RESEARCHREPORT

ENVIRONMENT

Title: Land application of animal manure and phosphorus cycling in soil.
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Abstract

Repeated land application of animal manure at rates to satisfy N requirements for crops may increase soil P to levels that cause environmental concern. Biochemical and microbiological parameters are important factors dictating P transformations in soil. Studies were conducted to investigate effects of animal manure application on activities of enzymes involved in P cycling in soils under a long-term continuous wheat experiment. Treatments included manure, P, NP, NPK, and NPK plus lime. Animal manure was applied every four years at 269 kg N ha^{-1} (approximately 103 kg P ha^{-1}) for over a century and chemical fertilizers were applied every year at 67 kg N, 14.6 kg P, and 28 kg K ha^{-1} for 69 years. The highest Mehlich-3 extractable P was found in soils treated with P, followed by NP, NPK, NPKL, and manure. The activities of enzymes involved in phosphorus transformations in soil, including alkaline phosphatase, inorganic pyrophosphatase, and phosphodiesterase, were significantly higher in soils treated with manure. Microbial biomass C and total microbial activities were also the highest in manure-treated soils. Results suggested that long-term application of manure at 269 kg N ha^{-1} every four years promoted biological activities and P cycling, but did not result in accumulation of excessive Mehlich-3 extractable P in soil.

Introduction

Numerous reports demonstrated that repeated application of animal manure led to accumulation of phosphorus (P) in soil that could result in soil P testing exceeding 220 lbs/acre to 1000 lbs/acre. Soil P testing is generally 65 lbs/acre or less to observe crop response to P fertilizer. Soil P testing of 120 lbs/acre is identified to be the crop responding index considering spatial variation in the field. Animal manure application leads to soil P levels exceeding crop response range. Thus, several states are calling for regulating manure land application based on P level in the soil and adjacent water bodies. However, the soil P testing value of 120 lbs/acre is an agronomic responding index. In addition, most reported data were obtained from short-term (one to two-years) field experiments. The fate of manure-P in the environment over an extended period of time is still unknown. Research data are needed to determine fate of the manure P compounds and factors affecting their mobility in the environment.

Phosphorus accumulation in manure-amended soil is mainly due to a compound called phytate (or phytic acid), a hexaphosphate ester. Phytate is an efficient P storage form. Approximately 75% of P in plant-based feeding stuff (cereal, legume, and seeds) exist as phytate-P. The release of P from phytate requires an enzyme, phytase. Phytate-P is essentially unavailable to monogastric animals, including humans, chickens, and pigs. They produce little or no phytase in the intestine and the phytate-P is excreted. In addition, inorganic phosphate is added in the animal feed for P nutrition. Attempts to decrease the amount of P being excreted from animal production systems have been to supplement diets only to required P levels, or to add microbial derived exogenous phytase enzymes to increase the P digestibility of grains, or use genetically engineered low-phytate maize as the feed.

Extensive research has been conducted in understanding P nutrition in animal production. Little effort has been made to understand the fate of P in animal manure following long-term application to the field. Although excess P in soil systems may not benefit crop production, this excess P may not be a threat to the environment. In order to determine the fate of manure-P in the environment, it is important to understand fate of phytate-P in soil and factors affecting P transformations.

Objectives

(1) To quantify soil microbial activity and activities of enzymes involved in P cycling in soils amended with animal manure
(2) To evaluate the mobility and transformation of phytate in soil.
(3) To determine phytase activity in lagoon effluent, and soils amended with lagoon effluent and the major sources of phytase in soil; and
(4) To assess the availability of phytate to plants.

NPPC funding was used to investigate the objective (1) and initiate experiments to launch full investigation on the subject.

Procedures

Soil samples were obtained from a century-long continuous winter wheat (*Triticum aestivum* L.) experiment located in a research station at Oklahoma State University. The experiment was initiated on a Kirkland silt loam with a mean particle-size distribution of 37.5% sand, 40.0% silt, and 22.5% clay. The manure treatment plot was initiated in 1899. The chemical fertilizer treatment plots were initiated in 1929. There were six plots continued under investigation, including manure, P, NP, NPK, NPK plus lime and an untreated check. Manure was applied every four years at 269 kg N ha^{-1} (approximately 103 kg P ha^{-1}). Chemical fertilizer plots received an annual application of 67 kg N, 14.6 kg P and 28 kg K ha^{-1}.

Composite soil samples, included 18 cores per plot at 0-10, 10-20, and 20-30 cm, were taken. The field-moist soil samples were sieved through a 2-mm screen and stored at 4°C. A portion of each sample was air-dried and a portion of air-dried samples was ground to pass an 80-mesh (180 μm) sieve. Soil pH values were determined using a combination glass electrode (soil:0.01 *M* $CaCl_2$ ratio = 1:2.5), and those of the organic C and total N by dry combustion using a Carlo-Erba NA 1500 Nitrogen/Carbon/Sulphur Analyzer. Particle size distribution was determined by a pipette analysis. Phosphorus levels in water and Mehlich-3 extracts were determined by both Murphy and Riley method (MRP), and by Inductively Coupled Plasma Spectrometer (ICP-P). The microbial biomass C was determined by the chloroform-fumigation-incubation method using a k_c factor of 0.45 with subtraction of the control. Methods used for assaying phosphatases and dehydrogenase are described in page 775, Methods of Soil Analysis, Part 2, 1994.

Organic C and total N were determined using 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 microbial biomass C, activities of dehydrogenase and phosphatases were determined using the < 2-mm field-moist samples. All results are expressed on a moisture-free basis. Moisture was determined after drying at 105^{o}C for 48 h. Statistical analyses were performed using Statistical Analysis System (SAS). All results reported are averages of duplicated assays and analyses.

Results and Discussion

To reach objective (1), water soluble P and Melich 3 P levels in these soils were determined because these P levels are commonly used as indexes of soil available P. Enzymes are catalysts for biochemical reactions. Conversion of organic P, such as phytate P, to inorganic P for plant uptake requires enzymes. Thus, the activities of enzymes involved in P cycling and transformations were determined. The main source of soil enzymes is microorganisms. Dehydrogenase activity was used as an indicator of total microbial activity because this enzyme is active only in active microbial cells. Microbial biomass C indicates total microbial biomass, including active and dormant microbial cells.

Effects on Soil pH, Mehlich-3 extractable P, Organic C and Total N

The pH values in the tested 0-10 cm surface soils ranged from 4.2 to 5.7 with that of the untreated check soil around 4.8 (Fig. 1). Manure application increased soil pH while chemical fertilizer application resulted in lower soil pH (Fig. 1). The pH values from manure-treated soils were approximately 0.9 units higher than that of the untreated check soil. With the exception of the P-treated soil, the pH values in soils treated with chemical fertilizers were 0.3 to 0.6 pH units lower than that of the check soil. Thus, there were 1.2 to 1.5 pH unit differences between manure- and chemical fertilizer-treated soils.

The levels of organic C and total N were the highest in 0-10 cm surface soils treated with NPKL, followed by NPK, NP, and manure (Fig. 1). These levels were significantly higher in soils treated with P than those of the untreated check soil. Organic C in the 0-10 cm surface soils ranged from 6.7 to 9.7 g C kg^{-1} soil (Fig. 1).

The pH values in manure treated soils were almost uniform in the soil profiles at 0 to

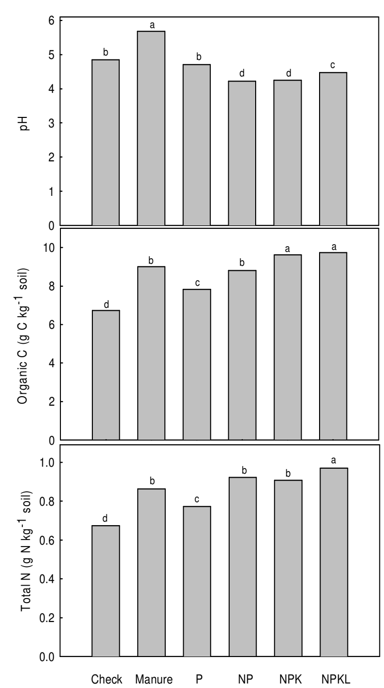

Fig. 1. Effect of long-term animal manure and chemical fertilizer applications on pH values, organic C content, and total N content of surface soils (0-10 cm). Different letters indicate significantly different means at $P < 0.05$ according to least significant difference test.

30 cm depth (Fig. 2). With the exception of lime and manure treated soils, there were little differences in pH values among soils at 20-30 cm. Liming increased pH value of the lower soil profile (20-30 cm) in the NPK-treated soil to a value close to that in the manure-treated soil.

Greater variations of organic C contents among the treatments were shown in the surface soils when compared with those in the subsurface soils (Fig. 2). Organic C in soils treated with NPK or NPKL decreased dramatically in the subsurface soil and fell below that in the manure treated soils (Fig. 2).

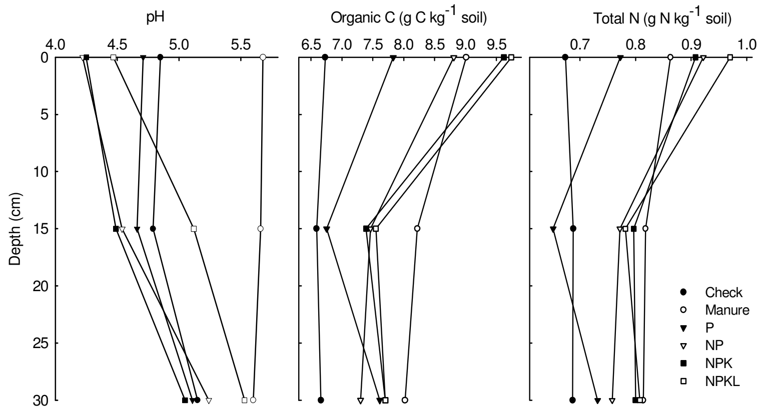

Fig. 2. Effect of long-term animal manure and chemical fertilizer applications on distribution of pH values, organic C and total N over soil profile 0-30 cm.

Water soluble P levels in these soils tested with Murphy and Riley method (1962) were low and did not exceed 1 mg P kg^{-1} soil with the exception of P- treated surface soils (0-10 cm) which was about 1.5 mg P kg^{-1} soil (data not shown). The levels of MRP in Mehlich-3 extracts varied significantly among the soils tested. The highest MRP levels were detected in 0-10 cm surface soils treated with P, which was approximately 115 mg P kg^{-1} soil (Fig. 3A). This level was almost 6 times of that detected in the surface check soil. Interestingly, MRP in the manure-treated soil was significantly lower than those in soils treated with chemical fertilizers. The level of MRP in the check soil was the lowest, which did not exceed 20 mg P kg^{-1} soil even in the top 0-10 cm surface soil (3A).

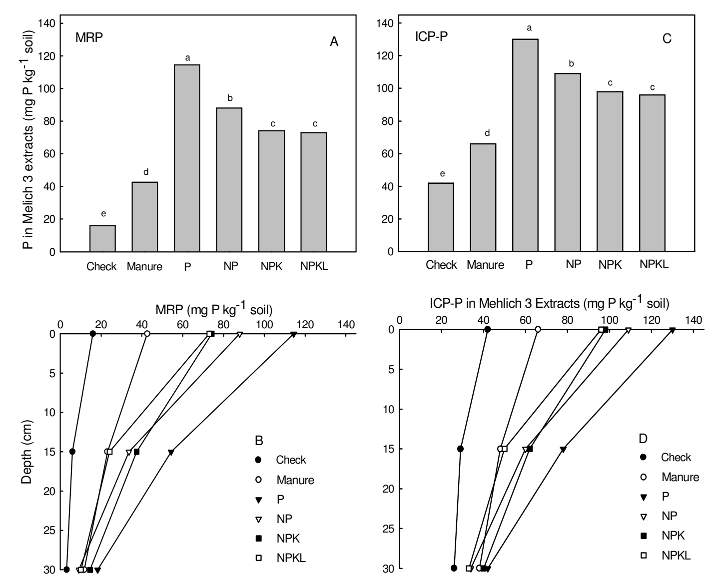

Fig. 3. Effect of long-term animal manure and chemical fertilizer applications on (A & C) MRP and ICP-P levels in surface 0-10 cm soils, and (B & D) distribution of MRP and ICP-P over soil profile 0-30 cm. Different letters in A indicate significantly different means at $P < 0.05$ according to least significant difference test.

The level of ICP-P in Mehlich-3 extracts varied among the soils tested with a trend similar to that of MRP (Fig. 3C). The distributions of MRP and ICP-P in soil profiles 0 to 30 cm were also similar, but both differed from those of pH, organic C and total N (Fig. 3B and D). In general, most of the variations in P contents were in the surface 0 to 10 cm.

When expressed MRP as percentages of ICP-P in Mehlich-3 extracts, this value varied among the soils, ranging from 38% to 88% in the 0-10 cm surface soils with the highest in the P-treated soil and the lowest in the untreated check soil (Table 1). In the 20-30 cm check soil, only 12% of ICP detectable P in Mehlich-3 extracts can be detected with Murphy and Riley method. This ratio ranged from 75% to 81% in other chemical fertilizer-treated surface soils and was 65% for manure-treated surface soil.

Table 1. MRP expressed as percentages of ICP-P†.

	Soil depth (cm)			LSD
Treatment	0 - 10	10 - 20	20 - 30	$P < 0.05$
	%			
Check	38	21	12	0.7
Manure	65	48	31	4.3
P	88	70	44	2.5
NP	81	56	27	4.9
NPK	76	60	37	4.1
NPKL	76	48	31	2.5
LSD $P < 0.05$	3.0	4.1	2.4	

† MRP and ICP-P are P concentrations (mg P kg^{-1} soil) in Melich-3 extracts determined by Murphy and Riley method and by Inductively Coupled Plasma Spectrometer (ICP), respectively.

Effect on Activities of Phosphatases

Among the soils tested, the activities of alkaline phosphatase, inorganic pyrophosphatase and phosphodiesterase were significantly greater in the soil treated with animal manure, while the activities of acid phosphatase were significantly greater in soils treated with chemical fertilizers (Fig. 4). The highest acid phosphatase activity was detected in the soil treated with NP, giving an average of 373 mg □-nitrophenol released kg^{-1} soil h^{-1}, followed by soils treated with NPK, NPKL, and P (Fig. 4). Acid phosphatase activities were similar in manure-treated and check soils. Activity of alkaline phosphatase in the manure-treated soil was more than 2-fold of those in the

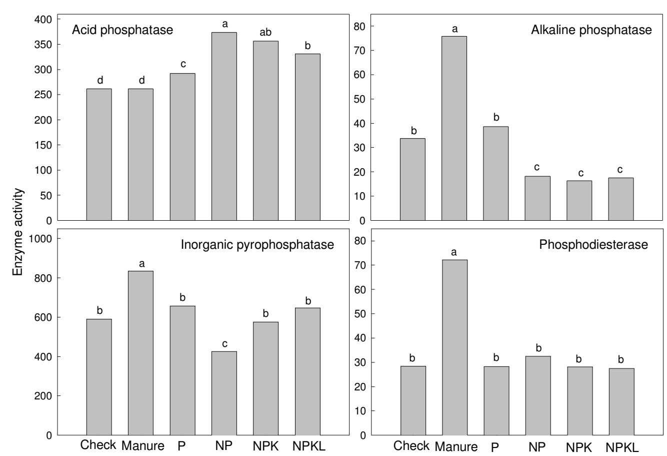

Fig. 4. Effect of long-term animal manure and chemical fertilizer applications on the activities of acid phosphatase (mg ρ-nitrophenol kg^{-1} soil h^{-1}), alkaline phosphatase (mg ρ-nitrophenol kg^{-1} soil h^{-1}), inorganic pyrophosphatase (mg PO_4^{-3} kg^{-1} soil h^{-1}), and phosphodiesterase (mg ρ-nitrophenol kg^{-1} soil h^{-1}) in surface soils (0-10 cm). Different letters in A indicate significantly different means at $P < 0.05$ according to least significant difference test.

check or P-treated soil, and exceeded 4-fold of those in the soils treated with NP, NPK, or NPKL (Fig. 4). Activity of inorganic pyrophosphatase was the lowest in the NP-treated soil, which was less than half of that in the manure-treated soil (Fig. 4). Activities of phosphodiesterase averaged around 29 mg □-nitrophenol released kg^{-1} soil h^{-1} for the soils tested with the exception of the manure-treated soil which showed more than twice the activity as the other soils (Fig. 4).

In the lower soil profiles (20 to 30 cm) tested, activities of phosphatases were always the highest in the manure-treated soil (Fig. 5). In addition, activities of enzymes involved in P cycling were generally more uniformly distributed over a 0-30 cm soil profile in the manure-treated soil (Fig. 5).

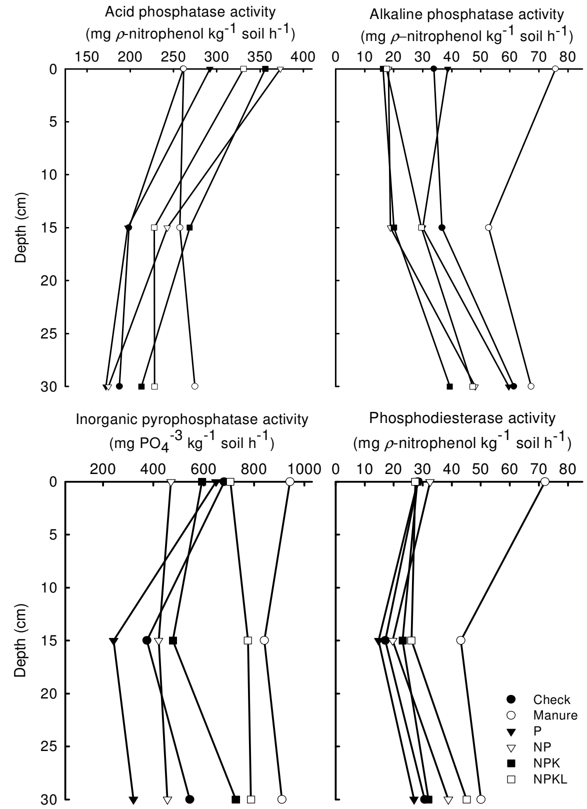

Fig. 5. Effect of long-term animal manure and chemical fertilizer applications on the activities of acid phosphatase, alkaline phosphatase, inorganic pyrophosphatase, and phosphodiesterase over soil profiles 0-30 cm

Effect on Microbial Biomass C and Dehydrogenase Activity

Microbial biomass C contents were the highest in the 0-10 cm surface soil treated with manure and the lowest in those of check and P-treated soils (Fig. 6). A similar trend was found in the lower soil profiles (20 to 30 cm, Fig. 6B). Microbial biomass C contents decreased with increasing soil depth in all the soils tested.

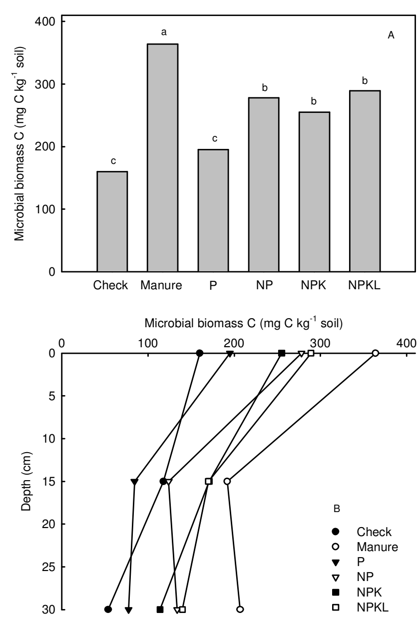

Fig. 6. Effect of long-term animal manure and chemical fertilizer applications on (A) microbial biomass C in surface soils (0-10 cm), and (B) distribution of microbial biomass C over soil profile 0-30 cm. Different letters in A indicate significantly different means at $P < 0.05$ according to least significant difference test.

The trends for dehydrogenase activity in the soils tested were similar to those for microbial biomass C contents (Fig. 7). The highest dehydrogenase activity was in soils treated with animal manure while the lowest was in the check soils. The activity of this enzyme is significantly higher in the surface and decreased with increasing soil depth with the exception of manure-treated soils. Interestingly, dehydrogenase activity was substantially higher in the 20-30 cm manure-treated soil than those in the 10-20 cm soil (Fig. 7B).

Discussion

One would expect that animal manure applications enrich soil organic matter and increase total N contents. Results from this study showed that soil organic matter and total N contents were actually lower in the 0-10 cm surface soil treated with animal manure when compared with some of the soils treated with chemical fertilizers. One explanation is that manure promoted biological and microbial activities, which accelerated break down of organic substances and resulted in reduced soil organic matter and total N content. The enhanced biological activities in manure-treated soils are evidenced by relatively high phosphatase activities, microbial biomass C and dehydrogenase activity.

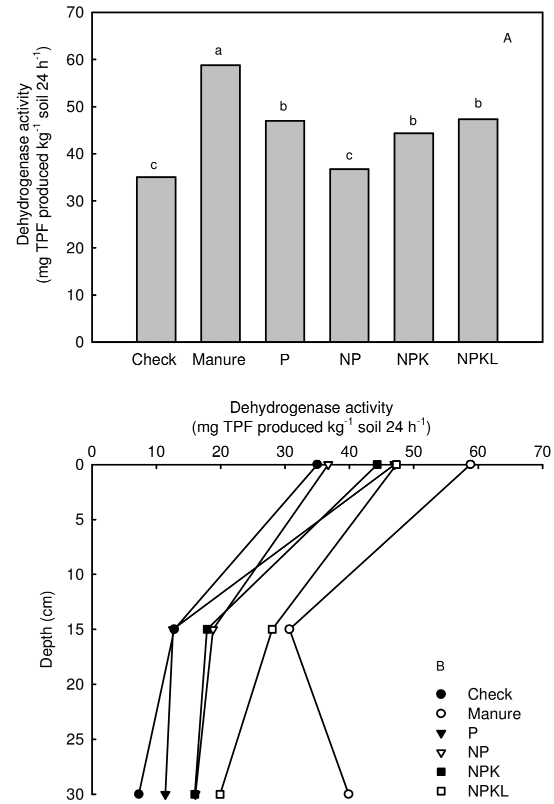

Fig. 7. Effect of long-term animal manure and chemical fertilizer applications on (A) dehydrogenase activity in surface soils (0-10 cm), and (B) distribution of dehydrogenase activity over soil profile 0-30 cm. Different letters in A indicate significantly different means at $P <$ 0.05 according to least significant difference test.

Phosphorus accumulation was observed in soils treated with P fertilizer alone. This demonstrated the importance of balancing nutritional needs for crop production. Mehlich-3 P levels in soil have been found to be highly correlated with dissolved P, a potential indicator of P enrichment due to runoff. Results from this study indicated that MRP in the manure-treated surface soil was only 43 kg P ha^{-1} after applied manure at 269 kg N ha^{-1} (approximately 103 kg P ha^{-1}) every four years for over a century. The P rate in manure application is almost double of that in chemical fertilizer treatments. However, the soil test P in the manure-treated soil is only 60% or less of those in chemical fertilizer-treated soils, and is far below 247 kg ha^{-1} to 1121 kg ha^{-1} (220 lbs/acre to 1000 lbs/acre) reported in the literature. Our result is consistent with those reported by Schlegel and Alam in 1999 Annual meeting for Agronomy Society of America. They found that P accumulation was observed in soils amended with animal manure for 3 years, but not for 10 or 30 years. One possible explanation is that the microbial community adapted to the conditions with time and mobilized P compounds in the animal waste. The released P can either be taken up by plants, transported to the subsoil, or transported in water for considerable distances.

Manure application showed a greater impact over the 0-30 cm soil profile while the influence of chemical fertilizer applications were mostly on the soil surface 0 to 10 cm. This is strongly demonstrated by phosphatase activities. Distributions of soil organic C and total N contents over the soil profiles indicated movement of soluble manure compounds from the surface to lower soil profiles. Deeper impact may result in a relatively longer-term effect. Poultry manure applied to a rice field at 120 or 180 kg N ha^{-1} showed a residual effect on wheat, which followed rice, and this residual effect was equivalent to 40 kg N ha^{-1}. The different trend for acid phosphatase activity in these soils may be accounted for by changes in soil pH. The highest pH value in soils tested did not exceed 5.25.

The higher microbial biomass C contents and dehydrogenase activity in manure-treated soils at 20-30 cm depth than those at 10-20 cm suggest possibly higher microbial population and activities in the lower soil profiles. Similar trends were also observed in phosphatase activities.

Manure application not only altered biological activities and P cycling in soil, but also provided adequate nutrients for wheat production. After a century long continuous wheat experiment, wheat yield in the past 64 years (1930-1994) was not significantly different between the manure-treated plot and plots treated with NP, NPK, or NPKL. These results also suggested that application of animal manure at 269 kg N ha^{-1} every four years for over a century did not result in accumulation of excessive amount of Mehlich-3 extractable P, but maintained wheat yield.

In conclusion, animal manure application may promote biological activities and P cycling in soil. The results obtained from this study may be useful to guide understanding the impact of long-term application of animal manure on P cycling and transformations in soil.