mg) over time.

P* < 0.05; *P* < 0.01

Table 3

Composition of soil P, degree of phosphate saturation (DPS) and NaOH-EDTA extraction efficiency (EE) at two depths during ten-week leaching of poultry-manure-amended soil columns (n = 3).

Depth (cm)	Week ^a					
	0	2	4	6	8	10
WEP ^b	-----mg P kg ⁻¹ dry weight-----					
0-5	0.8	1.6	9.2 ^{**}	3.2 [*]	3.7 [*]	5.6 [*]
10-15	1.2	4.7 [*]	9.3 [*]	2.9 [*]	4.4 [*]	4.0 [*]
TP_s ^c						
0-5	2529	2796	2811	2971	2764	620 [*]
10-15	2852	3574	2805	3106	1109 ^{*,+}	830 [*]
NEP ^c						
0-5	521 ^{**}	561 ^{**}	685	377 [*]	374 [*]	236 [*]
10-15	457 ^{**}	504 ^{**}	708	445 ^{**}	262 [*]	282 [*]
EE ^d	-----%-----					
0-5	21	20	24 [*]	13	14 [*]	56 ^{**}
10-15	16	14	25 [*]	14	24 [*]	34 ^{**}
DPS ^e						
0-5	41 ^{**}	64	80 [*]	65	68	74
10-15	49 ^{**}	70	80 [*]	62	70	67

^a Means with * ($P < 0.05$) and ** ($P < 0.001$) are significantly different across weeks; means with + are significantly different across depth ($P < 0.05$).

^b Water extractable P determined via molybdate colorimetry.

^c Total P in soil digests and NaOH-EDTA extracts (NEP) determined by ICP-OES.

^d EE is the percentage of TP_s extracted as NEP.

^e DPS is the degree of P saturation

747 **Table 4**748 Concentrations and relative percentages of P compounds determined by solution ^{31}P NMR spectroscopy of NaOH-EDTA.

Week	Depth (cm)	Organic P ^b																
		Inorganic P ^a			C-P	Orthophosphate monoesters										Orthophosphate diesters		
		OrthoP	PyroP	PolyP	Phos	<i>myo</i> IHP	G6P	α -glyc	β -glyc	Nucl	Chol	<i>scyllo</i>	Mono1	Mono2	Mono3	DNA	OD1	OD2
-----mg P kg ⁻¹ dry weight (relative percent)-----																		
2	0-5	387(72)	2	4(1)	4(1)	46(9)	4(1)	8(2)	8(2)	26(5)	4(1)	11(2)	4(1)	23(4)	4(1)	2	2	1
	10-15	350(76)	2	0	3(1)	23(5)	3(1)	6(1)	6(1)	16(4)	3(1)	10(2)	6(1)	20(4)	3(1)	2	2	2
4	0-5	711(80)	6(1)	0	0	50(6)	5(1)	5(1)	5(1)	23(3)	5(1)	17(2)	28(3)	23(3)	0	4	4	6(1)
	10-15	571(72)	2	6(1)	6(1)	65(8)	11(1)	11(1)	11(1)	26(3)	6(1)	16(2)	16(2)	32(4)	6(1)	5(1)	6(1)	2
6	0-5	245(72)	1	3(1)	2(1)	29(9)	2(1)	5(2)	5(2)	12(4)	2(1)	5(2)	7(2)	14(4)	2(1)	2(1)	3(1)	1
	10-15	352(72)	1	3(1)	3(1)	41(8)	3(1)	7(1)	7(1)	20(4)	3(1)	10(2)	3(1)	24(5)	3(1)	2	2	1
8	0-5	321(74)	3(1)	3(1)	3(1)	26(6)	3(1)	6(2)	6(2)	16(4)	3(1)	13(3)	3(1)	16(4)	3(1)	2	2(1)	2
	10-15	145(73)	1	1	1(1)	17(9)	1(1)	3(2)	3(2)	7(4)	1(1)	4(2)	1(1)	7(4)	1(1)	1(1)	2	1
10	0-5	236(69)	1	0	2(1)	28(8)	2(1)	5(1)	5(1)	16(5)	2(1)	7(2)	7(2)	21(6)	2(1)	2(1)	3(1)	1
	10-15	179(66)	1	4(2)	2(1)	25(9)	2(1)	4(2)	4(2)	12(5)	2(1)	6(2)	4(2)	14(5)	2(1)	2(1)	4(1)	1(1)
Initial	Soil	265(68)	2	3(1)	5(1)	34(9)	6(2)	6(2)	6(2)	17(4)	6(2)	9(2)	3(1)	17(4)	3(1)	3(1)	4(1)	2
	Manure	3668(58)	38(1)	0	0	1612(25)	89(1)	89(1)	89(1)	451(7)	44(1)	44(1)	0	133(2)	44(1)	13	25	6

^a OrthoP, orthophosphate; PyroP, pyrophosphate; PolyP, polyphosphate.^b Phos, phosphonates; *myo*-IHP, phytate; G6P, glucose 6-phosphate; α/β -glyc, glycerophosphates; Nucl, nucleic acid P; *scyllo*-IHP; Other monoesters: Mono1, 7-6 ppm; Mono2, 5.8-3.7 ppm; Mono3, 3.6-2.5 ppm; Other diesters: OD1, 2.5 – -0.6 ppm; OD2, -1.0 – -3.6 ppm.

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Figure 1

Figure 1. Relationship between soil water extractable P (WEP) and total dissolved P (TDP) losses in poultry manure-amended soil columns leached for ten weeks. Black symbols represent soils with degree of P saturation >60% or TDP loss >3 mg.

Figure 2 REVISED