

Title: Manure-Phosphorus in Soil: Detection and Mobility of Phytate – NPB #05-140

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Industry Summary

Annual swine effluent application in western Oklahoma for 11 consecutive years did not lead to significant accumulation of soil phosphorus. Similar trend was shown in a century-old field experiment in central Oklahoma. Although phytate is the main phosphorus form in animal feeding stuff, it has little nutritional value for monogastric animals and was suspected to be responsible for high P levels in animal manures. Laboratory studies showed that phytate added to soil did not remain in the solution and had limited mobility in soil columns that were constructed with acidic or alkaline soil. Therefore, solution phosphorus levels in soils were not increased appreciably by the addition of phytate to soil and manure-phosphorus would have limited mobility in the environment. Application of manure, however, enhanced soil biological activities and the capacity of soil to retain or cycle nutrients. Long-term manure application in central and western Oklahoma sustained agricultural production and did not result in P accumulation to levels close to those in inorganic P fertilizer-treated soils.

Abstract

Annual swine effluent application in western Oklahoma for 11 consecutive years did not lead to significant accumulation of soil phosphorus. Similar trend was shown in a century-old field experiment in central Oklahoma. Although phytate is the main phosphorus form in animal feeding stuff, it has little nutritional value for monogastric animals and was suspected to be responsible for high P levels in animal manures that may lead to P accumulation and contamination in the environment. Laboratory extraction, incubation and leaching studies indicated that mobility of phytate in soil was low and addition of phytate to soil did not appreciably increase extractable soil P. Phytate P also had limited mobility in soil columns that were constructed with acidic or alkaline soils. Therefore, solution phosphorus levels in soils were not increased appreciably by the addition of phytate to soil. This implies that manure-phosphorus would have limited mobility in the environment and leaching potential of phytate-P or phytate degradation intermediates would be limited. These findings suggest that phytate-P would likely accumulate in soil. However, significant P accumulation trend was not detected in two long-term field experiments located in central and western Oklahoma. On the other hand, application of swine effluent reduced microbial biomass C and P contents in soils, suggesting a reduction in the driving force of nutrient conversion in the soil ecosystem. High rates of swine effluent slightly reduced

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microbial biomass C/P ratios, suggesting microbial composition shifted toward a more bacterial dominating community with a greater proportion of P accumulated in the microbial biomass. When compared with the control soils, activities of alkaline phosphatase and inorganic pyrophosphatase were significantly greater in soil treated with swine effluent. Moreover, activity of acid phosphatase was not affected, and activity of phosphodiesterase was significantly lower in soils treated with swine effluent. In both long-term manure-applied field experimental sites, application of manure enhanced soil biological activities and the capacity of soil to retain or cycle nutrients. Long-term manure application in central and western Oklahoma sustained agricultural production and did not result in P accumulation to levels close to those in inorganic P fertilizer-treated soils.

Introduction

Approximately 8.23×10^7 kg of phosphorus (P) enters the environment from monogastric animals reared in the U.S. (Wodzinski and Ullah, 1996). Numerous reports demonstrated that repeated heavy application of animal manure led to accumulation of phosphorus (P) in soil, exceeding crop requirement for growth. Application of animal manure has led to soil P reaching up to $1121 \text{ kg P ha}^{-1}$ (approximately 220 lbs acre⁻¹) in Mehlich-3 extracts (Reed et al., 1998). Adding manure to soil can improve soil properties (Sommerfeldt and Chang, 1985) and crop yield (Ginting et al., 1998b; Sutton et al., 1986). However, repeated heavy application of manure may saturate the soil's capacity to retain manure-P (Nair et al., 1998) and result in P runoff and leaching that could potentially contaminate adjacent water bodies (James et al., 1996). Thus, several states are calling for regulating land application of manure based on P level in the soil and adjacent water bodies.

This is a national issue because pork production in the U.S. is on the rise. Modern production practices make food widely available and economically competitive. The United States has the lowest cost of production for pork in the world. The U.S. Department of Agriculture statistics show that the United States spends only 10% of its per capita income on food, compared to Japan at 18%, Mexico at 32%, and China at 48%. Without taking precautions to prevent possible environmental problems, these very competitive productions may be jeopardized.

Phosphorus accumulation in soils amended with animal waste is, in part, due to a compound called phytate (or phytic acid), a hexaphosphate ester. Phytate is an efficient P storage form in plant. Approximately 75% of P in plant-based feedstuffs (cereal, legume, and seeds) exists as phytate-P (i.e. de Boland et al., 1975; Lolas et al., 1976; O'Dell et al., 1972; Ravindran, et al., 1994). Of 13 to 16.6 million metric tonnes of P fertilizer used from all sources globally each year, about 9.3 million metric tonnes is incorporated into crop seeds, grains, and cereals (Reddy and Sathe, 2002). The release of P from phytate requires an enzyme, phytase. Phytate-P is essentially unavailable to monogastric animals, including humans, chickens, and pigs (Nelson et al., 1968a, b) because they produce little or no phytase by the microbes in the intestine and the phytate-P is excreted in the waste. To compensate for their inability to utilize phytate-P, producers often supplement inorganic P in animal feeds for P nutrition. It is estimated that P nutrition for all of the monogastric animals reared in the U.S. has a value of $\$1.68 \times 10^8$ each year (Wodzinski and Ullah, 1996). Addition of inorganic P in animal feeds also contributes to high P levels in animal waste products.

Attempts to increase the nutritional value of plant-based feedstuff and minimize the release of phytate-P into the environment include supplementing phytase in animal feed (Cromwell et al., 1993) or feeding animals with minimum phytate, such as genetically engineered corn with 33-66% phytate reduction (Raboy and Gerbasi, 1996). None of these attempts have been successful in application. Phytase is costly. Although total P concentration in swine effluent derived from low-phytate corn as the feed showed 42% P reduction when compared with swine effluent derived from regular corn (Gollany et al.,

2003), low-phytate corn produces relatively low yield. In addition, inorganic P supplementation is still required for animal production. Therefore, this approach is not economically competitive.

It is known that soil microorganisms have the ability to release phytate-P in animal wastes. Understanding the fate and transformation of phytate-P in soil is essential in developing management strategies to minimize potential negative environmental impact of animal waste land application. So far, there is limited information available about phytate in soil, due, in part, to the lack of effective methods to detect and quantify phytate in soil. Research articles in the literature on phytate are mostly related to food nutrition and quality. This is because phytate is a strong chelating agent, which could render availability and nutritional value of trace elements in food. Phytate is often quantified using a high performance liquid chromatography (HPLC) system in food science (i.e. de Boland et al., 1975; Maga, 1982). In soil science, limited research on organic P was reported in the 1970s (i.e. Anderson et al., 1974; Dalal, 1977). Organic P account for at least 50% of total soil P based on indirect subtraction method (Dalal, 1977). The major components of soil organic P are in the forms of inositol- penta- and hexa-phosphates (phytate and its derivatives) (Dalal, 1977; Anderson, 1980). This suggests that phytate is fairly stable in the soil environment. However, early studies indicated that phytate could be quite available for plant uptake in some soils while totally unavailable in others. Infrared spectroscopy studies revealed that P adsorption in soil occurs over a wide range pH from 3.3 to 11.9, and is especially high at low pH levels (Nanzyo and Watanabe, 1982). In calcareous soils, formation of Ca-P complexes reduces P solubility in soils and renders it unavailable to crops. Although excess P in soil systems may not benefit crop production, this excess P may not be a threat to the environment. Mechanisms by which phytate accumulates in soil are still unknown as well as the extent that phytate in soil is available to plants (Anderson et al., 1974). With the increasing concern of the environmental risk relating to land application of animal waste, it is important to illustrate and address some of the fundamental science behind the observed phenomenon and to evaluate phytate mobility and transformation in soil.

Objectives

- (1) To extract, detect and quantify phytate added to soil;
- (2) To evaluate transformation and mobility of phytate and manure-phytate in soil under laboratory and field environments; and
- (3) To reveal whether manure application led to increase in total soil P.

Materials & Methods

1. *Extraction and detection of soil phytate.* Two Oklahoma soils (from the two field experiments described below) were selected for this laboratory evaluation. One soil is acidic with pH around 5.0 (Magruda soil) and the other alkaline with pH around 7.8 (Panhandle soil). Two extractants, 0.5 M NaHCO₃ and 0.05 M CaCl₂ were tested. Briefly, a 10 g air-dried soil sample was placed in a 50 ml Erlenmeyer flask, treated +/- 1 mL Na-phytate (Sigma-Aldrich Co. P-3168) solution (100 mg phytate L⁻¹ with 20.11% P), and then extracted soil P by adding 200 mL of 0.5 M NaHCO₃ and shaking for 30 min. Extracts were obtained by filtering through a Whatman no 42 filter paper. An aliquot was analyzed for P concentrations using inductively coupled plasma spectrometer (ICP) or the Murphy-Riley colorimetric detection method (Murphy and Riley, 1962) following adjustment of extract pH. Three laboratory replications were conducted for each extract. The Murphy-Riley colorimetric method quantifies mostly inorganic P, while ICP method provides an estimate of total P in the solution. This procedure is similar to that used for the extraction and quantification of inorganic P in soil (Olsen and Sommers, 1982). Fractionation of organic P is needed if adding phytate to soil increased solution P concentrations.

2. *Transformation of phytate in soil.* The underlying hypothesis is that the added phytate in soil would break down by soil microorganisms during incubation, which would lead to increase in soil extractable P. The two selected soils (10 g) were treated with phytate (1 mL of 100 mg L⁻¹) and incubated under

aerobic conditions at 23°C and field-moisture content for 12, 24, 48, and 72 hours. Following incubation at the time and conditions specified, soils were extracted with 0.5 M NaHCO₃ and P in the extracts were determined as described above. Each treatment and analysis was replicated three times.

3. *Mobility of phytate in soil.* Soil leaching columns were constructed by mixing 20-g of soil with an equal weight of 20-mesh acid-washed silica sand thoroughly and transferring the mixture to a leaching tube (3.5 cm in diameter and 15 cm in length). One mL of phytate solution (100 mg L⁻¹) was added to each column and a 0.05M CaCl₂ was used to leach soluble phytate or phosphorus. Effluent from each of the leaching tubes was collected in 10 mL fractions with four fractions per collected column. Concentration of P in each fraction was determined by ICP. If total P concentrations in the effluents were higher due to addition of phytate to the leaching tubes, phytate and its degradation derivatives, including tri-, tetra-, penta-, and hexaphosphates, in the effluents would have been determined with HPLC with a reverse phase C-18 column using a Pulsed Electrochemical Detector (Sandberg and Ahderinne, 1986; Xu et al., 1992). The treatment and analysis was replicated three times.

4. *Manure-P accumulation and mobility in soil.* In addition to evaluating extraction, detection, and mobility of phytate in soil in the laboratory, we also evaluated the mobility and accumulation of phytate (more specifically manure-P at this point) in two long-term manure-applied field experiments, a continuous corn (*Zea mays* L.) field experiment that was initiated in 1995 in western Oklahoma, and a continuous winter wheat (*Triticum aestivum* L.) experiment located in central Oklahoma that was initiated in 1892. Sixty soil samples were taken from each of the two field experiments.

For the continuous corn experiment, soils have been applied swine effluent every year for the past 11 years at three different rates, 56, 168, and 504 kg N ha⁻¹ (referred to as Low, Medium, and High, respectively). There are three field replications for each treatment. These soils are Richfield (fine, smectic, mesic Aridic Argiustolls) clay loam, have a mean particle-size distribution of 30% sand, 42.5% silt, and 27.5% clay. Soil pH values are about 7.3 and organic carbon around 1.2% (data not shown). Each soil sample was a five core composite sample taken to depth 0-15, 15-30, 30-60, and 60-120 cm. Basic soil analyses included soil moisture, soil pH, soil texture, and soil organic carbon content. Total P and Mehlich-3 extractable P in some of these soils were also determined. As catalysts, microbial biomass (carbon and phosphorus) and activity of four phosphatase enzymes were quantified. Due to limited understanding on the fate and transformation of manure-P in the soil environment, we included several additional experiments to reveal the underline changes of P-cycling related activities in swine effluent treated soils. Contents of microbial biomass carbon and phosphorus and activities of four phosphatase enzymes were determined because these parameters served as catalysts in the conversion of organic P to inorganic P.

The continuous winter wheat experiment was initiated in 1892 with the manure treatment plot. Fertilizer treatment plots for this experiment, including NP and NPK, were initiated in 1929. The check plot has not been treated with fertilizer or manure for over a century, and served as a control for comparison. The experiment was initiated on a Kirkland (fine, mixed, thermic Udertic Paleustolls) silt loam with a mean particle-size distribution of 37.5% sand and 22.5% clay. Cattle manure from a feedlot was applied every four years at 269 kg N ha⁻¹ and was incorporated into soil immediately following application to reduce potential surface runoff. The average ratio of N:P of the applied manure is 3.3:1, which suggests that approximately 89.7 kg manure-P ha⁻¹ was applied every four years (22.4 kg P ha⁻¹ yr⁻¹). Chemical fertilizer plots received an annual application of 67 kg N, 14.6 kg P, and 28 kg K ha⁻¹ before planting in October. Long-term experiments could potentially provide considerable insights on the impacts of management practices on soil. Unfortunately, application of statistics on agricultural experiments was not yet in place at the time that the treatments in this study were initiated. To compensate for the no-replication restriction, the underlying changes in these soils were evaluated based on three random sample cores. Soil samples were taken in October of 2006 at 0-15, 15-30, 30-60, 60-120, and 120-180 cm depth.

Analyses for organic C, total N and total P were conducted using air-dried samples with particle size less than 180 μm . Soil pH and particle-size distribution were determined with air-dried samples that were less than 2-mm. The C_{mic} content, dehydrogenase activity and phosphatase activity were determined using the < 2-mm field-moist samples that were stored at 4°C. All results are expressed on a moisture-free basis. Moisture was determined after drying at 105°C for 48 h. Significant differences among treatments were determined using one-way analysis of variance (ANOVA). Comparison of treatment means was done using the least significant difference (LSD) test. All results reported are averages of duplicated assays and analyses.

Results

1. Laboratory study

As shown in Figure 1, Na-phytate was not recovered from the two selected Oklahoma soils by immediate NaHCO_3 extraction following addition of Na-phytate to the two selected soils. Theoretically, addition of Na-phytate at the concentration tested would increase solution P by 2 mg kg^{-1} soil. The Murphy-Riley colorimetric method quantifies mostly inorganic P, while ICP method provides an estimate of total P.

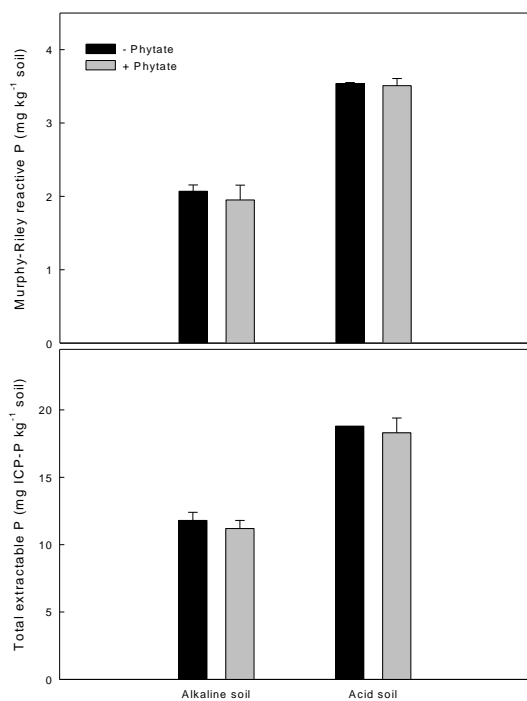


Fig. 1. Phytate solution was or was not added to an acid or alkaline soil. Phosphorus in the soil was extracted immediately using 0.5 M NaHCO_3 and shaking for 30 min. Extracts were analyzed for P concentrations using the Murphy-Riley colorimetric detection method (mostly inorganic P) and inductively coupled plasma spectrometer (ICP) (for total P).

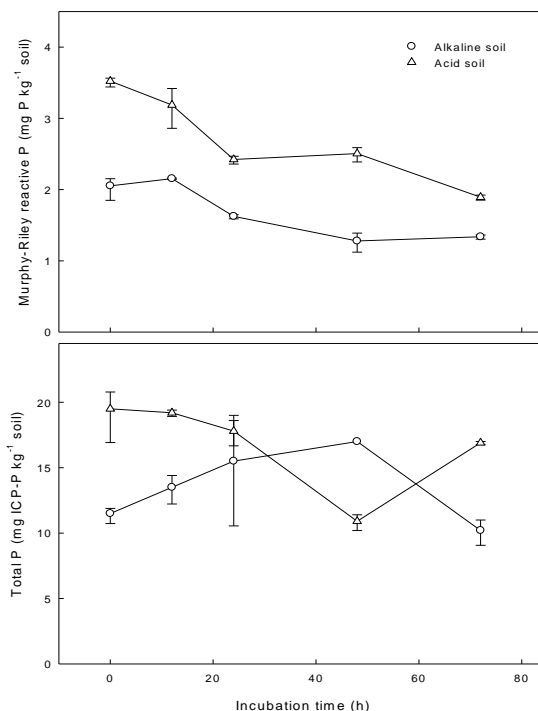


Fig. 2. Soil was treated +/- Na-phytate solution, and then incubated for time specified. Phosphorus in the soils were extracted by 0.5M NaHCO_3 , and quantified by the Murphy-Riley colorimetric detection method and ICP for total P.

We concluded that Na-phytate, though easily soluble in water, was not extracted from the soil by 0.5 M NaHCO_3 .

We hypothesized that phytate in soil would be degraded with time following addition to soil, thus increasing extractable soil P concentrations. Phytate solution was added to the two selected soils and incubated for different times up to 72 hours. Phosphorus was extracted using 0.5 M NaHCO₃ and quantified by the Murphy-Riley method and ICP. Data obtained suggested that phytate addition did not increase extractable soil P with time (Fig. 2). On the contrary, extractable P decreased slightly with incubation in both soils. This suggested that a portion of extractable P was converted to a non-extractable form during the incubation.

In subsequent studies, mobility of phytate in the two selected soils was evaluated. Based on data obtained in previous experiments, we decided to quantify effluent P by ICP only because this quantifies total P in the solution. Phytate and its degradation intermediates would be evaluated if this experiment showed that phytate leached out of the soil columns. During the leaching experiment, sodium in the phytate solution dispersed soil particles, making the leaching process slow for the phytate added to the column. Results obtained are summarized in Table 1.

Table 1. Phosphorus concentration (mg P L⁻¹) in 0.05 M CaCl₂ effluents leached out of soil

Soil type/ fraction number	Control	phytate added
Acid soil		
1	0.60	0.64
2	0.15	0.22
3	0.05	0.09
4	0.06	0.13
Alkaline soil		
1	0.41	0.28
2	0.18	0.18
3	0.14	0.13
4	0.14	0.09

Theoretically P concentration in the first effluent fraction could be as high as 2 mg P L⁻¹. Concentrations of P in the effluents of the phytate-added column that was made of the acid soil were slightly higher than those of the control column. Phosphorus concentrations in the effluents of the alkaline soil columns did not increase appreciably due to addition of phytate. This experiment was repeated by using water to leach the columns. Again, P concentrations did not increase appreciably due to addition of phytate. It appeared that mobility of phytate in alkaline soil was not detectable. Limited mobility of phytate was detected in the acid soil.

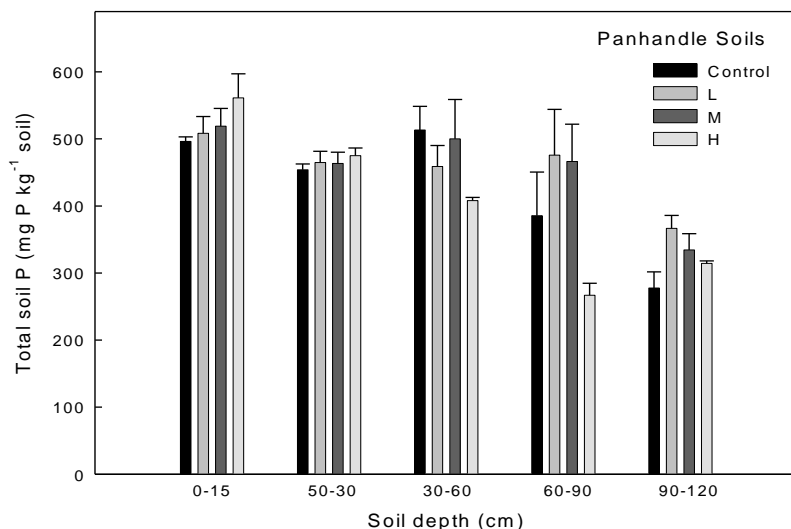


Fig. 3. Total soil P concentrations in soil sampled from a continuous corn field experiment that was initiated in 1995 in western Oklahoma. These soils have annual swine effluent application for the past 11 years at three different rates, 56, 168, and 504 kg N ha⁻¹ (referred to as Low, Medium, and High, respectively).

In summary, laboratory extraction, incubation, and leaching studies indicated that mobility of phytate in soil is low and addition of phytate to soil did not appreciably increase extractable soil P. Leaching potential of phytate-P or phytate degradation intermediates is limited, especially in alkaline soils. These findings suggest that phytate-P would likely accumulate in soil. Therefore, P accumulation in manure treated field soils was evaluated.

2. Field evaluation

For this study, we took soil samples up to 120 cm for one site and 180 cm for the other. Soil total P levels in the surface 0-15 cm increased following 11 years annual application of swine effluent at three different rates (Fig. 3). Soil total P levels at depth 15-30 were not affected by swine effluent application. At soil depth 30-60 cm and 60-90 cm, soil total P levels decreased significantly by high rates of swine effluent application. Deeper than 90 cm, soil total P levels were not affected significantly by swine effluent application. Similar trends were obtained for the levels of Mehlich-3 extractable P (data not shown). The highest Mehlich-3 P level was detected in the surface soils treated with high rates of swine effluent, which was approximately 50 mg P kg⁻¹ soil. This level was about 1.6 times of that detected in the surface soils from the control. However, the Mehlich-3 P contents in soils treated with low and medium rates of swine effluents were not significantly higher than those in the control soils. The obtained results were somewhat surprising, so the analyses were repeated three times.

Microbial biomass is the driving force of nutrient conversion in the soil ecosystem. Application of swine effluent reduced microbial biomass C and P contents in soils, though this reduction was not statistically significant (Fig. 4). In general, microbial biomass decreased with increasing soil depth. Biomass of microorganisms was detected mostly in the surface 30 cm depth of the soil profiles. As an indicator of microbial community structure and composition, microbial biomass C/P ratios were significantly higher in the surface soils (Table 2). High rates of swine effluent slightly reduced microbial biomass C/P ratios, suggesting microbial composition shifted toward a more bacterial dominating community. Phosphatase enzymes are responsible to convert organic P to inorganic P (available for plant uptake when soluble).

When compared with

the control soils, the activities of alkaline phosphatase and inorganic pyrophosphatase were significantly greater in soil treated with swine effluent, while the activity of acid phosphatase was not affected, and activity of phosphodiesterase was significantly lower in soils treated with swine effluent (Fig. 5).

These results suggested that annual application of swine effluent for over 11 years at rates evaluated did not promote accumulation of Mehlich-3 P or total P in these soils. Based on data obtained so far, there was no clear trend of P movement over a soil profile. Reduction of microbiological activities by swine effluent addition was detected, though this reduction was not statistically significant. Swine effluent application also led microbial community structure to shift toward a more bacterial dominating community with a greater proportion of P accumulated in the microbial biomass. The results obtained will be further confirmed by additional ongoing studies, and will be useful to guide understanding about the impact of long-term swine effluent application on cycling, accumulation, and mobility of manure P in soil.

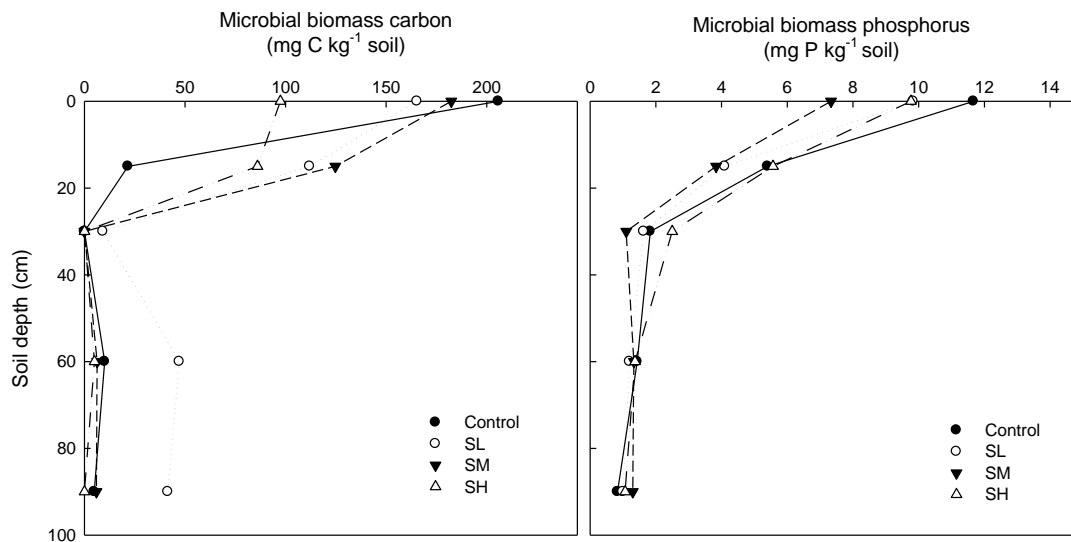


Fig. 4. Microbial biomass carbon and phosphorus in swine effluent applied soils at depth from 0-90 cm deep. Con=Control and S=Swine effluent. Application rates were L=Low (56 kg N ha⁻¹ yr⁻¹), M=Medium (168 kg N ha⁻¹ yr⁻¹), and H=High (504 kg N ha⁻¹ yr⁻¹).

Table 2. Average carbon to phosphorus content ratio in soil microorganisms. S=Swine effluent. Application rates were L=Low (56 kg N ha⁻¹ yr⁻¹), M=Medium (168 kg N ha⁻¹ yr⁻¹), and H=High (504 kg N ha⁻¹ yr⁻¹).

Soil depth (cm)	Microbial biomass C/P ratio in soils specified			
	Control	SL	SM	SH
0-15	17.64	16.82	24.87	9.97
15-30	4.01	27.31	32.54	15.45
30-60	4.36	5.66	4.73	3.2
60-90	7.03	3.93	4.75	3.64
90-120	5.84	4.15	4.59	1.88

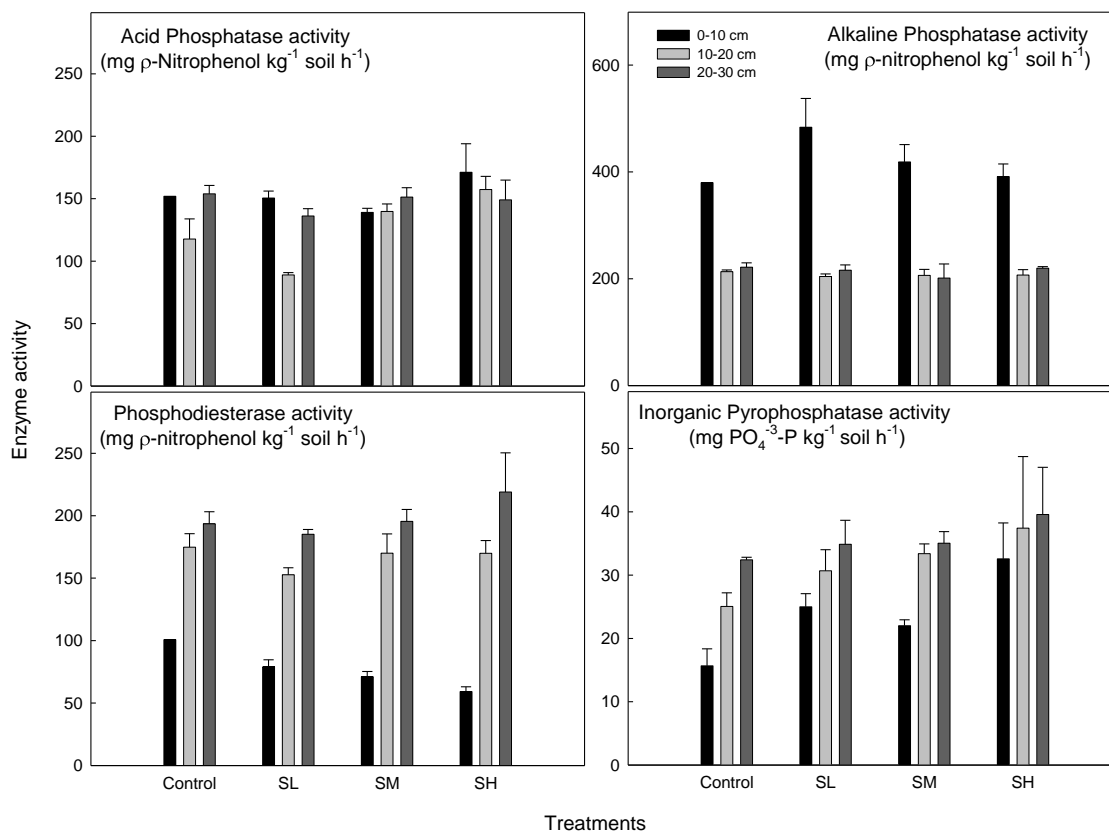


Fig. 5. Effect of different levels of swine effluent on activities of acid phosphatase, alkaline phosphatase, phosphodiesterase, and inorganic pyrophosphatase in soils at depths from 0-30 cm. Bars indicate standard error. S=Swine effluent. Application rates were L=Low (56 kg N ha^{-1} yr^{-1}), M=Medium (168 kg N ha^{-1} yr^{-1}), and H=High (504 kg N ha^{-1} yr^{-1}).

The obtained results were somewhat surprising. Similar evaluation was conducted in another long-term field experiment. Although manure used in this study was cattle manure (not swine effluent), there are common features on the impact of manure to the soil ecosystem. Moreover, this field study was established over 100 years ago. The detected changes in these soils would likely be due to long-term impact of management practices other than short-term changes due to other environmental variables. Moreover, in a previous study, we found that approximately 80% of chemical fertilizer-P applied over the course of 70 years was either recovered in the harvested grain or still remains in the top 30 cm soil depth, while only 32% of manure-P was recovered (Parham et al., 2002). We concluded that manure-P must have been mobilized to deeper soil profiles (below 30 cm depth) because there was limited P loss from runoff and leaching at this site.

For this study, we took soil samples up to 180 cm depth. Data obtained are shown in Figure 6. Again, significant P accumulation due to long-term manure application was not detected.

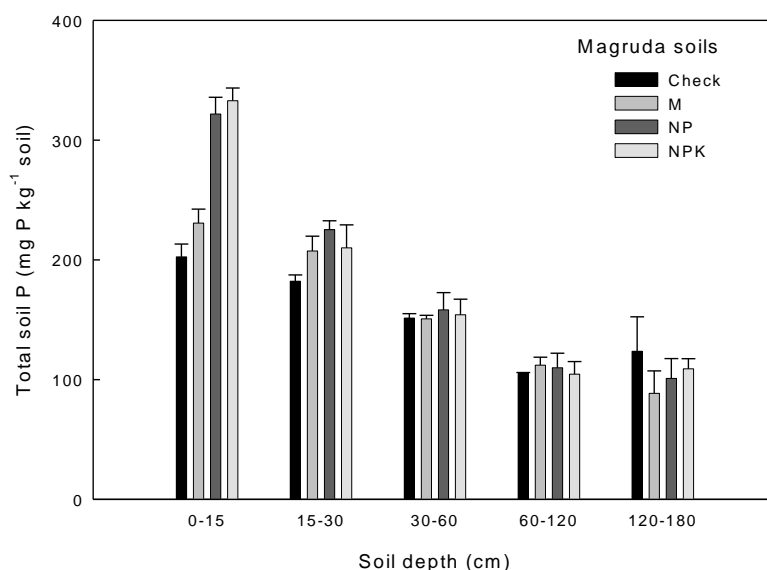


Fig. 6. Soil samples were taken from a century-long continuous winter wheat experiment located in central Oklahoma. The experiment was initiated on a Kirkland silt loam. The manure treatment plot was initiated in 1899. The chemical fertilizer treatment plots were initiated in 1929. There are six plots still under investigation, including manure, P, NP, NPK, NPK plus lime, and an untreated control plot. For this study, soil samples were taken from four different plots as indicated in the figure.

We conducted mass balance of P based on input and output annually and cumulatively over the course of the experiment for both sites. Fortunately we have great data support and record for these two sites. Concentrations of P in the applied swine effluent were measured every year before application. Grain yield, grain P content, and straw P content are quantified every year. Concentrations of total P in soil increased slightly in the top 0-30 cm soil in both sites due either to manure or P fertilizer application (Tables 3 and 4). Soils at 30-60 cm depth had P levels similar to or lower than the control in the two sites tested. When accounted all P in 0-120 cm depth soil profile, almost 100% of all chemical P added to the system could be accounted (Table 4). It appears that some of the applied manure-P was lost from the system (Tables 3 and 4).

Table 3. Phosphorus input/output (after subtracting the control) from soil that were treated with swine effluent in the continuous corn experiment.

Treatment	P increased (+) or decrease (-) in soil depth (cm) specified						P input in 11 yrs	P removal in corn from treatment in 11 yrs	P loss from the system in 11 yrs
	0-15	15-30	30-60	60-90	90-120	0-120			
	kg P ha ⁻¹								
Control	0	0	0	0	0	0	0		
SL	24	22	-220	366	360	552	411	30	-171
SM	46	19	-53	327	230	570	1232	-11	673
SH	131	43	-426	-480	148	-584	3696	48	4231

Swine effluent was applied at three different rates every years. The average N:P ratio in the applied manure was 3.2. Average yield and average corn P from 1991 to 2006 were used in the calculation. Conversion was based on 1 ha = 3.975 x 10⁶ kg per 15 cm soil.

Table 4. Phosphorus input/output (after subtracting the control) from soil treated with cattle manure and chemical fertilizers in the continuous winter wheat experiment.

Treatment	P increase (+) or decrease (-) in soil depth (cm) specified						P input in 70 yrs	P removal in grain from treatment in 70 yrs	P loss from the system in 70 yrs
	0-15	15-30	30-60	60-120	120-180	0-120			
	----- kg P ha ⁻¹ -----								
Control	0	0	0	0	0	0	0		
M	309	158	27	316	-371	810	1568	191	567
P	565	223	-44	207	-262	952	1022	64	6
NPK	535	200	-22	94	-82	806	1022	253	-38

Cattle manure was applied every four years at 269 kg N ha⁻¹. The average N:P ratio in the manure was 3:3, suggesting an annual application rate of 22.4 kg P ha⁻¹. Inorganic fertilizers were applied annually in September. Average yield for 1948-1998 and average grain P for 1991-1995 were used in the calculation. Conversion was based on 1 ha = 3.975 x 10⁶ kg per 15 cm soil.

Discussion

Although phytate-P may be a main form of manure P and studies showed that manure P was more mobile in the environment than inorganic fertilizer P, laboratory extraction, incubation, and leaching studies indicated that mobility of phytate in soil was low and addition of phytate to soil did not appreciably increase extractable soil P. The obtained results are consistent with observations from field experiments (Parham et al., 2002). The availability of manure-P was significantly lower than that of the inorganic fertilizer-P, evidenced by significantly lower P recovered in grain from manure treatment (12%) in comparison with those from chemical fertilizer treatments (23 to 31%). Therefore, leaching potential of phytate-P or phytate degradation intermediates would be limited. These findings suggest that phytate-P would likely accumulate in soil. However, annual swine effluent application in the field at three different rates for 11 consecutive years did not lead to significant accumulation of total P in soil. Small accumulation of P was observed in the surface 0-30 cm soil profiles, but lower P levels than those of the control soils were observed in 30-60 cm soil profiles. Similar results were obtained in another long-term winter wheat experiment that had received cattle manure application every four years for over a century. Mass balance of P input and output from the systems at both field sites suggested that some added manure-P was not recovered from the system, even though all P in 0-120 cm depth of soil profiles were accounted. Interestingly, almost 100% of all chemical fertilizer P added to the system in the course of 70 years was recovered. This concurs with our previous report that inorganic P in soil is essentially immobile, while organic P is somewhat mobile. However, the unaccounted manure P in the previous studies (Parham et al., 2002) was not found in the deeper soil profiles as suspected. It seemed that some added manure P was lost from the system, and P loss from the system was marked higher in soils receiving higher rates of manure application. These findings deserve special attention with targeted research effort for further confirmation. The obtained results suggest that long-term manure application may not lead to significant P accumulation in the soil ecosystem. Therefore, there would be limited potential for the swine effluent P to reach adjacent water bodies and cause eutrophication.

On the other hand, manure application altered microbiological activities, P cycling, and provided adequate nutrients for crop production. The enhanced microbiological activity by long-term manure treatment was also evidenced by increased enzyme activity. After a century long continuous winter wheat experiment, wheat yield in 64 years (1930-1994) was not significantly different between the manure-treated plot and plots treated with chemical fertilizers (Boman et al., 1996).

In summary, annual swine effluent application in western Oklahoma for 11 consecutive years did not lead to significant accumulation of soil phosphorus. Similar trend was shown in a century-old field experiment with manure application in central Oklahoma. Although phytate is the main phosphorus form in animal feed, has little nutritional value for monogastric animals, and was suspected to be responsible for high P levels in animal manure, laboratory studies showed that phytate added to soil did not stay in the solution and had limited mobility in soil columns that were constructed with acidic or alkaline soil. Therefore, solution phosphorus levels in soils were not increased appreciably by the addition of phytate to soil. This implies that manure-phosphorus would have limited mobility in the environment. Manure application, however, did enhance soil biological activities and the capacity of soil to retain and cycle nutrients. Long-term manure application in central and western Oklahoma sustained agricultural production and did not result in P accumulation to levels close to those in inorganic P fertilizer-treated soils.

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