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Soil phosphorus bioavailability as influenced by long-term management and applied phosphorus source

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Abstract

Soil phosphorus (P) availability may be impacted by management practices, thereby affecting plant P uptake and plant response to P amendments. The aim of this study was to determine the effects of long-term management on soil P pools and to assess the response of P bioavailability, plant growth and P uptake to mineral versus manure P treatments. Soils were collected from plots under organic (ORG), organic with composted manure (ORG+M), conventional (CONV) and restored prairie (PRA) management. Italian ryegrass (*Lolium multiflorum* L.) seedlings were grown in the greenhouse for 106 d in soils amended with varying rates of manure or mineral P. The ORG soil had lower concentrations of labile P (resin-P and $\text{NaHCO}_3\text{-P}$) compared to the CONV and PRA soils as determined by sequential P fractionation prior to planting. Ryegrass biomass (root + shoot) and shoot P uptake from soils receiving no P were significantly lower for the ORG than all other management systems. While apparent P use efficiency of the whole plant was increased by low P rate in the ORG management system, the source of applied P, manure > mineral, only influenced Olsen test P.

Keywords

Phosphorus, long term management, mineral P, manure P, apparent P use efficiency

Introduction

Long-term agricultural management can influence soil phosphorus (P) fractions and bioavailability. Since P is an essential macronutrient limiting plant productivity in many ecosystems, agricultural production is heavily dependent on P fertilizers for crop yields. Organic production systems are often perceived as more sustainable, since they avoid the use of chemical fertilizers and pesticides, but management and productivity can vary widely. Mäder et al. (2002) found a 20% decrease in crop yields in organic compared to conventionally managed systems over a 21-yr period. During this time there was a corresponding 34 to 53% decrease in fertilizer and energy consumption.

Phosphorus management in organic systems is especially challenging. Unlike N, which may be added to the soil through organic materials (e.g. manure, compost, meals, etc.) or by incorporating N-fixing legumes into the crop rotation, there are less options available for P inputs under organic certification. Rock phosphate and bone meal are mostly insoluble in the Northern Great Plains since the soils are typically alkaline and often calcareous (Arcand et al. 2010). Although manure can be an adequate source of P for maintaining yields in many organic systems, application in the Northern Great Plains region is limited by large farm size and long distances to a manure source (Lynch 2014). These limitations to P replenishment have led to lower soil test P in the organic cropping systems in both on-farm and long-term research trials (Entz et al. 2001; Knight et al. 2010; Malhi et al. 2006; Schneider et al. 2017; Welsh et al. 2009). However, corresponding plant P deficiencies have not been reported despite the continuous export of P at harvest, suggesting that the labile P pools are being replenished from less labile pools and play an important role in maintaining adequate plant available P. Soil tests are routinely used to estimate the concentration of available P in soil; however, this may not

effectively take into account the less available forms that potentially become available over the growing season. In Ontario, Schneider et al. (2016) found that total organic P and the proportion of P as orthophosphate diesters were greater in organic versus conventional dairy farm forage fields.

On the other hand, the over application of P in mineral fertilizer or animal manure treated soils has created serious environmental problems worldwide (Sharpley et al. 2001). The fate of excess P in soil is largely dependent on soil properties that may result in sorption to soil particles (McLaughlin et al. 2011; Wang et al. 2015), immobilization in microbial biomass (Bünemann et al. 2012), conversion to less available forms (MacDonald et al. 2012), or removal in runoff (Bünemann et al. 2012; Wang et al. 2010). This latter has been identified as a major source of eutrophication of water bodies (Schindler et al. 2012; Sharpley et al. 1992), causing potentially toxic algal blooms. Moreover, manure application has traditionally been applied based on recommended N fertilizer rates (Miller et al. 2011; Sharpley et al. 1997), resulting in a P supply that largely exceeds crop needs. Both the previous site management and the P source being applied should be considered to maximize plant P utilization, while minimizing potential negative environmental impacts.

Since anthropogenic practices influence soil P availability, and ultimately plant P uptake and yields, we collected soils from a long-term field experiment near Winnipeg, Canada for a greenhouse experiment. Previous studies at this location reported that the soil test P levels were below the agronomic threshold in the organic grain-forage rotation (Bell et al. 2012; Welsh et al. 2009). In comparison, total N throughout the profile (120 cm depth) did not reveal any significant differences among the alfalfa (*Medicago sativa* L.)/crop or annual crop rotations under organic or conventional management or the re-established grassland after 18 years (Bell et

al. 2012). The low long-term yields in the organic/alfalfa crop may be caused by P deficiencies, which could also affect N fixation during the alfalfa phase and subsequent availability to the wheat (*Triticum aestivum* L.) crop. A previous study in Switzerland comparing organic and conventional management systems suggested a differential response of soils from the different systems to contrasting P amendments (Oberson et al. 2010).

The aim of this study was to determine differences in P bioavailability and apparent P recovery in ryegrass among soils managed with long-term organic (ORG), organic with a one-time manure application (ORG+M), conventional (CONV) and restored prairie (PRA) when amended with varying rates of manure or mineral P. The objectives of this study were to: (1) evaluate initial operationally defined (extractable) soil P pools; (2) examine the relationships among soil test P (Olsen P), plant P uptake and yield in response to manure and mineral P amendments; and (3) determine the effect of long-term management on the apparent P recovery of manure and mineral P amendments. Ryegrass was chosen as the test plant because of its rapid growth and potential to extract excess P from the soil (Starnes et al. 2008). We hypothesized that contrasting management systems with different initial labile P levels would result in a differential crop response to rate and type (manure or mineral) of P application.

Materials and methods

Description of long-term management experiment

The study was conducted at the Glenlea farming system experiment, established in 1992, as part of the University of Manitoba Glenlea Research Station located in southern Manitoba, Canada in the Red River Valley (49°38'25"N, 97° 8'28"W). The experiment is a randomized complete block design with split plots (fully phased) and three replicates. The plots that were

used in this study were a forage-grain rotation with organic no input (ORG), organic-manure amended (applied in 2007 only; ORG+M), and conventional (CONV) management (Table 1) with a 4-yr rotation of flax-alfalfa-alfalfa-wheat (*Linum usitatissimum* L., *Medicago sativa* L., *Triticum aestivum* L.). In addition, each block included a restored prairie plot (PRA; 45 x 60 m) seeded to *Agropyron dasytachum* L., *Andropogon gerardii* Vitman, *Elymus lanceolatus* (Scribn. and Smith) Gould, *Elymus trachycaulus* (Link) Gould ex Shinnars, *Panicum virgatum* (L.), *Pascopyrum smithii* (Rydb.) A. Löve, and *Sorghastrum nutans* (L.) (Bell et al. 2012) to be used as a reference. The PRA plots did not receive any fertilizer application. The soil is a Humic Vertisol of the Scantbury and Hoddinott series with 9% sand, 26% silt and 66% clay, an average pH_{H2O} of 7.4 and 7.7% organic matter content (Welsh et al. 2009). Further details of the site and experimental design are given by Welsh et al. (2009) and Bell et al. (2012).

Soil sampling and initial soil P analysis

In May 2011, 20 soil samples (0-15 cm) were collected randomly from across each plot and pooled prior to the wheat phase for initial soil chemical analysis and soil P fractionation. The soils were air-dried and finely ground (<1 mm) prior to analysis. Additional bulk soil (0-15 cm depth) was collected (~12.0 kg per plot), dried and mechanically ground (< 4 mm) for the greenhouse study. The soils for this trial were pooled together and mixed to make one composite sample for each management system.

The soil samples (0.5 g) were sequentially extracted in duplicate to fractionate total P into molybdate-reactive P (MRP) and molybdate-unreactive P (MUP) pools [total P (Pt) = MRP + MUP] differing in bioavailability using a modified procedure by Hedley et al. (1982), where the microbial P extraction was eliminated. Although the reactive P is mostly orthophosphate, it may

contain some organic compounds and the unreactive P may include inorganic polyphosphates (Shand et al. 2000). These fractions have been operationally defined with decreasing availability potential as: (1) Resin P- P_{MRP} , exchangeable with solution; (2) 0.5 M $NaHCO_3$ (pH 8.5) P_{MRP} and P_{MUP} , sorbed on soil minerals and some microbial P; (3) 0.1 M NaOH- P_{MRP} , P_{MUP} , associated with Fe and Al oxyhydroxides; (4) 0.1 M NaOH after ultrasonification- P_{MRP} , P_{MUP} , removing P at internal surfaces of aggregates (sNaOH P_{MRP} and P_{MUP}); (5) 1 M HCl- P_{MRP} apatite mineral associated with Ca and some occluded P and (6) residual P by dry ashing. To determine MRP in the extracts, an aliquot was first acidified with 3 M H_2SO_4 and molybdate-reactive P was measured colorimetrically with an UV/vis spectrophotometer 6405 (Jenway, Staffordshire, UK) by acid-molybdate reaction at 880 nm (Murphy and Riley 1962). Total P was determined on an aliquot of the extract by autoclave digestion with ammonium persulfate and 0.9 M H_2SO_4 , with MUP calculated as the difference between total P and MRP.

Bicarbonate extractable P (Olsen-P) was determined on the soil in a 1:20 ratio with 0.5 M $NaHCO_3$ (pH 8.5) and shaken for 30 min (Olsen et al. 1954). Olsen-MRP and Olsen-MUP in the extracts were determined as described above. The Olsen P extraction differs from the $NaHCO_3$ -P fraction described above since the Resin P has already been removed from the soil during the sequential extraction. Total soil P was determined by heating samples at 500°C for 4 h and extracting with 0.5 M H_2SO_4 (Saunders and Williams 1955). Total soil C and N were measured using an Elementar Vario Max N/C Analyzer (ELEMENTAR Analysensysteme, Hanau, Germany).

Manure characterization

Composted well-decomposed cattle manure was supplied by the Agriculture and Agri-

Food Canada Beef Research Unit at Brandon, Manitoba. It was from the same manure batch as applied to the field (Welsh et al. 2009). The composted manure was air-dried and had been stored at room temperature since 2007 and was analysed prior to the greenhouse study by Nova Scotia Agriculture Laboratory Services (Table 2).

Greenhouse experiment

Soils collected in bulk were pre-incubated for 14 d at 40% gravimetric moisture in the greenhouse and 0.9 kg dry weight equivalent was filled in 12.7 cm diameter pots. The experimental design was a completely randomized design with four management systems (ORG, ORG+M, CONV, PRA), eight P treatments (Table 3), and four replicates.

Mineral fertilizer as KH_2PO_4 was applied in solution to pots prior to planting. Composted manure was mixed well with soil before adding to the pot. Taking into consideration the nutrient concentrations in the manure amendments (Table 2), macronutrients (other than P) were added as a solution of NH_4NO_3 , CaNO_3 , MgSO_4 , and K_2SO_4 to all treatments, including the control, for a final level of: 150 mg N kg^{-1} , 200 mg K kg^{-1} , 35 mg Ca kg^{-1} and 15 mg Mg kg^{-1} . Microelements were also added at a rate (mg kg^{-1}) of: 2 Cu, 1 Mn, 1 B, and 0.1 Mo (Oberson et al. 2010).

Fifteen Italian ryegrass (*cv. Danergo*) seeds were sown to each pot and thinned to 10 plants after emergence. Soil moisture was maintained at 60% field capacity, as determined by saturating soil and allowing water to drain for 48 h. The greenhouse conditions were set to 25°C day and 18°C night with a 16 h photoperiod. Plants received soluble nutrients (except P) after each ryegrass cut (days 30, 62, 75 and 89) to ensure that other nutrients were not limiting plant growth.

Soil and plant sampling and analysis

Ryegrass plants were cut approximately 2 cm above the soil at 30, 62, 75 and 89 d with the final harvest at 106 d after planting (DAP), combining dried plant samples from all sampling dates into one composite sample per pot. At the final harvest, pots were destructively sampled and soil was separated from the roots, which were washed before analysis. Plant shoots and roots were oven dried (60°C for 48 h), weighed and ground (<1 mm) prior to analysis. Plant tissue P was determined by ashing at 500°C for 4 h and extracted by heating in 10% HNO₃/30% HCl (Jones and Case 1990). Total P in plant tissue extracts was measured by inductively coupled plasma spectroscopy by the Nova Scotia Agriculture Laboratory Services lab. Total C and N contents of soil and N content of plant tissue were measured using an Elementar Vario Max N/C Analyzer (ELEMENTAR Analysensysteme, Hanau, Germany). Soil Olsen P was also determined at final harvest (106 DAP) with the same procedure as described above. Total plant P uptake (shoot and root) was determined for each pot and used to determine apparent P use efficiency (APUE), calculated as:

$$\text{APUE} = [(\text{Plant P uptake}_{\text{treatment}} - \text{Plant P uptake}_{\text{control}}) / \text{total P applied}] * 100$$

(modified from Eghball and Power 1999 to include shoot and root).

Statistical analyses

Statistical analyses were completed using the GLM procedure of JMP 11 (SAS Institute Inc., Cary, NC). For the field soils, the significance of the effects of management system was tested with two-way analysis of variance. For the greenhouse trial, significance of block, management system, P treatment, and management x P treatment interaction was included in the model. All variables were tested for normality using the Shapiro-Wilk test and log transformed

when required. Significant differences among managements or P treatments were determined using Tukey's test ($P < 0.05$). Regression analysis of variables and total P added were analysed in JMP 11 and multiple slopes and intercepts were compared using an indicator variable regression for multiple linear fits.

Results

Soil extractable P fractions

The sequential P extraction revealed lower concentrations of both soil labile (Resin P, NaHCO_3 -MRP and MUP) and moderately labile (NaOH-MRP) P fractions in the ORG soil compared to the PRA and CONV systems (Table 4). The greatest differences were seen in the MRP of these fractions when the CONV soil had 111%, 176% and 133% higher values than the ORG soil, while the PRA had P concentrations 277%, 447% and 325% more than ORG. Moreover, the ORG soil was significantly lower than PRA for the NaOH-MUP and sNaOH-MRP fractions. In contrast, the residual P fraction was higher in the ORG soil compared to the PRA soil. On the other hand, there were no significant differences among management systems for the HCl-MRP or sNaOH-MUP fractions and for total P in the soil. In the soil sampled from the PRA plots, the NaOH-MUP and all MRP except HCl-MRP were nearly double that of the conventionally-managed soils, equivalent to the difference obtained in the residual P pool. The sum of P fractions represents a P recovery of between 78% and 89% when compared to total P obtained by ignition (Table 4). The Olsen MRP values were significantly higher in the PRA system compared to all others, with a concentration two to three times higher.

Plant biomass and nutrient P uptake

The management by treatment interaction was significant for all plant responses except root P concentration and APUE (Table 5). The shoot biomass had the lowest value for the control (0P) in the ORG soil as compared with all other treatment and management combinations (Table 6). This was also the case for the shoot P uptake. In the soil from ORG management, the medium and high manure treatments and the 160 mineral P treatment resulted in significantly higher plant P uptake compared to ryegrass grown in the no P control pots. The same trend was reported in the soils from CONV management; however, in the ORG+M soils only the high manure treatment resulted in a significant increase in ryegrass P uptake compared to the control. The shoot P uptake increased at a factor than biomass production. In the CONV soil, for example, shoot biomass was ~12% higher in the 160 mg kg⁻¹ mineral P compared to the 15 mg kg⁻¹ mineral P treatment, with a corresponding 119% increase in shoot P uptake. Shoot P concentrations were consistently higher than the control treatment at the medium and high manure rates and 160 mg kg⁻¹ mineral P in all management systems (Fig. 1a to d).

Soil chemical properties in the greenhouse study

Soil chemical properties assessed at 106 DAP differed considerably among the applied P treatments and management systems (Table 5). The soil from ORG management was significantly lower than all other systems for the Olsen-MRP, and the PRA soil was significantly higher (Fig. 2b). The Olsen-MUP values were significantly lower in the ORG and ORG+M systems than the CONV and PRA (Fig. 2d). The Olsen-MRP concentrations increased with increasing rates of P applied. These increases, however, were much more important with manure than with mineral fertilizer. Total soil C and N concentrations were significantly different for both management and treatment but there was a significant interaction for total N only (Table 5).

Soil N was significantly lower in PRA for the control, 40 and 160 KH_2PO_4 treatment compared to ORG+M and CONV (Table 7). There were no difference in soil C for the control, low manure, medium manure and 80 KH_2PO_4 treatments but the ORG was lower than the PRA in the high manure treatment and the PRA was significantly lower than ORG+M at 40 KH_2PO_4 and 160 KH_2PO_4 (Table 7).

Relationship between P added and growth attributes

A regression analysis of the relationship between shoot biomass and total P added for the manure P treatments showed a linear relationship that did not level off even at the highest manure application (Fig. 3a). In comparison, the mineral P treatments resulted in a shoot biomass peaking at 80 mg P kg^{-1} soil, except in the soils from ORG management where they peaked around 160 mg P kg^{-1} (Fig. 3b).

The shoot P uptake increased with increasing P applications of manure P (quadratic function) and mineral P (linear function) for all management systems. Moreover, the manure P was superior to mineral P for same available P added (Fig. 3c,d). For the manure P treatments, the shoot P uptake was not affected by the management systems (Table 6) and there were no differences among the slopes of P uptake vs total P applied ($P=0.37$; Fig. 3c). By contrast, the slopes were significantly different for the mineral P treatments ($P=0.003$; Fig. 3d), and all plots receiving mineral P fertilizer had significantly lower shoot P uptake in the ORG system (Table 6). The shoot P uptake was significantly correlated to shoot biomass for both the manure ($r^2=0.60$) and the mineral ($r^2=0.70$) treatments (Fig. 3e,f).

The management system, P treatment and the interaction between P treatment and management were significant for shoot and root P uptake in ryegrass after 106 d (Table 5). The

high manure rate and 160 KH_2PO_4 resulted in significantly higher shoot P uptake than the control in the ORG and CONV management (Table 6). For the ORG+M system, only the high manure treatment was significantly higher than the control. Interestingly, there was no increase in root biomass or root P uptake for the ORG or ORG+M systems but there was an increase in root P uptake for the medium and high manure P treatments in the CONV systems. In the PRA system, there was no increase in the shoot biomass, root biomass or shoot P uptake with addition of mineral or manure P compared to the control. However, there was an increase in the root P uptake with the high manure treatment only. The mean plant APUEs differed significantly among P treatments and management (Table 5, 6), with the ORG higher for low manure and mineral P additions.

Discussion

The sequential P fractionation of the Glenlea Long-term Rotation trial soils demonstrated that the P concentrations of labile and moderately labile fractions (resin, NaHCO_3 and NaOH) were being depleted in the ORG management system when compared to the CONV and PRA system, except where manure was once (2007) applied previously (ORG+M). Although P was applied regularly to the CONV plots, there were no significant differences among management systems for total P. Higher yields in the CONV plots would have resulted in more P being exported during forage and grain harvest (e.g. grain yields were 1.6 to 2.4 times higher in CONV than in ORG plots; Welsh et al. 2009). The lower labile P in soils under ORG management agrees with other studies conducted in the same area (Entz et al. 2001; Knight et al. 2010; Welsh et al. 2009). Lower concentrations of labile P could be a result of less organic matter being returned to the ORG system with an increase in mineralization potential or less organic P being

stabilized in the soil. However, there was no difference in soil C for soils from different management systems for our initial soil characterization. Bell et al. (2012) also reported lower soil test P in the ORG vs CONV plots at this site, along with a significant reduction in organic C in the 0-15 cm depth. Although we used a modified Hedley fractionation that did not include P held in microbial biomass, Braman et al. (2016) reported no significant difference in microbial biomass P between the ORG and CONV management in these same plots in 2011.

While abundant total P exists in the soil, replacement of P in long-term organic systems is essential for maintaining yields, considering both labile and moderately labile P pools may be depleted. This is especially relevant where alfalfa biomass is continuously removed with no P returned, as in the case of the ORG rotation sequence for the soils used herein. For example, Schneider et al. (2017) found that alfalfa yields did not differ between organic and conventional dairy farms in Ontario where manure was routinely applied to fields, despite lower soil test P values on the organic farm fields. In our greenhouse study, the soil Olsen-P concentrations were significantly different at final harvest for both management and P application rate but no interaction was observed. The Olsen-MRP values after 106 d in the 0P treatment are very similar to the initial values, indicating that the labile pool was being replenished. Considering that 160 mg P kg⁻¹ was added to the pots for the very high mineral P treatment and the values of the total Olsen-P increased only by 31.4, 35.4, 28.7 and 35.6 mg P kg⁻¹ soil for the ORG, ORG+M, CONV, and PRA soils, respectively, some of the available P was not removed by the Olsen P extraction or it is being immobilized or converted to less available pools.

Extractions to measure soil test P are assumed to give an indication of how much P will be available immediately or in the short-term (i.e. days to weeks), with these values often used to make fertilizer recommendations for producers. Although several limitations have been reported

with different extractions and sequential P fractionation procedures (Condon and Newman 2011), it does provide us information on the potential bioavailability of P in soils under different management systems. Because we were not looking for the particular P species, which could be provided by ^{31}P NMR, the operationally defined pools of Hedley procedure provided a useful indication of bioavailability. In agreement with other studies (Entz et al. 2001; Knight et al. 2010; Welsh et al. 2009), we found that reported deficiencies in soil test P are not representative of all of the P that may become available by microbial and/or chemical processes. However, the Olsen-P extraction used in this greenhouse study does seem to adequately predict the yield response to P fertilization, especially for the mineral P treatments.

It has been documented that fertilizer application can change the relative concentrations of inorganic P and organic P pools in soils (Hedley et al. 1982; Keller et al. 2012; Malik et al. 2012). In our experiment, the rates of manure P were applied with the assumption that 60% of composted manure P would be potentially available (Larney et al. 2006). This value is in line with our greenhouse pot study where the manure P was 60-90% as effective as mineral P, if assuming the mineral P is 100% available. In the medium and high manure treatments, 48% - 78% of the P was either removed by plant biomass or detected in the post-harvest soil Olsen-MRP extracts, as demonstrated by the regression analysis for the plant P uptake and total P added, with a linear trend continuing even at the highest application rates. Interestingly, the regression analysis of the shoot biomass to total P applied demonstrated a linear increase in shoot biomass for the soils under ORG management even at 280 mg P kg⁻¹, while the biomass was levelling off at a much lower rate (160 mg P kg⁻¹) of mineral P. These P supply rates for the manure are likely higher than what would occur in the field, considering the optimum temperature and moisture conditions in the greenhouse, but it may also be an indication of higher

P availability than assumed above. In addition, there may be a priming effect of the manure with added C, enzyme and organisms further promoting the growth rate.

Compared to the control, there was a greater response of ryegrass shoot biomass in the ORG and ORG+M soils than the CONV and PRA soils to both the manure and mineral P treatments. This was expected considering the lower levels of labile P in the ORG and ORG+M systems. In a previous study reporting on biochemical and microbial properties of the soils sampled 30 d after planting the ryegrass in the greenhouse, there was a significantly higher alkaline phosphatase activity and *phoD* gene abundance in soils from ORG management, corresponding to lower concentrations of NaHCO₃-extractable MRP (Fraser et al. 2015). However, it was unclear whether these differences observed after 30 d would result in increased P uptake and/or improved yields. Shoot biomass yields for the CONV soil were significantly higher than the ORG in the control and 15, 40 and 80 mg P kg⁻¹ mineral P treatment. From 80 to 160 mg P kg⁻¹ soil, the shoot biomass did not increase more than 0.4 g in any soil except the ORG (from 8.8 to 10.7 g), indicating that application at the higher rates did not result in a corresponding yield increase with the exception of the latter.

The concept of sufficiency level of soil nutrients assumes that P addition will increase yields to a maximum level after which point there is no positive response to additional rates (Bray 1944). The larger differences in plant P uptake than biomass, especially in the medium and high manure treatments, and highest rate of fertilizer P for all but the ORG treatment, may indicate a luxury uptake of P in these plants. It is worth noting that we were continuously harvesting the shoot biomass, which may affect the uptake of P differently than with an annual crop that is harvested at maturity. However, the linear regressions of total P applied and shoot P uptake resulted in r^2 values between 0.94 to 0.99 in the manure P treatments and 0.98 to 0.99 in

the mineral P treatments, demonstrating a linear increase in P uptake even at the high application rates. Although manure showed a lower slope of regression with plant P uptake than mineral P, the higher rate of P in the manure treatment may cause the slope to decrease. In a similar study comparing P uptake by ryegrass in soil amended with turkey litter compost and mineral P, Audette et al. (2016) found higher shoot P uptake in the compost amended pots despite N and K being equalized among treatments. In addition, they observed that both the quantity and thickness of the roots appeared much greater in the compost amended soil compared to that receiving P fertilizer.

Other studies have also found higher plant P uptake in manure treated soils compared to mineral fertilizer (Eghball and Power 1999), whereas O'Halloran and Sigrist (1993) reported no difference in ryegrass shoot P content with soils incubated with liquid hog manure with and without mineral P added. In contrast, Oberson et al. (2010) reported that ryegrass grown in the greenhouse amended with mineral P had more biomass and higher P uptake than the same rate of manure P. In our study, the higher shoot P uptake in the manure P treatments than mineral P was likely a result of the underestimation of the potentially available P in the composted cattle manure. Considering that the source (manure vs mineral P) did not have any effect on soil test P, the rate was the determining factor.

Excessive application of P fertilizer, whether in the form of mineral P or manure P, may result in negative environmental consequences and should be avoided. The results here indicated that with high rates of manure (140 mg P kg⁻¹ and higher) and mineral P (160 mg P kg⁻¹), plant biomass did not increase at the same rate as P uptake. This was true for soils from the ORG+M, CONV, and PRA systems, but not the ORG, thus highlighting the importance of considering the previous management and P application rate to maximize plant P utilization and minimize

negative environment impacts.

Conclusions

Long-term management significantly affected plant responses to the different types and rates of P amendment. The initial characterization of the soils by sequential P fractionation indicated that the levels of labile P were depleted in the ORG soil compared to the CONV and PRA soils. When each soil was amended with mineral or manure P and planted to ryegrass, manure treatments resulted in higher plant P uptake due to higher rates of P addition for the high manure rate. The P uptake in the mineral P treatments resulted in a linear trend, with plants in the PRA soil having very similar P uptake to the ORG+M soil, despite the much higher initial $\text{NaHCO}_3\text{-P}$ values as demonstrated by the fractionation method. Contrary to our initial hypothesis, the overall influence of long-term management history with the contrasting source of applied P did not have a significant effect on the apparent P recovery of ryegrass in the greenhouse, suggesting no differential response of soils as affected by farming system to P application. The differences among plant and soil variables measured in soils from the ORG, ORG+M, CONV and PRA management systems substantiate the need to maintain long-term management trials for monitoring the effects of anthropogenic activities.

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Figure 1: Italian ryegrass plant P concentrations in roots and shoots after 106 days in soils under long-term a) organic (ORG), b) manure-amended organic (ORG+M), c) conventional (CONV), and d) prairie (PRA) management system. Values are treatment means $\pm$ standard deviation (n=4) with same letters within a management system representing no significant difference at $P = 0.05$ as determined by Tukey's test.

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Figure 3: Relationships between Italian ryegrass shoot biomass and total P added to pots (a, b), shoot P uptake and total P added to pots (c, d) and shoot P uptake and biomass (e, f) after 106 days in soils collected from field plots under long-term organic (ORG), manure-amended organic (ORG+M), conventional (CONV), and prairie (PRA) management. Each point represents the treatment mean (n=4). The P-values shown in plots c and d represent the difference between the slopes of the management systems.

Table 1: Management systems at the Glenlea Long Term Crop rotation and Management site.

Organic – no input ^a	ORG	No fertilizers or pesticides.
Organic + manure ^a	ORG+M	Composted cattle manure applied in fall 2007 (10 t ha ⁻¹ fresh weight) with total N and P at 63 and 24 kg ha ⁻¹ , respectively.
Conventional ^a	CONV	Fertilizer application based on soil test recommendations and pesticide application based on economic thresholds.
Restored native prairie	PRA	Established in 1992. Undisturbed except for burning in 1998, 2002, 2005.

^aFlax – alfalfa- alfalfa-wheat

Table 2: Main selected properties for composted beef cattle manure (expressed on a dry basis except dry matter).

Parameter	Value ^a
Dry matter (%)	58.0 (7.5)
pH	7.1 (0.06)
Nutrient Content (g kg ⁻¹)	
Total P	1.7 (0.3)
Total N	6.7 (0.9)
NH ₄ ⁺ -N	0.4 (0.05)
K	2.3 (0.2)
Mg	3.5 (0.4)
Ca	16.8 (2.9)

^aMean +/- standard deviation, n=3.

Table 3: Phosphorus amendments and application rates for greenhouse study assessing the response of Italian ryegrass in soils collected from organic, organic with manure, conventional and prairie management.

Treatment	Addition	Total P (mg P kg ⁻¹ soil)	Potentially available P ^a (mg P kg ⁻¹ soil)
Control	No P		0
Low manure	Composted cattle manure	21	12
Medium manure	Composted cattle manure	140	84
High manure	Composted cattle manure	280	168
Low mineral P	KH ₂ PO ₄	15	15
Medium mineral P	KH ₂ PO ₄	40	40
High mineral P	KH ₂ PO ₄	80	80
Very high mineral P	KH ₂ PO ₄	160	160

^aAssuming 60% of total P in the compost is available (Larney et al. 2006).

Table 4: Sequentially-extracted P (modified Hedley method) and total P, C and N concentrations in composite soil samples (0-15 cm) taken in May 2011. Means with same letters within a soil property represent no significant treatment effect at P=0.05 as determined by Tukey’s test. An absence of letters indicates no significant treatment effect at P<0.05.

Soil Property	ORG	ORG+M	CONV	PRA	ANOVA (P value)
	----- mg P kg ⁻¹ -----				
<u>Molybdate-reactive P (MRP)</u>					
Resin-MRP	10.2 c	16.4 bc	21.5 b	38.5 a	***
NaHCO ₃ -MRP	4.6 c	7.0 bc	12.7 b	25.2 a	***
NaOH-MRP	14.3 c	17.6 c	33.3 b	60.8 a	***
SNaOH-MRP	4.8 b	6.2 b	10.1 b	17.6 a	***
HCl-MRP	114.4 a	114.4 a	98.6 a	105.5 a	ns
<u>Molybdate-unreactive P (MUP)</u>					
NaHCO ₃ -MUP	16.1 c	17.8 bc	24.0 ab	25.5 a	**
NaOH-MUP	79.5 b	82.1 b	79.1 b	133.4 a	**
sNaOH-MUP	23.1 a	22.6 a	25.9 a	19.4 a	**
<u>Residual P</u>					**
	272.7 a	266.5 ab	254.6 ab	186.5 b	
Σ P Fractions (mg kg ⁻¹)	539.6 a	550.6 a	559.8 a	612.3 a	ns
Total P (mg kg ⁻¹) ^a	693.8 a	702.9 a	668.9 a	688.0 a	ns
Fraction P Recovery (%) ^b	78	78	84	89	
Olsen MRP (mg kg ⁻¹)	5.8 b	7.2 b	10.4 b	19.1 a	***
Total C (g kg ⁻¹)	30.8 a	28.5 a	29.2 a	28.6 a	ns
Total N (g kg ⁻¹)	3.0 a	2.8 a	3.1 a	2.8 a	ns

Note: *, **, *** significant at 0.05, 0.01 and 0.001 probability, respectively. ns Not significant.

^aTotal soil P by ignition (Saunders and Williams 1955).

^b% of total P recovered in P fractions.

Table 5: Analysis of variance for the effect of previous soil management (M; organic, organic-manure amended, conventional and restored prairie management) and treatments (T; control, low manure, medium manure, high manure, 15, 40, 80 and 160 mg P kg⁻¹).

ANOVA (p-value)	Soil				Plant ^a						
	Olsen-MRP	Olsen-MUP	Total C	Total N	Shoot biomass	Root biomass	Shoot P concentration	Root P concentration	Shoot P uptake	Root P uptake	APUE ^c %
Management (M)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.02	0.05
Treatment (T)	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.04
M x T	<0.01	<0.001	ns ^b	<0.001	0.02	0.01	<0.001	ns	<0.001	0.01	ns

^a Harvest was 106 d after the ryegrass was planted (DAP). Shoot biomass and shoot P concentration and uptake are based on composite sample of harvests on 30, 62, 75, 89 and 106 DAP.

^b ns Not significant.

^c Apparent P use efficiency (root and shoot)

Table 6: Ryegrass biomass, plant P uptake, and apparent P use efficiency for shoots collected at 30, 62, 75, 89 and 106 days and roots collected at 106 days as affected by management systems and P treatments. Means with same letters within a row (management system) or a column (P treatment) represent no significant difference at $p < 0.05$ as determined by Tukey's test. An absence of letters indicates no significant effect at $p < 0.05$.

		ORG	ORG+M	CONV	PRA
Shoot Biomass (g DM pot ⁻¹)	Control	4.1 bB	8.2 A	11.0 A	9.3 A
	Low Manure	7.0 ab	9.1	10.8	7.8
	Medium Manure	9.0 ab	9.5	11.2	10.0
	High Manure	8.7 ab	10.8	11.7	9.7
	15 KH ₂ PO ₄	5.8 abB	8.8 AB	11.8 A	9.3 AB
	40 KH ₂ PO ₄	7.7 abB	10.4 AB	12.7 A	10.2 AB
	80 KH ₂ PO ₄	8.8 abB	11.3 AB	13.1 A	10.6 AB
	160 KH ₂ PO ₄	10.7 a	11.6	13.2	11.0
Root Biomass (g DM pot ⁻¹)	Control	0.9 B	1.4 AB	1.8 abA	1.6 A
	Low Manure	1.1	1.4	1.5 b	1.3
	Medium Manure	1.4	1.4	1.7 ab	1.8
	High Manure	1.7	1.7	1.5 b	1.1
	15 KH ₂ PO ₄	1.2	1.5	1.9 ab	1.6
	40 KH ₂ PO ₄	1.6	1.8	2.2 a	1.6
	80 KH ₂ PO ₄	1.7	1.7	1.9 ab	2.0
	160 KH ₂ PO ₄	1.7	1.6	1.7 ab	1.7
Shoot P (mg P pot ⁻¹)	Control	6.7 dB	19.9 bA	22.0 eA	22.4 A
	Low Manure	15.3 cd	21.7 b	25.3 e	20.2
	Medium Manure	28.5 abc	37.0 ab	48.6 bc	43.0
	High Manure	40.7 a	48.3 a	61.7 a	52.3
	15 KH ₂ PO ₄	11.8 cdB	20.7 bA	25.4 eA	22.5 A
	40 KH ₂ PO ₄	17.0 cdB	27.1 abA	29.4 deA	26.3 A
	80 KH ₂ PO ₄	21.2 bcdB	37.4 abA	40.5 cdA	31.7 A
	160 KH ₂ PO ₄	33.0 abB	43.6 abAB	55.7 abA	44.2 AB
Root P (mg P pot ⁻¹)	Control	31.6	27.5	17.5 cd	20.5 b
	Low Manure	22.4	24.9	22.2 cd	31.1 ab
	Medium Manure	32.8	29.2	36.3 b	35.6 ab
	High Manure	39.7	34.0	50.7 a	51.3 a
	15 KH ₂ PO ₄	24.1	23.4	13.7 cd	21.3 b
	40 KH ₂ PO ₄	20.5	20.2	12.7 d	18.8 b
	80 KH ₂ PO ₄	19.1	29.8	20.4 cd	17.0 b
	160 KH ₂ PO ₄	28.5	30.0	26.9 bc	29.7 ab
Apparent P use efficiency (%) ^a	Control				
	Low Manure	71.9 A	29.0 AB	20.6 B	0
	Medium Manure	23.0	16.6	27.1	20.0
	High Manure	17.4	14.2	19.6	14.0
	15 KH ₂ PO ₄	48.4 A	20.0 AB	24.7 AB	16.2 B
	40 KH ₂ PO ₄	26.8	15.4	23.8	10.7
	80 KH ₂ PO ₄	23.5	15.3	28.1	14.0
	160 KH ₂ PO ₄	20.5	17.2	24.3	12.3

^aApparent P use efficiency (APUE) was calculated as $APUE = [(plant\ P\ uptake_{treatment} - plant\ P\ uptake_{control}) / total\ P\ applied] * 100$

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Table 7. Soil C and N concentrations (0-15 cm) from organic (ORG), organic+manure (ORG+M), conventional (CONV) and prairie (PRA) management after 106 day greenhouse trial. Means (n=4) with same letters within a row (P treatment) represent no significant difference at $p < 0.05$ as determined by Tukey's test.

	ORG	ORG+M	CONV	PRA	ORG	ORG+M	CONV	PRA
	Soil N ($\mu\text{g g}^{-1}$)				Soil C ($\mu\text{g g}^{-1}$)			
Control	3.5a	3.4a	3.3a	3.1b	31.8a	32.6a	31.9a	31.2a
Low Manure	3.3a	3.5a	3.4a	3.2a	33.2a	35.1a	32.7a	31.9a
Med Manure	3.5a	3.7a	3.7a	3.6a	37.1a	37.3a	37.6a	37.4a
High Manure	3.9a	4.0a	4.1a	4.0a	40.8b	41.9ab	42.4ab	42.8a
15 KH_2PO_4	3.3a	3.5a	3.4a	3.2a	32.8a	33.7a	32.1a	31.9a
40 KH_2PO_4	3.2ab	3.3a	3.3a	3.0b	32.5ab	33.0a	31.8ab	31.2b
80 KH_2PO_4	3.2a	3.3a	3.3a	3.0a	32.1a	33.6a	32.1a	31.0a
160 KH_2PO_4	3.1b	3.3a	3.3a	2.9b	31.8ab	33.4a	32.0ab	30.5b

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