Characterizing the Behaviour and Efficacy of Struvite Fertilizer for Organically Managed Crops in Manitoba Soils

by

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ABSTRACT

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Recycling phosphorus (P) from wastewater for use in fertilizers can contribute to an improved circular economy for P. Struvite recovered from municipal wastewater is a sparingly soluble fertilizer that has been recommended as a suitable P source for organic production, where soil P deficiencies can be particularly severe. Optimizing the use of struvite fertilizer in such systems requires greater understanding of its effects on crop performance, soil P dynamics, and soil organisms such as arbuscular mycorrhizal fungi (AMF).

Field studies on an alkaline clay soil demonstrated increases in yield and P uptake for organic spring wheat (*Triticum aestivum* L.) and alfalfa (*Medicago sativa* L.)–grass forage, whereas flax (*Linum usitatissimum* L.) showed no response. Forage response to struvite increased in the second and third years after struvite application, demonstrating the potential of struvite as a multi-year P source.

A soil incubation using the same alkaline clay and a neutral-pH sandy clay loam showed that P transformation processes were delayed in soil amended with struvite rather than monoammonium phosphate (MAP), and that P concentrations in labile P pools were also affected by soil type and application rate. However, water-extractable P was unexpectedly high after incubating struvite-amended soils only a few days, possibly due to sample grinding. A pot experiment on alfalfa in the same soils showed no differences between struvite and MAP on alfalfa performance or labile soil P pools. Plant P uptake increased over the unfertilized control only in the neutral-pH soil at a high P application rate. At this application rate, struvite showed a smaller inhibitory effect than MAP on root colonization with mycorrhizal arbuscular fungi.

Overall, this research shows that struvite can be an effective P fertilizer, even in alkaline soils, but that struvite dissolution and transformation processes and the resulting effects on plants and soil organisms vary with application rate and soil type. Further research is needed to determine appropriate methods for assessing P availability in struvite-amended soils.

FOREWORD

This thesis was prepared in manuscript format according to the guidelines established by the Department of Soil Science, University of Manitoba, and consists of five chapters. Chapter 1 is a general introduction to the thesis, including relevant background information for the rationale and approaches used in this research. Chapters 2 to 4 are research chapters, each written as manuscripts suitable for publication in refereed journals. Chapter 5 is an overall synthesis of the research, including practical implications and recommendations for further study.

Chapter 2 describes a set of field experiments assessing the effect of struvite application rate on the performance of spring wheat, flax, and alfalfa–grass forage in an alkaline soil in Manitoba. Chapter 3 focuses on the soil P dynamics in a soil incubation study without plants, comparing the effects of struvite and monoammonium phosphate (MAP) application on labile soil P pools over time in two contrasting Manitoba soils. Chapter 4 reports the findings of a plant growth study conducted under controlled conditions examining the effects of struvite application on alfalfa performance, labile soil P pools, and colonization of alfalfa roots with arbuscular mycorrhizal fungi.

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The contributions of all authors are as follows:

- Joanne Thiessen Martens: Conceptualized and conducted experiments; collected, curated, and analyzed data; interpreted results; wrote the original manuscript draft and completed revisions based on peer-review feedback.
- Martin Entz: Conceptualized experiments; administered and supervised the project; reviewed and edited the manuscript draft; acquired funding.
- Kimberley Schneider: Conceptualized experiments; administered and supervised the project; reviewed and edited the manuscript draft; acquired funding.
- Francis Zvomuya: Administered and supervised the project; reviewed and edited the manuscript draft.
- Henry Wilson: Administered the project; acquired funding.

Chapters 3 and 4 are currently being prepared for submission to peer-reviewed journals for publication. The contributions of authors to those manuscripts are as follows:

- Joanne Thiessen Martens: Conceptualized and conducted experiments; collected, curated, and analyzed data; interpreted results; wrote original manuscript drafts.
- Kimberley Schneider: Conceptualized experiments; administered and supervised the project; reviewed and edited manuscript drafts; acquired funding.
- Francis Zvomuya: Conceptualized experiments; administered and supervised the project; reviewed and edited manuscript drafts.
- Henry Wilson: Administered the project; acquired funding.
- Don Flaten: Conceptualized experiments.

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1. INTRODUCTION

1.1 Phosphorus in Agriculture and the Environment

Phosphorus (P) management in agricultural systems presents a set of intertwined challenges arising from a disconnected P cycle. Globally, nearly 60 Tg of P was mined in 2013, about 20 Tg of which was applied to soils as synthetic fertilizers along with about 15 Tg of P from organic fertilizers such as manure; however, only 6.2 Tg of this P was actually consumed by humans (Chen and Graedel 2016). Applying P fertilizers is necessary to replace nutrients exported in harvested products and to prevent or correct soil P deficiencies, but fertilizers are often applied in excess of crop requirements due to the many physical, chemical, and biological processes that limit P supply from soil to crops (Zhang et al. 2004; Ziadi et al. 2013; Roberts and Johnston 2015). Inappropriate fertilizer management practices can increase non-point-source P losses from agricultural land to aquatic ecosystems (Withers et al. 2014; Jarvie et al. 2017). Meanwhile, nutrients exported in agricultural products are consumed and excreted by livestock or humans, often contributing to excess P in the environment far from where they were sourced due to point-source losses from livestock operations and wastewater treatment facilities (Cordell et al. 2009; Chen and Graedel 2016). Surface waters, particularly in freshwater systems, can be sensitive to additions of very low concentrations of dissolved P and suffer disproportionately great damage from P losses that are agronomically insignificant (Withers et al. 2014; Jarvie et al. 2017; Sharpley et al. 2018). Thus, we are faced with what Sharpley et al. (2018) describe as a “conundrum, derived from simultaneous deficiencies and excesses of P across local, regional, and national scales” (p. 775).

1.2 P in Organic Systems

The issue of P deficiency is particularly acute in organic crop production systems that do not have adequate access to suitable P sources (Martin et al. 2007; Paulsen et al. 2016). The main commercially available P sources that are permitted in organic agriculture in Canada are manure, compost, phosphate rock, and plant- and animal-based products such as alfalfa pellets and bone meal (Canadian General Standards Board 2021a). Phosphate rock and bone meal are poorly soluble, especially under alkaline soil conditions (Arcand and Schneider 2006; Nelson and Janke 2007); manure is not readily available in regions dominated by stockless farms, such as certain parts of the Canadian prairies (Potter et al. 2010); and other amendments are too expensive for field-scale crop production. These constraints leave organic farmers with few options for supplying P to crops. The resulting P deficits and eventual soil P deficiencies can limit crop yields through direct P deficiency and through nitrogen (N) deficiency induced by poor growth and biological N fixation by P-limited legume green manures, ultimately threatening the sustainability of organic crop production systems (Martin et al. 2007; Welsh et al. 2009; Snyder and Spaner 2010; Paulsen et al. 2016). Thus, exploring novel P amendments suitable for organic production is a priority.

The characteristics that make an amendment suitable for organic production are determined by the four general principles of organic production—health, ecology, fairness, and care (IFOAM n.d.). Key aspects of these principles that guide regulation of organic management practices and permitted substances for soil fertility management include concern for human, animal, and soil health; emphasis on ecological processes and recycling of materials and energy; management that safeguards future generations; and a precautionary approach to adopting new technologies (Table 1.1). Thus, a soil amendment ideally suited for organic production would be from a renewable resource and/or be recycled, would have moderate solubility in soil so as to provide an adequate nutrient supply without drastically modifying nutrient cycling processes, would not have a negative impact on soil biological communities, would ensure the supply of key resources for future generations, and would not pose a human health risk. Organic certification systems, regulated at a national level in Canada since 1999 (Canadian General Standards Board 2020), have been developed based on these principles, along with practical considerations that sometimes involve a certain degree of compromise. For example, the need for soil fertility amendments in organic crop production has led to conventional livestock manure being permitted for use, even though it may contain some undesirable substances, as long as certain animal welfare conditions are met (Canadian General Standards Board 2021b).

Table 1.1 Excerpts from the internationally recognized principles of organic agriculture relevant to soil fertility management practices and permitted substances

Principle	**Relevant Excerpts** [a]
Health	Health is the wholeness and integrity of living systems. The role of organic agriculture … is to sustain and enhance the health of ecosystems and organisms from the smallest in the soil to human beings.
Ecology	This principle roots organic agriculture within living ecological systems. It states that production is to be based on ecological processes, and recycling. Organic farming, pastoral and wild harvest systems should fit the cycles and ecological balances in nature. Organic management must be adapted to local conditions, ecology, culture and scale. Inputs should be reduced by reuse, recycling and efficient management of materials and energy in order to maintain and improve environmental quality and conserve resources.
Fairness	Natural and environmental resources that are used for production and consumption should be managed in a way that is socially and ecologically just and should be held in trust for future generations. Fairness requires systems of production, distribution and trade that are open and equitable and account for real environmental and social costs.
Care	Organic agriculture should be managed in a precautionary and responsible manner to protect the health and well-being of current and future generations and the environment. Practitioners of organic agriculture can enhance efficiency and increase productivity, but this should not be at the risk of jeopardizing health and well-being.

[a] Excerpted from *The Four Principles of Organic Agriculture* (IFOAM n.d.).

1.3 Advancing the Circular Economy for P

Recognition of concurrent P deficiencies and excesses has led to growing interest in reconnecting broken nutrient cycles and creating a circular economy for P, in which the P already circulating in biogeochemical cycles is redistributed to where it is needed and is used more efficiently (Childers et al. 2011; Withers et al. 2018; Schneider et al. 2019; Nicksy and Entz 2021). This circular system, in which P is recovered from waste streams for reuse as a fertilizer, reduces the P load to the environment while also replacing a portion of the demand for non-renewable mined phosphate rock. The feasibility of such an approach is evident in the 100-plus P recovery systems currently in operation in wastewater treatment facilities around the world (Kabbe 2021).

Of the P recovery processes developed to date, extraction of struvite (magnesium ammonium phosphate hexahydrate, $NH_4MgPO_4 \cdot 6H_2O$) is one of the most promising options and is the process used by the majority of existing P recovery facilities (Kabbe 2021). Struvite is a crystalline compound containing equal molar concentrations of P, N, and magnesium (Mg). Compared to monoammonium phosphate, the form of P used in some common synthetic fertilizers (solubility of 404 g L^{-1} at 25 °C; Lide 2007), the solubility of struvite is low in water (0.18 g L^{-1} at 25 °C) but increases in acids (1.78 g L^{-1} in 0.01 M HCl at 25 °C) (Le Corre et al. 2009). In the nutrient-rich, alkaline conditions present in the pipes of wastewater treatment facilities, struvite can precipitate or crystallize spontaneously, clogging the pipes (Le Corre et al. 2009). Controlled extraction processes help to prevent pipe clogging, are economically viable, and yield a struvite product that is relatively plant-available and free of contaminants (Egle et al. 2016; Weissengruber et al. 2018; Wollmann et al. 2018; Huygens and Saveyn 2018). In Canada, high-purity struvite fertilizer derived from municipal wastewater using the Pearl® process is commercially available as the granular product Crystal Green®, with a nutrient analysis (N-P_2O_5-K_2O, %) of 5-28-0 plus

10% Mg (Ostara Nutrient Recovery Technologies Inc.). Crystal Green has been the focus of several studies assessing the solubility and agronomic value of struvite (Talboys et al. 2016; Degryse et al. 2017; Hall et al. 2020; Anderson et al. 2020), though struvites produced through other processes and from sources other than municipal wastewater have also been investigated (Johnston and Richards 2003; Gell et al. 2011; Ackerman et al. 2013; Katanda et al. 2016; Hilt et al. 2016; Mackey et al. 2021).

Currently, soil amendments derived from human waste are not permitted in certified organic production systems anywhere in the world, due to concerns regarding the safety of such products for human health. However, several authors have recommended struvite as a P source suitable for organic production systems due to its low level of contamination relative to many other P fertilizers, its greater solubility than rock P amendments currently permitted in organic production, and its recycled nature (Rahman et al. 2014; Möller et al. 2018; Weissengruber et al. 2018). High-purity struvite from human waste has been provisionally approved for use in organic production in the EU (Expert Group for Technical Advice on Organic Production 2016). Struvite derived from plant or livestock sources was added to the most recent permitted substances list in the Canadian Organic Production Standard, but struvite from human sources is not currently permitted (Canadian General Standards Board 2021a).

1.4 Struvite as a Potential P Source in Canadian Organic Cropping Systems

Little is currently known about the agronomic potential of struvite fertilizer in Canadian organic cropping systems, its behaviour in the soils and climate of the Canadian prairie region or other analogous growing conditions, or its effects on soil organisms. Even in conventional systems, studies that investigate management practices to optimize struvite use under realistic conditions are lacking as most studies have been conducted under controlled conditions, have often used unrealistic P application rates and/or soils that have high background soil test P, and have only rarely assessed crop yield (Hertzberger et al. 2020).

1.4.1 Struvite Solubility

Struvite is considered a slow-release fertilizer due to its relatively low solubility in water (0.4–4.4% of total P, as summarized by Kratz et al. (2019)) relative to common P fertilizers such as monoammonium phosphate (MAP) and triple super phosphate (TSP), which are highly soluble in water (Meyer et al. 2018; Kratz et al. 2019). Upon application to soil, soluble fertilizers such as MAP dissolve rapidly and are then transformed to less-soluble forms through adsorption or precipitation (Sample et al. 1980). In calcareous soils, a primary reaction product formed within days of MAP application is dicalcium phosphate dihydrate (DCPD) (Beaton et al. 1963), with a solubility constant (pK_{sp}) of 6.55 (Bennett and Adams 1976). In comparison to DCPD, struvite is less soluble in water, with a pK_{sp} of 13.26 (Ohlinger et al. 1998). However, the solubility of struvite is greater than that of phosphate rock fertilizers, which are virtually insoluble in water (e.g., 0.1% of total P; Wollman et al. 2018) and provide little to no P benefit to crops in neutral to alkaline soils (Möller et al. 2018). The slow dissolution of struvite relative to MAP has implications for P diffusion and retention in soil (Degryse et al. 2017; Everaert et al. 2017; do Nascimento et al. 2018), but the longer-term effect on P supply to crops from struvite, relative to the reaction products of soluble P fertilizers, is not clear. If struvite-P is released in synchrony with crop uptake, perhaps retention processes do not play a major role in struvite P dynamics. However, if P precipitation does occur, the reaction products formed from addition of magnesium phosphates such as struvite to soil may be more soluble than those formed from addition of calcium phosphates

(Bell and Black 1970). Speciation of P in struvite-amended soils would provide greater insights on these P transformation dynamics.

To understand the behaviour of struvite in soils, it is necessary to know how struvite interacts with the various extractants commonly used to characterize soil P pools for agronomic and environmental management. Common soil extractions have been found to dissolve a greater proportion of the P in struvite than is water soluble. For example, in a sequential fractionation procedure performed on finely ground struvite, nearly all the P in struvite (>95%) was extracted in the labile pools (resin and bicarbonate extraction steps, 16 h extraction time each), despite only 4.4% of total P being extractable in water (Meyer et al. 2018). A recent study in which granular struvite was added to washed quartz and immediately extracted with Bray, Mehlich-3, or Olsen soil tests (5–30 min extraction times) reported that about 16–24% of the total P was extracted from struvite, depending on the test (Gu et al. 2021). Extraction of P from struvite in the acidic Bray and Mehlich-3 tests is not surprising given struvite's enhanced solubility in acids, but the extractability of struvite-P in the alkaline Olsen test is contrary to expectations. The Olsen test is thought to extract solution P, weakly adsorbed P, slightly soluble Ca-P (presumably DCPD), and readily hydrolyzed organic P from soils; the mechanisms of P extraction are through precipitation of bicarbonate ions with Ca^{2+}, thus increasing orthophosphate solubility, and substitution of bicarbonate ions for orthophosphate ions on adsorption sites (Olsen et al. 1954; Schoenau and O'Halloran 2008). Gu et al. (2021) speculated that exposed Mg-OH on the surface of struvite crystals reacted with the bicarbonate ions of the Olsen extractant, releasing P from struvite crystals into solution. If this mechanism is also active in soil, for example through the activity of organic anions such as citrate, it could help to explain the relatively good performance of struvite in neutral to alkaline soils in some cases.

Some less-common soil extractions are thought to mimic the activity of organic acids or anions in soil. For example, soil extractions using low concentrations of citric, malic, and oxalic acids (at pH <3.5 and 7.5; 1–100 mM) have been tested for their ability to provide insights on the mobilization of P, Ca, and Fe by organic anions exuded from roots in calcareous soils (Ström et al. 2005). Others have used a weak citric acid extraction (10 mM) to characterize the P availability in soils under differing land use or with contrasting properties (DeLuca et al. 2015; Jalali and Jalali 2016). The H3A test is a mixture of low-molecular-weight organic acids also designed to mimic root exudate activity in soil (Haney et al. 2016). Theoretically, the P in struvite should be detected by such tests, as it is thought to be released through the activity of organic acids (Talboys et al. 2016). However, Gu et al. (2021) found that extraction of P directly from struvite using the acidic H3A test was lower than with the alkaline Olsen-P test, suggesting an alternative mechanism of P dissolution. Weak citrate extractions have not been tested on struvite or struvite-amended soils, to my knowledge.

Soil processes appear to promote struvite dissolution, based on the increased solubility of struvite when incubated with soil relative to its solubility in water or other weak extractants (Duboc et al. 2017; Meyer et al. 2018; Hall et al. 2020). Duboc et al. (2017) found that an Olsen-P extraction from soil incubated 5 d with ground recycled fertilizers, including struvite, was a better predictor ($R^2 = 0.88$) of crop P uptake than the solubility of these fertilizers according to standard fertilizer characterization extractions (water, $R^2 = 0.37$; 2% citric acid, $R^2 = 0.39$). However, extractions from incubated soils amended with struvite can also yield unexpected and perhaps misleading results. Previous studies have reported higher concentrations and recovery of soluble soil P (water-extractable, $CaCl_2$-extractable, or isotopically exchangeable P) in soils containing remnants of applied struvite granules than in MAP-amended soils, attributing these results to the sample preparation and/or extraction processes (Degryse et al. 2017; Everaert et al. 2017). A rainfall simulation study showed that runoff loss of P from struvite was vastly smaller than that from MAP (Everaert et al. 2018), despite greater solution P in soil amended with struvite rather than MAP in an incubation study (Everaert et al. 2017). Similarly, including residual granules of struvite in soil samples analyzed with agronomic soil tests could result in overestimation of available soil P, while removing residual granules would underestimate available soil P by ignoring future P release from struvite (Gu et al. 2021). These results highlight the need for better understanding of how struvite P interacts with various standard tests and whether the agronomic and environmental management recommendations stemming from such tests are applicable for struvite-amended soils.

1.4.2 The Fertilizer Value of Struvite

Despite struvite's low solubility in water, research conducted to date (much of it under controlled conditions) shows that struvite is a relatively effective P fertilizer for crops. Past research has shown similar crop response with struvite and soluble P fertilizers, especially in acidic to neutral-pH soils (Cabeza et al. 2011; Talboys et al. 2016; Vogel et al. 2017; Horta 2017). Several studies have indicated that crop response to struvite was greater than crop response to most other recovered P fertilizers or phosphate rock across a range of soils (Meyer et al. 2018; Wollmann et al. 2018). Thus, struvite may be a relatively effective P source for use in organic crop production systems, especially in alkaline soils, where phosphate rock is ineffective (Rahman et al. 2014). However, results among and sometimes within individual studies vary considerably, highlighting the importance of interactions among the properties of the struvite itself, the soil to which it is applied, and the crop grown.

1.4.3 Selected Factors Affecting Soil–Struvite–Crop Dynamics

1.4.3.1 Struvite Particle Size

Dissolution of struvite in soil and the subsequent transformation of P to other forms are affected by the characteristics of the struvite itself, sometimes in interaction with soil properties. The importance of struvite particle size has been recognized for decades, with Bridger et al. (1962) recommending pulverized struvite for short-duration crops such as radishes and large granules of struvite for trees. Although commercial struvite fertilizers such as Crystal Green are applied in granular form (available sizes of Crystal Green range from 0.9 to 3 mm diameter), much of the past research demonstrating relatively high solubility of struvite in soil has been conducted using finely ground struvite (e.g., Achat et al. 2014; Duboc et al. 2017; Meyer et al. 2018; Wollmann et al. 2018). Meta-analysis showed no overall effect of struvite particle size on relative crop performance (Hertzberger et al. 2020), but individual studies have demonstrated differences in the patterns of P release from granular vs. ground struvite, especially in certain soil types. For example, in four Arkansas soils with slightly acidic pH but a wide range of clay content (9–37%), ground struvite showed rapid release to the water-soluble P pool, especially in the soil with greatest clay content, followed by a decline in water-soluble P over time. In contrast, granular struvite gradually increased the concentration of water-soluble P in the high-clay soil over six months, with no differences over time in the other soils (Anderson et al. 2020). Studies on Australian soils with a range of pH and P retention capacity showed that granular struvite dissolved more slowly than ground struvite, but the difference was much greater in alkaline soils than in acidic soils (Degryse et al. 2017; Everaert et al. 2017); in these studies, crop response was also smaller with granular relative to ground struvite, especially in alkaline soils. Intermediate granule sizes have received little research attention, but a recent study comparing 1.5- and 3.0-mm Crystal Green granules in blends with MAP in an acidic Illinois soil reported no effect of struvite granule size on maize or soybean growth or P uptake, but generally greater concentrations of soil Mehlich-3 P with 1.5-mm rather than 3.0-mm granules (Hertzberger et al. 2021). The findings on the differences in behaviour among different struvite particle sizes have implications for the interpretation of results from other studies using ground struvite.

1.4.3.2 Soil Properties

Soil pH is expected to have a large impact on struvite solubility and agronomic performance, based on the solubility properties of struvite itself. Several studies have reported poorer crop performance with struvite relative to soluble fertilizers in alkaline soils (Massey et al. 2009; Ackerman et al. 2013; Hilt et al. 2016; Robles-Aguilar et al. 2019), especially when granular fertilizers were used, as previously noted (Degryse et al. 2017; Everaert et al. 2017). According to a recent meta-analysis, crop biomass and P uptake from struvite were generally similar to those attained with soluble fertilizers in acidic soils and declined slightly with increasing soil pH; however, soil pH explained only 0.5% and 8% of the variation in crop biomass and P uptake, respectively (Hertzberger et al. 2020).

Along with pH, soil P retention capacity is expected to have an impact on struvite dissolution and agronomic performance, as occurs with other P sources (e.g., Duminda et al. 2017). Soil texture is correlated with P retention capacity (Ige et al. 2011) and could therefore affect struvite behaviour in soil. Several studies comparing the P dynamics associated with struvite in contrasting soils have noted possible effects of P retention capacity or texture (Katanda et al. 2016; Degryse et al. 2017; Everaert et al. 2017; do Nascimento et al. 2018; Anderson et al. 2020), but have not used a systematic approach to isolate the effect of soil texture or P retention capacity from other soil properties and thus do not demonstrate clear implications for crop P supply. For example, greater P retention in soil could promote struvite dissolution by reducing the P concentration in soil solution (Degryse et al. 2017) but this mechanism could also reduce P diffusion away from struvite granules (Everaert et al. 2017). In a wheat–canola crop sequence study under controlled conditions, greater P recovery efficiency was observed in a sandy soil relative to a clay loam in the first crop after struvite application, but the opposite pattern emerged in subsequent crop phases (Katanda et al. 2016). The meta-analysis of Hertzberger et al. (2020) showed no effect of soil texture on crop response to struvite; however, it is noteworthy that most studies included in that analysis were conducted on loamy and sandy soils rather than on clay soils.

1.4.4 Crop P Acquisition Mechanisms

The effect of struvite on crop performance has been assessed in a range of crops including annual cereals and oilseeds, annual grain legumes, forage grasses and legumes, and vegetables (Hertzberger et al. 2020). In direct comparisons of different crop species, previous research has shown that the response to struvite of canola (Katanda et al. 2016), buckwheat (Talboys et al. 2016), and soybean (Rech et al. 2019) was larger than that of wheat. In contrast, maize showed a larger response to struvite than soybean in a study assessing struvite–MAP blends (Hertzberger et al. 2021). Other research has reported that P uptake by plants fertilized with struvite was equal to or greater than that of plants fertilized with TSP for all crops tested—maize, sorghum, amaranth, forage rye, and sunflower (Vogel et al. 2015). The reason for differences among crop types has sometimes been attributed to production of organic acids or anions by crop roots, as demonstrated in vitro by Talboys et al. (2016). However, Rech et al. (2019) were unable to reproduce this effect using weaker concentrations of organic acids. The relationship between organic acid/anion production and response of crops to struvite has not been tested directly in crop plants, to my knowledge.

Many crop species associate with arbuscular mycorrhizal fungi (AMF) and rely on this symbiotic relationship for a portion of their P acquisition. Although AMF generally access the same pool of soil P as plant roots, there is some evidence that AMF can enhance dissolution of sparingly soluble P sources such as rock phosphate (Ramirez et al. 2009) while others have shown no effect (Antunes et al. 2007). A study comparing mycorrhizal and non-mycorrhizal tomato plants demonstrated greater P uptake in mycorrhizal plants fertilized with struvite rather than MAP but no difference between fertilizer treatments in the non-mycorrhizal plants (Di Tomassi et al. 2021). These authors concluded that AMF enhanced the ability of plants to take up P from struvite; however, the mechanism did not appear to be through additional dissolution of struvite, but rather though effective scavenging of P released from struvite via other processes. In contrast, a study on cereal rye grown in struvite-amended sand, with and without a commercial AMF inoculant, reported greater P uptake from struvite in uninoculated plants than in inoculated plants due to greater root biomass and soil exploration in the uninoculated plants (Schwalb et al. 2021). From these two studies, we can speculate that the P-scavenging ability of plants (possibly but not necessarily in partnership with AMF) is important for P acquisition from struvite, but further research is required to test this hypothesis.

Though AMF may or may not enhance P uptake from struvite, use of struvite as a P source may help to support this (usually) beneficial symbiosis by limiting the rate of P release to soluble soil P pools. Establishment of the AMF symbiosis is particularly sensitive to P supply very early in plant development (Balzergue et al. 2011, 2013), so the relatively slow dissolution of struvite could reduce the suppression of AMF root colonization often seen with soluble P fertilizers (Grant et al. 2005; Bittman et al. 2006). Even if AMF colonization does not increase P supply from struvite to crops, reducing the negative effects of P fertilizers on AMF can support the other beneficial functions of AMF in plant and soil health, such as crop disease resistance, drought tolerance, soil aggregation, and soil organic matter accumulation (Hamel and Strullu 2006). Di Tomassi et al. (2021) found no difference in AMF colonization between struvite- and MAP-amended tomato plants; however, plant–AMF relationships are likely to differ with crop type and in different soils and thus deserve further research.

1.5 Management of Struvite Fertilizer in Crop Production

Despite promising results on the potential of struvite as an effective P fertilizer, little attention has been devoted to development of management guidelines for struvite, especially in organic cropping systems where the recommended practice of blending struvite with a soluble fertilizer (Benjannet et al. 2020; Hertzberger et al. 2021) is not an option. The frequent occurrence of interactions among crop species and/or soil types with struvite vs. soluble fertilizers suggests that current fertilizer recommendations are not necessarily applicable for struvite. In addition, the presence of undissolved granules after plant growth (Talboys et al. 2016; Degryse et al. 2017; Rech et al. 2019) or observed residual effects of struvite beyond the life cycle of the first crop (Katanda et al. 2016; Wollmann et al. 2018; Szymańska et al. 2020) indicate that assessments of P supply to crops beyond a single growing season are needed. Although comparisons to other fertilizers continue to be useful, optimal use of struvite fertilizer will only be possible with a thorough understanding of its behaviour in diverse growing conditions and under various management practices.

1.6 Objectives

Although struvite shows promise as an effective P fertilizer, information on the interacting factors that contribute to optimization of a holistic set of goals, including productivity and soil health considerations, is lacking. Therefore, the overall objective of this study was to assess the impact of using struvite fertilizer as a P source on soil–fertilizer–crop dynamics in the context of organic cropping systems on soils with low soil test P, characteristic of the Northern Great Plains region. Specific objectives of this research were to (i) evaluate the effect of struvite application rate on the productivity and P use efficiency of organic crops under field conditions in an alkaline soil (Chapter 2); (ii) characterize the changes in labile P pools due to struvite application over time in soils incubated without plants (Chapter 3); and (iii) evaluate the effects of struvite fertilizer on crop productivity, root colonization with AMF, and labile soil P pools in soils planted to alfalfa under controlled conditions (Chapter 4). By investigating soil P dynamics in controlled-environment experiments using soil from the field study site, along with a contrasting soil type, this study aims to qualitatively link findings across experiments to provide context for interpretation of results, guide recommendations for use of struvite under a broader range of field conditions, and identify areas requiring further research.

1.7 Thesis Outline

The structure of this thesis follows the guidelines established by the Department of Soil Science, University of Manitoba, for a manuscript-based thesis. The research chapters (Chapters 2 through 4) were prepared as individual manuscripts suitable for publication and are as follows:

Chapter 2: Response of organic grain and forage crops to struvite application in an alkaline soil

Chapter 3: Effect of struvite application on labile phosphorus pools in contrasting Manitoba soils

Chapter 4: The potential of struvite fertilizer to optimize plant growth, labile soil phosphorus, and mycorrhizal fungi in alfalfa

1.8 References

Achat, D.L., Sperandio, M., Daumer, M.-L., Santellani, A.-C., Prud'Homme, L., Akhtar, M., and Morel, C. 2014. Plant-availability of phosphorus recycled from pig manures and dairy effluents as assessed by isotopic labeling techniques. Geoderma **232–234**: 24–33. doi:10.1016/j.geoderma.2014.04.028.

Ackerman, J.N., Zvomuya, F., Cicek, N., and Flaten, D. 2013. Evaluation of manure-derived struvite as a phosphorus source for canola. Can. J. Plant Sci. **93**: 419–424. doi:10.4141/cjps2012-207.

Anderson, R., Brye, K.R., Greenlee, L., and Gbur, E. 2020. Chemically precipitated struvite dissolution dynamics over time in various soil textures. Agric. Sci. **11**: 567–591. doi:10.4236/as.2020.116036.

Antunes, P.M., Schneider, K., Hillis, D., and Klironomos, J.N. 2007. Can the arbuscular mycorrhizal fungus *Glomus intraradices* actively mobilize P from rock phosphates? Pedobiologia **51**: 281–286. doi:10.1016/j.pedobi.2007.04.007.

Arcand, M.M., and Schneider, K.D. 2006. Plant- and microbial-based mechanisms to improve the agronomic effectiveness of phosphate rock: a review. An. Acad. Bras. Ciênc. **78**: 791–807. doi:10.1590/S0001-37652006000400013.

Balzergue, C., Chabaud, M., Barker, D.G., Bécard, G., and Rochange, S.F. 2013. High phosphate reduces host ability to develop arbuscular mycorrhizal symbiosis without affecting root calcium spiking responses to the fungus. Front. Plant Sci. **4**: article 426. doi:10.3389/fpls.2013.00426.

Balzergue, C., Puech-Pagès, V., Bécard, G., and Rochange, S.F. 2011. The regulation of arbuscular mycorrhizal symbiosis by phosphate in pea involves early and systemic signalling events. J. Exp. Bot. **62**: 1049–1060. doi:10.1093/jxb/erq335.

Beaton, J.D., Speer, R., Read, D.W.L., and Dueck, A. 1963. Distribution of monocalcium and monoammonium phosphate reaction products in a calcareous Saskatchewan soil. Nature **198**: 813–814. doi:10.1038/198813b0.

Bell, L.C., and Black, C.A. 1970. Crystalline phosphates produced by interaction of orthophosphate fertilizers with slightly acid and alkaline soils. Soil Sci. Soc. Am. J. **34**: 735–740. doi:10.2136/sssaj1970.03615995003400050020x.

Benjannet, R., Nyiraneza, J., Khiari, L., Cambouris, A., Fuller, K., Hann, S., and Ziadi, N. 2020. Potato response to struvite compared with conventional phosphorus fertilizer in Eastern Canada. Agron. J. **112**: 1360–1376. doi:10.1002/agj2.20118.

Bennett, A.C., and Adams, F. 1976. Solubility and solubility product of dicalcium phosphate dihydrate in aqueous solutions and soil solutions. Soil Sci. Soc. Am. J. **40**: 39–42. doi:10.2136/sssaj1976.03615995004000010014x.

Bittman, S., Kowalenko, C.G., Hunt, D.E., Forge, T.A., and Wu, X. 2006. Starter phosphorus and broadcast nutrients on corn with contrasting colonization by mycorrhizae. Agron. J. **98**: 394–401. doi:10.2134/agronj2005.0093.

Bridger, G.L., Salutsky, M.L., and Starostka, R.W. 1962. Micronutrient sources, metal ammonium phosphates as fertilizers. J. Agric. Food Chem. **10**: 181–188. doi:10.1021/jf60121a006.

Cabeza, R., Steingrobe, B., Römer, W., and Claassen, N. 2011. Effectiveness of recycled P products as P fertilizers, as evaluated in pot experiments. Nutr. Cycl. Agroecosystems **91**: 173–184. doi:10.1007/s10705-011-9454-0.

Canadian General Standards Board 2020.December 16. Organic agriculture. [Online] Available: https://www.tpsgc-pwgsc.gc.ca/ongc-cgsb/programme-program/normes-standards/comm/32-20-agriculture-eng.html [2022 Apr. 1].

Canadian General Standards Board 2021a. Organic production systems: Permitted substances lists, CAN/CGSB-32.311-2020. Canadian General Standards Board. [Online] Available: http://publications.gc.ca/collections/collection_2020/ongc-cgsb/P29-32-311-2020-eng.pdf [2021 Apr. 8].

Canadian General Standards Board 2021b. Organic production systems: General principles and management standards, CAN/CGSB-32.311-2020. Canadian General Standards Board. [Online] Available: http://publications.gc.ca/collections/collection_2020/ongc-cgsb/P29-32-310-2020-eng.pdf [2021 Apr. 8].

Chen, M., and Graedel, T.E. 2016. A half-century of global phosphorus flows, stocks, production, consumption, recycling, and environmental impacts. Glob. Environ. Change **36**: 139–152. doi:10.1016/j.gloenvcha.2015.12.005.

Childers, D.L., Corman, J., Edwards, M., and Elser, J.J. 2011. Sustainability challenges of phosphorus and food: solutions from closing the human phosphorus cycle. BioScience **61**: 117–124. doi:10.1525/bio.2011.61.2.6.

Cordell, D., Drangert, J.-O., and White, S. 2009. The story of phosphorus: Global food security and food for thought. Glob. Environ. Change **19**: 292–305.

Degryse, F., Baird, R., Silva, R., and McLaughlin, M. 2017. Dissolution rate and agronomic effectiveness of struvite fertilizers - effect of soil pH, granulation and base excess. Plant Soil **410**: 139–152. doi:10.1007/s11104-016-2990-2.

DeLuca, T.H., Glanville, H.C., Harris, M., Emmett, B.A., Pingree, M.R.A., de Sosa, L.L., Cerdá-Moreno, C., and Jones, D.L. 2015. A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. Soil Biol. Biochem. **88**: 110–119. doi:10.1016/j.soilbio.2015.05.016.

Di Tomassi, I., Chatterjee, N., Barrios-Masias, F.H., Zhou, Q., Gu, C., and Margenot, A.J. 2021. Arbuscular mycorrhizae increase biomass and nutrient uptake of tomato fertilized with struvite compared to monoammonium phosphate. Plant Soil **464**: 321–333. doi:10.1007/s11104-021-04957-2.

Duboc, O., Santner, J., Golestani Fard, A., Zehetner, F., Tacconi, J., and Wenzel, W.W. 2017. Predicting phosphorus availability from chemically diverse conventional and recycling fertilizers. Sci. Total Environ. **599–600**: 1160–1170. doi:10.1016/j.scitotenv.2017.05.054.

Duminda, D.M.S., Kumaragamage, D., Indraratne, S.P., and Flaten, D. 2017. Fertilizer-induced phosphorus dynamics in alkaline-calcareous soils as influenced by soil chemical properties. Can. J. Soil Sci. **97**: 159–170. doi:10.1139/cjss-2016-0055.

Egle, L., Rechberger, H., Krampe, J., and Zessner, M. 2016. Phosphorus recovery from municipal wastewater: An integrated comparative technological, environmental and economic assessment of P recovery technologies. Sci. Total Environ. **571**: 522–542. doi:10.1016/j.scitotenv.2016.07.019.

Everaert, M., Degryse, F., McLaughlin, M.J., De Vos, D., and Smolders, E. 2017. Agronomic effectiveness of granulated and powdered P-exchanged Mg–Al LDH relative to struvite and MAP. J. Agric. Food Chem. **65**: 6736–6744. doi:10.1021/acs.jafc.7b01031.

Everaert, M., da Silva, R.C., Degryse, F., McLaughlin, M.J., and Smolders, E. 2018. Limited dissolved phosphorus runoff losses from layered double hydroxide and struvite fertilizers in a rainfall simulation study. J. Environ. Qual. **47**: 371–377. doi:10.2134/jeq2017.07.0282.

Expert Group for Technical Advice on Organic Production 2016. Final report on organic fertilizers and soil conditioners (II). European Commission Directote-General for Agriculture and Rural Development. [Online] Available: https://ec.europa.eu/info/sites/info/files/food-farming-fisheries/farming/documents/final-report-egtop-fertilizers-ii_en.pdf [2020 Jun. 9].

Gell, K., Ruijter, F.J. de, Kuntke, P., Graaff, M. de, and Smit, A.L. 2011. Safety and effectiveness of struvite from black water and urine as a phosphorus fertilizer. J. Agric. Sci. **3**: p67. doi:10.5539/jas.v3n3p67.

Grant, C., Bittman, S., Montreal, M., Plenchette, C., and Morel, C. 2005. Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. Can. J. Plant Sci. **85**: 3–14. doi:10.4141/P03-182.

Gu, C., Zhou, Q., Cusick, R.D., and Margenot, A.J. 2021. Evaluating agronomic soil phosphorus tests for soils amended with struvite. Geoderma **399**: 115093. doi:10.1016/j.geoderma.2021.115093.

Hall, R.L., Boisen Staal, L., Macintosh, K.A., McGrath, J.W., Bailey, J., Black, L., Gro Nielsen, U., Reitzel, K., and Williams, P.N. 2020. Phosphorus speciation and fertiliser performance characteristics: A comparison of waste recovered struvites from global sources. Geoderma **362**: 114096. doi:10.1016/j.geoderma.2019.114096.

Hamel, C., and Strullu, D.-G. 2006. Arbuscular mycorrhizal fungi in field crop production: Potential and new direction. Can. J. Plant Sci. **86**: 941–950. doi:10.4141/P05-099.

Haney, R.L., Haney, E.B., Harmel, R.D., Smith, D.R., and White, M.J. 2016. Evaluation of H3A for determination of plant available P vs. FeAlO strips. Open J. Soil Sci. **6**: 175–187. doi:10.4236/ojss.2016.611017.

Hertzberger, A.J., Cusick, R.D., and Margenot, A.J. 2020. A review and meta-analysis of the agricultural potential of struvite as a phosphorus fertilizer. Soil Sci. Soc. Am. J. **84**: 653–671. doi:10.1002/saj2.20065.

Hertzberger, A.J., Cusick, R.D., and Margenot, A.J. 2021. Maize and soybean response to phosphorus fertilization with blends of struvite and monoammonium phosphate. Plant Soil **461**: 547–563. doi:10.1007/s11104-021-04830-2.

Hilt, K., Harrison, J., Bowers, K., Stevens, R., Bary, A., and Harrison, K. 2016. Agronomic response of crops fertilized with struvite derived from dairy manure. Water. Air. Soil Pollut. **227**: 388. doi:10.1007/s11270-016-3093-7.

Horta, C. 2017. Bioavailability of phosphorus from composts and struvite in acid soils. Rev. Bras. Eng. Agríc. E Ambient. **21**: 459–464. doi:10.1590/1807-1929/agriambi.v21n7p459-464.

Huygens, D., and Saveyn, H.G.M. 2018. Agronomic efficiency of selected phosphorus fertilisers derived from secondary raw materials for European agriculture. A meta-analysis. Agron. Sustain. Dev. **38**: 52. doi:10.1007/s13593-018-0527-1.

IFOAM n.d. The four principles of organic agriculture. [Online] Available: https://www.ifoam.bio/en/organic-landmarks/principles-organic-agriculture [2022 Mar. 28].

Ige, D.V., Akinremi, O.O., Flaten, D.N., Ajiboye, B., and Kashem, M.A. 2011. Phosphorus sorption capacity of alkaline Manitoba soils and its relationship to soil properties. Can. J. Soil Sci. doi:10.4141/S04-064.

Jalali, M., and Jalali, M. 2016. Relation between various soil phosphorus extraction methods and sorption parameters in calcareous soils with different texture. Sci. Total Environ. **566–567**: 1080–1093. doi:10.1016/j.scitotenv.2016.05.133.

Jarvie, H.P., Johnson, L.T., Sharpley, A.N., Smith, D.R., Baker, D.B., Bruulsema, T.W., and Confesor, R. 2017. Increased soluble phosphorus loads to Lake Erie: Unintended consequences of conservation practices? J. Environ. Qual. **46**: 123–132. doi:10.2134/jeq2016.07.0248.

Johnston, A.E., and Richards, I.R. 2003. Effectiveness of different precipitated phosphates as phosphorus sources for plants. Soil Use and Management 19: 45–49. doi:10.1111/j.1475-2743.2003.tb00278.x.

Kabbe, C. 2021. Inventory of phosphorus “recovery and/or recycling” facilities operating or under construction at or downstream of wastewater treatment installations, v.10. P-Rex Environment. [Online] Available: https://www.phosphorusplatform.eu/images/download/Kabbe_P-recorecy_tech_implementation%20Table_2021_10_31.pdf [2022 Apr. 2].

Katanda, Y., Zvomuya, F., Flaten, D., and Cicek, N. 2016. Hog-manure-recovered struvite: Effects on canola and wheat biomass yield and phosphorus use efficiencies. Soil Sci. Soc. Am. J. **80**: 135–146. doi:10.2136/sssaj2015.07.0280.

Kratz, S., Vogel, C., and Adam, C. 2019. Agronomic performance of P recycling fertilizers and methods to predict it: A review. Nutr. Cycl. Agroecosystems **115**: 1–39. doi:10.1007/s10705-019-10010-7.

Le Corre, K.S., Valsami-Jones, E., Hobbs, P., and Parsons, S.A. 2009. Phosphorus recovery from wastewater by struvite crystallization: A review. Crit. Rev. Environ. Sci. Technol. **39**: 433–477. doi:10.1080/10643380701640573.

Lide, D.R. 2007. CRC Handbook of Chemistry and Physics 88TH Edition 2007-2008. CRC Press, Taylor & Francis, Boca Raton, FL.

Mackey, E., Harrison, J.H., Tao, H., Norberg, S., Adams-Progar, A., and Whitefield, E. 2021. Effect of application season on release of available phosphorus from soil receiving mono-ammonium phosphate or struvite. Water. Air. Soil Pollut. **232**: 467. doi:10.1007/s11270-021-05396-6.

Martin, R.C., Lynch, D.H., Frick, B., and Straaten, P. van 2007. Phosphorus status on Canadian organic farms. J. Sci. Food Agric. **87**: 2737–2740. doi:10.1002/jsfa.3077.

Massey, M.S., Davis, J.G., Ippolito, J.A., and Sheffield, R.E. 2009. Effectiveness of recovered magnesium phosphates as fertilizers in neutral and slightly alkaline soils. Agron. J. **101**: 323. doi:10.2134/agronj2008.0144.

Meyer, G., Frossard, E., Mäder, P., Nanzer, S., Randall, D.G., Udert, K.M., and Oberson, A. 2018. Water soluble phosphate fertilizers for crops grown in calcareous soils – an outdated paradigm for recycled phosphorus fertilizers? Plant Soil **424**: 367–388. doi:10.1007/s11104-017-3545-x.

Möller, K., Oberson, A., Bünemann, E.K., Cooper, J., Friedel, J.K., Glæsner, N., Hörtenhuber, S., Løes, A.-K., Mäder, P., Meyer, G., Müller, T., Symanczik, S., Weissengruber, L., Wollmann, I., and Magid, J. 2018. Improved phosphorus recycling in organic farming: Navigating between constraints. Adv. Agron. **147**: 159–237. doi:10.1016/bs.agron.2017.10.004.

do Nascimento, C.A.C., Pagliari, P.H., Faria, L. de A., and Vitti, G.C. 2018. Phosphorus mobility and behavior in soils treated with calcium, ammonium, and magnesium phosphates. Soil Sci. Soc. Am. J. **82**: 622. doi:10.2136/sssaj2017.06.0211.

Nelson, N.O., and Janke, R.R. 2007. Phosphorus sources and management in organic production systems. HortTechnology **17**: 442–454.

Nicksy, J., and Entz, M.H. 2021. Recycled nutrients as a phosphorus source for Canadian organic agriculture: a perspective. Can. J. Soil Sci. **101**: 571–580. NRC Research Press. doi:10.1139/cjss-2021-0014.

Ohlinger, K.N., Young, T.M., and Schroeder, E.D. 1998. Predicting struvite formation in digestion. Water Res. **32**: 3607–3614. doi:10.1016/S0043-1354(98)00123-7.

Olsen, S.R., Cole, C.V., F. S. Watanabe, and Dean, L.A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture, Washington, DC. [Online] Available: http://hdl.handle.net/2027/uiug.30112019277042.

Paulsen, H.M., Köpke, U., Oberson, A., and Rahmann, G. 2016. Phosphorus—The predicament of organic farming. Pages 195–213 *in* E. Schnug and L.J. De Kok, eds. Phosphorus in Agriculture: 100 % Zero. Springer Netherlands, Dordrecht. doi:10.1007/978-94-017-7612-7_10.

Potter, P., Ramankutty, N., Bennett, E.M., and Donner, S.D. 2010. Characterizing the spatial patterns of global fertilizer application and manure production. Earth Interact. **14**: 1–22. doi:10.1175/2009EI288.1.

Rahman, Md.M., Salleh, M.A.Mohd., Rashid, U., Ahsan, A., Hossain, M.M., and Ra, C.S. 2014. Production of slow release crystal fertilizer from wastewaters through struvite crystallization – A review. Arab. J. Chem. **7**: 139–155. doi:10.1016/j.arabjc.2013.10.007.

Ramirez, R., Mendoza, B., and Lizaso, J.I. 2009. Mycorrhiza effect on maize P uptake from phosphate rock and superphosphate. Commun. Soil Sci. Plant Anal. **40**: 2058–2071. doi:10.1080/00103620902960583.

Rech, I., Withers, P.J.A., Jones, D.L., and Pavinato, P.S. 2019. Solubility, diffusion and crop uptake of phosphorus in three different struvites. Sustainability **11**: 134. doi:10.3390/su11010134.

Roberts, T.L., and Johnston, A.E. 2015. Phosphorus use efficiency and management in agriculture. Resour. Conserv. Recycl. **105**: 275–281. doi:10.1016/j.resconrec.2015.09.013.

Robles-Aguilar, A.A., Pang, J., Postma, J.A., Schrey, S.D., Lambers, H., and Jablonowski, N.D. 2019. The effect of pH on morphological and physiological root traits of *Lupinus angustifolius* treated with struvite as a recycled phosphorus source. Plant Soil **434**: 65–78. doi:10.1007/s11104-018-3787-2.

Sample, E.C., Soper, R.J., and Racz, G.J. 1980. Reactions of phosphate fertilizers in soils. Pages 263–310 *in* F.E. Khasawneg, E.C. Sample, and E.J. Kamprath, eds. The Role of Phosphorus in Agriculture. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI. doi:10.2134/1980.roleofphosphorus.c12.

Schneider, K.D., Thiessen Martens, J.R., Zvomuya, F., Reid, D.K., Fraser, T.D., Lynch, D.H., O'Halloran, I.P., and Wilson, H.F. 2019. Options for improved phosphorus cycling and use in agriculture at the field and regional scales. J. Environ. Qual. **48**: 1247–1264. doi:10.2134/jeq2019.02.0070.

Schoenau, J.J., and O'Halloran, I.P. 2008. Sodium bicarbonate-extractable phosphorus. Pages 89–94 *in* M.R. Carter and E.G. Gregorich, eds. Soil Sampling and Methods of Analysis, 2nd edition. CRC Press, Boca Raton, FL, USA.

Schwalb, S.A., Hemkemeyer, M., Watson, C., and Wichern, F. 2021. Mycorrhiza reduces phosphorus uptake from struvite in rye (*Secale cereale* L.) plants. J. Soil Sci. Plant Nutr. **21**: 3451–3460. doi:10.1007/s42729-021-00619-5.

Sharpley, A., Jarvie, H., Flaten, D., and Kleinman, P. 2018. Celebrating the 350th anniversary of phosphorus discovery: A conundrum of deficiency and excess. J. Environ. Qual. **47**: 774–777. doi:10.2134/jeq2018.05.0170.

Snyder, C., and Spaner, D. 2010. The sustainability of organic grain production on the Canadian prairies – A review. Sustain. Basel **2**: 1016–1034. doi:10.3390/su2041016.

Ström, L., Owen, A.G., Godbold, D.L., and Jones, D.L. 2005. Organic acid behaviour in a calcareous soil: implications for rhizosphere nutrient cycling. Soil Biol. Biochem. **37**: 2046–2054. doi:10.1016/j.soilbio.2005.03.009.

Szymańska, M., Sosulski, T., Bożętka, A., Dawidowicz, U., Wąs, A., Szara, E., Malak-Rawlikowska, A., Sulewski, P., van Pruissen, G.W.P., and Cornelissen, R.L. 2020. Evaluating the struvite recovered from anaerobic digestate in a farm bio-refinery as a slow-release fertiliser. Energies **13**: 5342. doi:10.3390/en13205342.

Talboys, P.J., Heppell, J., Roose, T., Healey, J.R., Jones, D.L., and Withers, P.J.A. 2016. Struvite: a slow-release fertiliser for sustainable phosphorus management? Plant Soil **401**: 109–123. doi:10.1007/s11104-015-2747-3.

Vogel, T., Kruse, J., Siebers, N., Nelles, M., and Eichler-Löbermann, B. 2017. Recycled products from municipal wastewater: Composition and effects on phosphorus mobility in a sandy soil. J. Environ. Qual. **46**: 443–451. doi:10.2134/jeq2016.10.0392.

Vogel, T., Nelles, M., and Eichler-Löbermann, B. 2015. Phosphorus application with recycled products from municipal waste water to different crop species. Ecol. Eng. **83**: 466–475. doi:10.1016/j.ecoleng.2015.06.044.

Weissengruber, L., Möller, K., Puschenreiter, M., and Friedel, J.K. 2018. Long-term soil accumulation of potentially toxic elements and selected organic pollutants through application of recycled phosphorus fertilizers for organic farming conditions. Nutr. Cycl. Agroecosystems **110**: 427–449. doi:10.1007/s10705-018-9907-9.

Welsh, C., Tenuta, M., Flaten, D.N., Thiessen-Martens, J.R., and Entz, M.H. 2009. High yielding organic crop management decreases plant-available but not recalcitrant soil phosphorus. Agron. J. **101**: 1027–1035. doi:10.2134/agronj2009.0043.

Withers, P., Doody, D., and Sylvester-Bradley, R. 2018. Achieving sustainable phosphorus use in food systems through circularisation. Sustainability **10**: 1804. doi:10.3390/su10061804.

Withers, P.J.A., Neal, C., Jarvie, H.P., and Doody, D.G. 2014. Agriculture and eutrophication: Where do we go from here? Sustainability **6**: 5853–5875. doi:10.3390/su6095853.

Wollmann, I., Gauro, A., Müller, T., and Möller, K. 2018. Phosphorus bioavailability of sewage sludge-based recycled fertilizers. J. Plant Nutr. Soil Sci. **181**: 158–166. doi:10.1002/jpln.201700111.

Zhang, T.Q., MacKenzie, A.F., Liang, B.C., and Drury, C.F. 2004. Soil test phosphorus and phosphorus fractions with long-term phosphorus addition and depletion. Soil Sci. Soc. Am. J. Madison **68**: 519–528.

Ziadi, N., Whalen, J.K., Messiga, A.J., and Morel, C. 2013. Assessment and modeling of soil available phosphorus in sustainable cropping systems. Advances in Agronomy 122: 85–126. doi:10.1016/B978-0-12-417187-9.00002-4.

2. RESPONSE OF ORGANIC GRAIN AND FORAGE CROPS TO STRUVITE APPLICATION IN AN ALKALINE SOIL

2.1 Abstract

Struvite ($NH_4MgPO_4 \cdot 6H_2O$) may be an appropriate fertilizer to address phosphorus (P) deficiencies in organic cropping systems, but field studies assessing crop response to struvite are lacking. Field experiments were conducted over 3 yr on a low-P, alkaline soil in Manitoba to assess the effect of struvite application rate on the yield and P accumulation of organically managed grain and forage crops. Struvite was applied to spring wheat (*Triticum aestivum* L.) and flax (*Linum usitatissimum* L.) at 0, 20, 30, and 40 kg P ha^{-1} in separate experiments each year and to alfalfa (*Medicago sativa* L.)–grass forage at 0, 30, 60, and 90 kg P ha^{-1} in a single application in a 3-yr experiment. Wheat grain yield, P concentration, and P accumulation increased linearly with increasing struvite rate, whereas flax showed little to no response. Forage yield, P concentration, and P accumulation also increased with struvite rate. Benefits to forage yield and P accumulation were greatest in the second year, demonstrating important residual effects of struvite application. Struvite application shifted forage composition to become dominated by alfalfa whereas the unfertilized treatment was dominated by grasses. Annual P recovery efficiency was 4–7% for wheat, 1–2% for flax, and 7–12% for forage and did not vary significantly with struvite application rate. Our findings demonstrate that struvite applied at a relatively high rate is an effective P source for wheat and alfalfa-based forage under organic management, but not for flax.

2.2 Introduction

Phosphorus management in agricultural systems faces several intertwined challenges, including the projected depletion of mined P reserves, environmental degradation due to excess P, especially in freshwater systems, and the need to satisfy crop P requirements to support food production (Withers et al. 2014; Cordell and White 2014; Filippelli 2018; Withers 2019). Concentration of intensive livestock production operations and human populations in geographically separate regions from much of the world's cropland means that P has become concentrated in some regions and depleted in others (Chen and Graedel 2016; Reid et al. 2019). Stockless cropping systems following organic production standards are particularly prone to developing P deficits and soil P deficiencies (Entz et al. 2001; Welsh et al. 2009; Knight et al. 2010) due in part to the scarcity or poor bioavailability of P inputs currently permitted in organic production (Martin et al. 2007; Möller et al. 2018).

Creating a circular economy for P, in which P is recovered from waste streams and used as a fertilizer, is a priority for sustainable P management (Chen and Graedel 2016; Schneider et al. 2019; Withers 2019; Nicksy and Entz 2021). While many processes for P recovery exist, the extraction of struvite ($NH_4MgPO_4 \cdot 6H_2O$) from nutrient-rich waste streams, including municipal wastewater, has gained considerable attention as an economically viable process that can yield a product with low levels of contamination, high P concentration, and high P bioavailability relative to other recovered P products (Ahmed et al. 2018; Huygens and Saveyn 2018).

Due to its low solubility in water, struvite is often characterized as a slow-release fertilizer (Talboys et al. 2016), but its solubility characteristics in a laboratory do not necessarily provide an accurate prediction of its value as a nutrient source for crops (Meyer et al. 2018). The small but growing body of published research on the agronomic performance of struvite fertilizers demonstrates that crop response to struvite is superior to that of phosphate rock (Weissengruber et al. 2018; Wollmann et al. 2018) and is often similar to soluble P fertilizers, especially in acidic to neutral pH soils (as reviewed by Hertzberger et al., 2020). In alkaline soils, however, crop response to struvite is often inferior relative to soluble fertilizers (Ackerman et al. 2013; Hilt et al. 2016; Hertzberger et al. 2020), especially with granular rather than finely ground struvite (Degryse et al. 2017). Crop response to struvite may also vary with crop species (Katanda et al. 2016; Talboys et al. 2016; Rech et al. 2019) and soil type (Katanda et al. 2016; Everaert et al. 2017), demonstrating the need to conduct thorough investigations into soil–struvite–plant interactions under a wide variety of conditions. Much of the knowledge generated so far regarding struvite fertilizer management comes from controlled-environment experiments comparing struvite to other P sources, often in short-term studies that do not assess impact on crop grain yields (Hertzberger et al. 2020). Little attention has been paid to assessment of agronomic practices for struvite use under field conditions, a necessary step for the development of appropriate management recommendations.

Several authors have identified struvite as a P source well-suited to organic crop production due to its low concentration of potentially toxic elements and good agronomic value relative to phosphate rock, especially under neutral to alkaline soil conditions (Weissengruber et al. 2018; Wollmann et al. 2018; Huygens and Saveyn 2018). Use of a recycled product such as struvite also supports the principles of organic agriculture emphasizing nutrient recycling and minimal reliance on non-renewable inputs (IFOAM n.d.). In Canada, struvite made from livestock manure or plant-derived waste streams has been added to the Permitted Substances List of the most recent Organic Production Standard but struvite derived from human waste is not currently permitted (Canadian General Standards Board 2021).

Agronomic studies assessing crop response to struvite under organic management are required to guide organic production recommendations but are extremely scarce to date. In particular, a greater understanding is needed of crop response to granular struvite in the challenging environment of the calcareous, highly P-retaining soils with low soil test P (STP) that commonly occur in organic cropping systems in the Northern Great Plains region. Although struvite may optimally be used in a blend with a soluble fertilizer (Benjannet et al. 2020), assessing struvite as a sole P source is necessary for relevance to organic crop production, where soluble P fertilizers are not permitted. While studies comparing struvite to other fertilizers continue to be useful, the unique soil–struvite–plant interactions observed in the literature demonstrate the need for greater examination of struvite fertilizer management practices, such as application rate, timing, and placement, in a wide range of crops and soils. Assessment of crop yield, P accumulation in biomass or grain, and P use efficiency of annual and perennial crops in response to differing struvite application rates under realistic field conditions will provide a foundation for future research exploring underlying mechanisms and refining fertilizer management practices.

As a step toward addressing this research gap, we conducted field studies in Manitoba, Canada, assessing the effect of granular struvite application on the response of spring wheat (*Triticum aestivum* L.), flax (*Linum usitatissimum* L.), and perennial alfalfa (*Medicago sativa* L.)–grass forage under organic management. These three crop types represent a diversity of plant families and are important contributors to the organic sector of the region. Our objective was to evaluate the effect of struvite application rate on organic crop productivity and P use efficiency under field conditions in a calcareous soil with low STP. We hypothesized that (1) crop yield, P concentration, and P accumulation in grain or forage biomass would increase with struvite application rate for all crops; (2) the beneficial effect of a one-time struvite application to a multi-year forage stand would increase over time due to the slow-release qualities of struvite; and (3) P recovery efficiency in grain or forage biomass would decline with increasing struvite application rates.

2.3 Materials and Methods

2.3.1 Study Site

Field plot experiments were conducted near Libau, MB (50° 14' 34", -96° 43' 44"), in 2017, 2018, and 2019. The site is near the northeastern edge of the Northern Great Plains and has a humid continental climate with mild summers and precipitation occurring year-round (Dfb designation; PlantMaps, n.d.). Long-term (1981-2010) average annual temperature and precipitation are 2.8°C and 570 mm, respectively (Table 2.1; Environment and Climate Change Canada, 2021). Weather data for the study years were collected at a provincially operated weather station 8 km south of the study site (Manitoba Agriculture 2021).

Table 2.1 Monthly, growing season (May to August), and annual precipitation and air temperature for the study years and the 30-yr average

	Total Precipitation				**Average Temperature**			
Period	**2017**	**2018**	**2019**	**30-yr Average**	**2017**	**2018**	**2019**	**30-yr Average**
	mm				°C			
May	22.1	38.8	20.4	58.1	11.3	14.2	9.5	11.4
June	47.9	93.1	24.0	87.5	17.3	18.0	17.2	16.7
July	71.8	31.7	61.4	87.1	19.2	20.0	19.6	19.3
Aug.	38.1	63.0	43.5	76.3	17.3	19.0	17.5	18.5
May–Aug.	179.9	226.6	149.3	309.0	16.3	17.8	16.0	16.5
Annual (Jan.–Dec.)	371.8	397.9	437.3	570.3	3.2	2.2	1.6	2.8

Note. Weather data for study years were recorded at Selkirk, MB, 8 km from the study site (Manitoba Agriculture 2021), and 30-year average (1981-2010) weather data were recorded at Beausejour, MB, 30 km from the study site (Environment and Climate Change Canada 2021).

Soil at the study site is a Udic Boroll in the USDA soil classification and a Gleyed Rego Black Chernozem in the Canadian soil classification, belonging to the Dencross Series. It has clay texture (~45% clay), alkaline pH, and relatively high total organic matter content (Table 2.2).

Table 2.2 Selected soil chemical properties based on soil tests conducted prior to establishment of each experiment

	Wheat			**Flax**			**Alfalfa**
Soil property (unit)	**2017**	**2018**	**2019** [a]	**2017**	**2018**	**2019** [a]	**2016** [b]
Nitrate-N, 0–60 cm (kg ha^{-1}) [c]	142	142	59	95	76	59	16
P, 0–15 cm (mg kg^{-1}) [d]	2	2	6	3	2	6	2.6
K, 0–15 cm (mg kg^{-1}) [e]	345	335	352	297	300	352	333
Sulphate-S, 0–60 cm (kg ha^{-1}) [f]	56	20	72	58	22	72	31
Organic Matter, 0–15 cm (%) [g]	5.5	5.9	4.8	5.3	5.5	4.8	5.9
pH, 0–15 cm [h]	8.1	8.2	8.1	8.1	8.2	8.1	8.1
pH, 15–60 cm [h]	8.3	8.3	8.3	8.4	8.4	8.3	8.4

[a] A single soil test was completed for the wheat and flax experiments in 2019 as they were immediately adjacent and had identical history.

[b] The alfalfa–grass experimental area was sampled in 2016, prior to experiment establishment in 2017.

[c] Extraction in 0.2 M KCl using the Cd reduction determination method (Gelderman and Beegle 2015). Conversion to mass-per-area was performed by the soil analysis lab based on assumptions of typical soil bulk density in the region.

[d] Extraction in 0.5 M $NaHCO_3$ at pH 8.5 (Olsen et al. 1954) using the ascorbic acid–molybdate determination method (Murphy and Riley 1962).

[e] Extraction in 1.0 M NH_4OAc at pH 7.0 with atomic emission spectroscopy (Warncke and Brown 2015).

[f] Extraction in 0.2 M KCl at room temperature with the turbidimetric determination method (Cihacek et al. 2015). Conversion to mass-per-area was performed by the soil analysis lab based on assumptions of typical soil bulk density in the region.

[g] Total organic matter by loss on ignition (Combs and Nathan 2015).

[h] Determined in 1:1 soil:water (Peters et al. 2015).

Although not certified organic, the study site had been managed according to organic production standards since 2008, with no use of crop protection products or fertilizers during this period, including inputs permitted in organic production. This lack of nutrient addition to soil is not optimal organic management but was used to create the low-STP soil conditions often found on organic farms in this region. Crop history at the study site included long-term alfalfa–grass hay production (established in 2005, with one to two hay cuts each year). Locations for individual wheat and flax experiments were transitioned to annual cropping 2–3 yr prior to the present experiments. Each wheat and flax experiment was preceded by a full-season legume–cereal green manure crop in the previous year.

2.3.2 Baseline Soil Sampling and Analyses

Soil samples were collected from each experiment location in the spring prior to seeding, except for the alfalfa–grass experiment, which was sampled in an area immediately adjacent to the experiment location in the previous year. The samples were submitted to a commercial soil testing laboratory (AGVISE Laboratories, Northwood, SD) for analysis of selected properties according to the methods described in Table 2.2. One composite sample was submitted for each wheat and flax experiment (2017 and 2018) or set of adjacent experiments (2019). For the alfalfa–grass experiment, multiple samples were analyzed and results were averaged. Soil particle size distribution was determined using the hydrometer method (Gee and Or 2002) on soil samples collected from the site for a related experiment.

2.3.3 Experimental Design, Management, and Data Collection

2.3.3.1 Wheat and Flax Experiments

Separate experiments were established for ‘Cardale’ hard red spring wheat and ‘CDC Bethune’ flax in 2017, 2018, and 2019, using a randomized complete block design with four replications (blocks) per treatment in each year. The treatments in each experiment were 0, 20, 30, and 40 kg P ha^{-1} (designated 0P, 20P, 30P, and 40P, respectively) applied as the granular struvite fertilizer Crystal Green® (5-28-0 plus 10% Mg guaranteed nutrient analysis, approx. 3 mm diameter; Ostara

Nutrient Recovery Technologies Inc., Vancouver, BC). The 20P application rate was approximately equivalent to the general P recommendations for cereals and flax in low-P soils in Manitoba (Manitoba Agriculture 2007) and to the specific P fertilizer recommendations for target yields of 3.5 and 2.5 Mg ha^{-1} for wheat and flax, respectively, based on soil tests for the experiment locations (16–21 kg P ha^{-1}). Individual experimental units (plots) were 1.8 × 8 m in size in all cases.

Crops were managed according to the Canadian Organic Standard in place at the time (Canadian General Standards Board 2018), with the exception of struvite application, which was not permitted under the standard. No other nutrient sources were applied and no crop protection products such as herbicides were used, to maintain growing conditions as similar as possible to typical certified organic production systems in the region.

Plots were seeded on 15 May 2017, 11 May 2018, and 21 May 2019 with a Fabro plot-scale double-disc drill (Swift Machinery Co., Swift Current, SK) with 15 cm row spacing. Seeding rates targeted relatively high plant populations of 300 and 700 plants m^{-2} for wheat and flax, respectively, with adjustments for seed germination rates and expected seedling mortality. Struvite fertilizer was placed in the furrow with the seed at the specified rates for each treatment. Pre-seeding tillage operations included the use of a field cultivator and light harrows to control spring weed growth. In 2017, annual weeds (primarily wild mustard [*Sinapis arvensis* L.]) were controlled using inter-row tillage approximately 4 wk after seeding. In 2018, no weed control was required due to very low weed populations. In 2019, hand-weeding was used during early crop growth to manage perennial weeds persisting across the experiments from previous years. One block was excluded from analysis in the wheat and flax experiments in 2019 due to very high weed pressure.

To determine whether crops established adequately, plant populations for each crop were assessed 2–3 wk after seeding by counting established crop seedlings in a 1-m length of two adjacent crop rows, at two locations in each plot.

Crop response to addition of struvite fertilizer was evaluated by assessing crop grain yield and grain P concentration in each experiment. Wheat grain yield in all years and flax grain yield in 2017 were determined by harvesting mature grain from the center 1.5 m of each plot with a plot combine. Due to unsuitable weather in 2018 and 2019, flax grain was harvested by hand-cutting plants from quadrats (1.8 and 2.7 m^2 in 2018 and 2019, respectively) in each plot, air-drying samples, and later threshing them with a plot combine used as a stationary thresher. Harvested grain samples were cleaned using a dockage tester to remove chaff and weed seeds prior to weighing.

Subsamples of grain from each plot were ground using a coffee grinder and 0.5 g portions were analyzed for total P concentration at a commercial agricultural testing laboratory (AGVISE Laboratories, Northwood, ND) using inductively coupled plasma–optical emission spectroscopy (Perkin Elmer, Waltham, MA) following digestion with HNO_3 and H_2O_2 at 150°C (Havlin and Soltanpour 1980).

Total P accumulation in grain was calculated as the product of grain yield and P concentration. Apparent P recovery efficiency in grain from applied struvite was calculated for each fertilized plot using the difference method (Syers et al. 2008):

$$\text{P recovery efficiency (\%)} = 100 \times (PA_f - PA_{uf})/P_{app}, \qquad (1)$$

where PA_f and PA_{uf} are the P accumulation in grain in the fertilized and unfertilized treatments, respectively, and P_{app} is the quantity of P applied, all in kg ha^{-1}.

In addition, concentration of P in labile soil pools was assessed in 2018 and 2019 and mycorrhizal colonization in wheat and flax roots was assessed in 2018; methods and results for these variables are reported in Appendix I.

2.3.3.2 Alfalfa–Grass Forage Experiment

A multi-year experiment was conducted in 2017–2019 to assess the effect of struvite application rate on alfalfa–grass forage productivity and P use efficiency over time. The experiment was laid out as a randomized complete block design with four replications (blocks) and a one-way treatment design with four levels. One block was excluded from analysis due to missing data. The treatments were struvite application rates of 0, 30, 60, and 90 kg P ha^{-1} (designated as 0P, 30P, 60P, and 90P, respectively). The 30P rate corresponded approximately to the general P fertilizer recommendation for legume forages in very low-STP soils in Manitoba (Manitoba Agriculture 2007) and to the specific P fertilizer recommendations based on soil test results to achieve alfalfa forage yield of 9 Mg ha^{-1} (27 kg P ha^{-1}). Experimental units (plots) were 4 × 8 m in size.

Commercial granular struvite fertilizer (Crystal Green®, approx. 3 mm diameter) was banded into an existing alfalfa–grass stand (established in 2005) on 15 May 2017 at the specified rates using a Fabro plot-scale double-disc seeder to a depth of 2–3 cm and with a band spacing of 15 cm. No additional struvite was applied in subsequent years. The experiment was managed using organic techniques typical of the region with no additional nutrient sources or crop protection products used. Hay was cut twice annually when alfalfa was in full flower (in late June or July and in late August) using a tractor-mounted rotary mower and then removed from the plots by hand.

Forage response to differing rates of struvite fertilizer was evaluated by assessing above-ground dry matter yield and P concentration of alfalfa–grass forage just prior to hay cutting in each of the 3 yr of the study. Forage yield samples were collected twice during each growing season (18 Jul. and 28 Aug. 2017; 27 Jun. and 24 Aug. 2018; 2 Jul. and 19 Aug. 2019) by clipping above-ground growth about 1 cm above the soil surface within 75 × 75 cm or 100 × 50 cm quadrats in two locations in each plot, avoiding plot edges and areas within plots affected by forage winterkill. Samples were dried to constant weight at ~60 °C in a forced-air oven for at least 48 h and were subsequently weighed. In 2019, samples were hand-separated into alfalfa, grass, and weed components prior to drying to determine effects of struvite application on forage composition. Weights of the separated components were used to calculate the proportion of each component in the forage mix and were summed to obtain total forage yield in each hay cut.

Dried forage samples from each hay cut were ground to pass a 1-mm screen using a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) and subsamples were analyzed for P concentration as described for wheat and flax. In 2019, previously separated legume, grass, and weed components for each plot were recombined into a single sample prior to grinding, subsampling, and analysis.

Above-ground forage P accumulation at each hay harvest was calculated as the product of forage yield and P concentration. For each plot, forage yield and P accumulation values for the two harvests within each year were summed to obtain totals for each year. Average forage P concentration for each year was calculated by dividing total annual forage P accumulation by total annual yield. Cumulative forage yield and P accumulation for the entire 3-yr study were calculated based on individual harvests.

Apparent P recovery efficiency of forage was calculated for each year and for the entire 3-yr experiment using the difference method as described above for wheat and flax but in this case using above-ground forage P accumulation in fertilized and unfertilized treatments.

Labile soil P pools (2018 and 2019) and mycorrhizal colonization of alfalfa roots (2018) were also assessed as reported in Appendix I.

2.3.4 Statistical Analyses

Analysis of variance (ANOVA) was conducted using PROC GLIMMIX of SAS® software (version 9.4, SAS OnDemand for Academics; SAS Institute, Cary, NC). Data from wheat and flax experiments were analyzed with P application rate as a fixed effect and block and year as random effects. Data from the alfalfa–grass experiment were analyzed with P application rate and year and their interaction as fixed effects, using a repeated measures statement for year, and block as a random effect. For the alfalfa–grass experiment, cumulative forage P accumulation and total forage P recovery efficiency data over the whole study period were analyzed with P application rate as a fixed effect and block as a random effect. Treatment means were compared using Tukey's multiple comparison procedure at $\alpha = 0.05$. When the rate (or rate by year interaction for alfalfa–grass) effect was significant, orthogonal polynomial contrasts were performed, followed by linear regression analysis using PROC MIXED of SAS software.

Data for grain and forage yield, P concentration, and P accumulation were analyzed as normal distributions based on the Shapiro-Wilk test (W-statistic > 0.9). For plant population density in the wheat and flax experiments, a Poisson distribution was specified in the model statement in conjunction with the ILINK function in the LSMEANS statement. A beta distribution was specified for forage P recovery efficiency and for forage component proportions. Grain P recovery efficiency data for wheat and flax were analyzed as a Gaussian distribution due to presence of negative values, precluding analysis as a beta distribution. Where a test of homoscedasticity indicated unequal variances among treatment groups, the RANDOM _residual_/GROUP statement and the Satterthwaite denominator degrees of freedom were specified in the GLIMMIX procedure.

2.4 Results and Discussion

2.4.1 Weather and Soil Conditions

All three study years were drier than the long-term average (Table 2.1). Monthly average air temperatures were generally near normal, although May was relatively warm in 2018 and relatively cool in 2019 (Table 2.1).

Soil test P at all experiment locations was low to very low, ranging from 2 to 6 mg kg^{-1} Olsen-P (Table 2.2). In wheat and flax experiments, all other crop macronutrients were adequate for crop growth (medium or high rating in AGVISE Laboratories soil test results), except for sulphate-S in 2018 (low rating) and nitrate-N in 2019 (low rating). Additional N mineralization during the growing season from preceding annual green manures was expected in the wheat and flax experiments. In the alfalfa–grass experiment, laboratory ratings for nitrate-N, sulphate-S, and K were very low, low, and high, respectively. Low nitrate-N was not expected to limit productivity of the alfalfa–grass forage due to biological N fixation by the alfalfa, which was inoculated with the appropriate *Rhizobium* strain during establishment.

2.4.2 Wheat

2.4.2.1 Plant Population

Mean wheat plant populations in individual treatments and years (242–296 plants m^{-2}) were somewhat lower than our target plant population (300 plants m^{-2}) but were generally within the recommended range of 250–300 plants m^{-2} for spring wheat in Manitoba (Manitoba Agriculture n.d.). Struvite application rate did not have a significant effect on wheat plant population (Table 2.3). The highest application rate used in the present study is 67% greater than the maximum safe rate (24 kg P ha^{-1}) of seed-placed monoammonium phosphate (MAP) application recommended for cereal crops in Manitoba (Manitoba Agriculture 2007). Seed-placed struvite has been shown to pose less toxicity risk to canola than MAP (Katanda et al. 2019), but to the best of our knowledge, no research has been published reporting effects of seed-placed granular struvite on wheat seedling establishment.

Table 2.3 Wheat plant population density, grain yield, P concentration, P accumulation, and apparent P recovery efficiency in grain as affected by struvite application rate across three experimental years

Struvite rate	**Plant population**	**Grain yield**	**Grain P conc.**	**Grain P accum.**	**P recovery efficiency**
	plants m^{-2}	Mg ha^{-1}	mg g^{-1}	kg ha^{-1}	%
0 kg P ha^{-1}	281 (9.0) [a]	1.92 (0.35) c [b]	3.4 (0.24) b	6.5 (1.2) c	-
20 kg P ha^{-1}	269 (8.7)	2.14 (0.35) bc	3.5 (0.24) ab	7.3 (1.2) bc	4.0 (3.3)
30 kg P ha^{-1}	278 (8.9)	2.33 (0.35) ab	3.6 (0.24) ab	8.2 (1.2) ab	5.4 (3.3)
40 kg P ha^{-1}	265 (8.6)	2.62 (0.35) a	3.7 (0.24) a	9.5 (1.2) a	7.4 (3.3)
			p-values		
ANOVA	0.10	0.0001	0.005	<0.0001	0.21
Linear [c]	-	<0.0001	0.0005	<0.0001	-
Quadratic [c]	-	0.20	0.56	0.14	-

[a] Means presented are estimated using the least squares method, with SEM shown in parentheses.

[b] Where results of ANOVA are significant, means within columns followed by the same letter are not significantly different according to Tukey's test ($\alpha = 0.05$).

[c] Results of orthogonal polynomial contrasts, conducted only after identifying a significant effect of struvite rate according to ANOVA.

2.4.2.2 Grain Yield, P Concentration, and P Accumulation

Mean wheat grain yield in individual treatments and years ranged from 1.37 to 3.26 Mg ha^{-1}, similar to organic spring wheat yields in other field research trials in the region (Vaisman et al. 2011; Carkner et al. 2020) and similar to or greater than wheat yields on commercial organic farms in the region (Entz et al. 2001). Grain P concentration ranged from 2.9 to 4.0 mg g^{-1}, slightly lower than the range of 3.8 to 4.3 mg g^{-1} observed by Turmel et al. (2009) in organic spring wheat in the same region. Grain P accumulation ranged from 4.8 to 11.2 kg ha^{-1}.

Wheat grain yield, P concentration, and P accumulation across the three study years increased linearly with struvite P rate (Table 2.3; Figure 2.1), supporting our hypothesis regarding the effect of struvite addition on wheat. Grain yield and P accumulation were significantly greater than the unfertilized treatment only in the 30P and 40P treatments (Table 2.3), indicating that the recommended fertilizer P application rate of 20 kg P ha^{-1} was not sufficient to optimize crop response. Several previous studies have shown significant increases in wheat biomass and above-ground tissue P accumulation from struvite application relative to unfertilized treatments in pot experiments (Talboys et al. 2016; Degryse et al. 2017; Everaert et al. 2017; Rech et al. 2019).

The relationships between struvite application rate and grain yield and P accumulation remained linear at higher struvite application rates, as indicated by the non-significant quadratic trends (Table 2.3) and visual examination of data points (Figure 2.1). This finding suggests that the added P in struvite did not reach the critical level required to maximize wheat yield and P accumulation at this study site, despite these rates containing much more P than required by a spring wheat crop. Low-STP soils such as those at the study site may retain P released from applied fertilizers, thus requiring relatively high P application rates to overcome P deficiency and meet crop requirements, as observed in recent research on maize (*Zea mays* L.) in China (Ibrahim et al. 2021). Alternatively, a slow rate of struvite dissolution may have restricted P supply to the crop. Additional struvite application rates greater than those used in this study, along with applications of all other nutrients at rates that meet or exceed crop requirements, would be required to establish a yield curve indicating the maximum wheat response to struvite application and the optimum application rate. The potential to optimize crop P supply by applying struvite in combination with another P source permitted in organic production, such as composted manure, is also worthy of investigation.

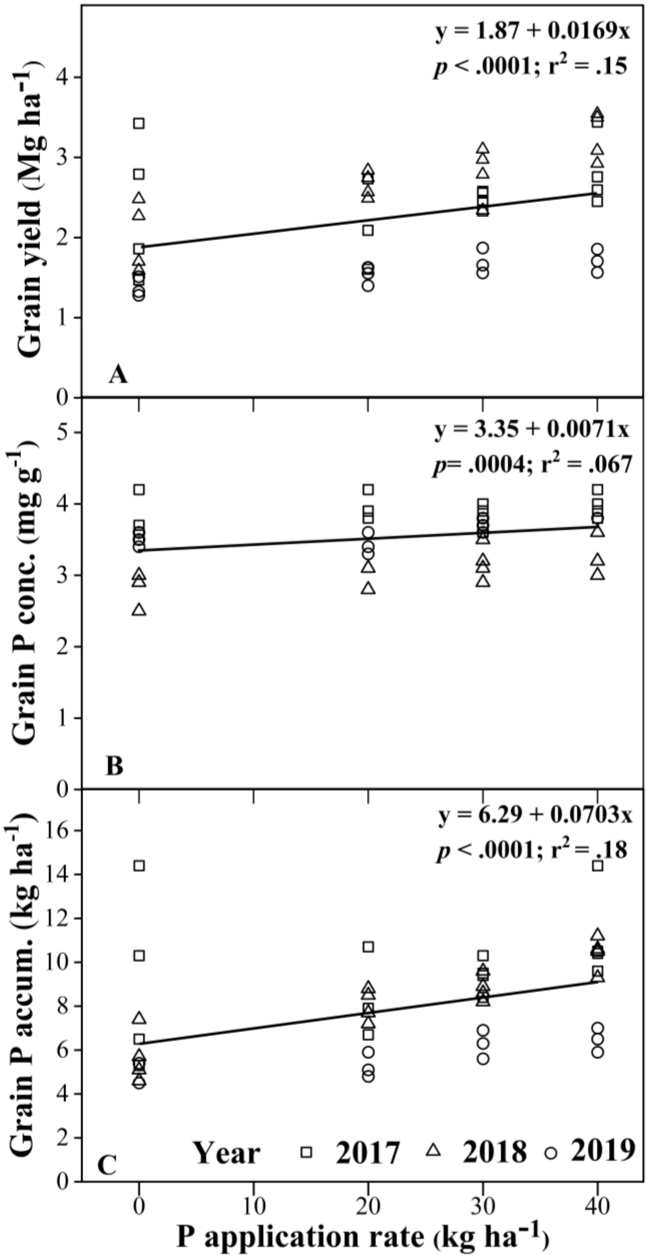

Figure 2.1 Regression relationships between P application rate as granular struvite and wheat grain yield (A), grain P concentration (B), and grain P accumulation (C) across the three study years (2017, 2018, and 2019). Data points represent individual observations in the three years of the study.

2.4.2.3 Phosphorus Recovery Efficiency

Struvite application rate did not have a significant effect on the P recovery efficiency of wheat (Table 2.3). Although this finding does not support our hypothesis that P recovery efficiency would decline with higher application rates, it is consistent with the linear grain yield and P accumulation responses, where application of higher rates of struvite continued to elicit greater responses. If P had become non-limiting at higher application rates, we would have expected a decline in P recovery efficiency at the higher rates, a trend that tends to occur with P application rates that are in excess of crop needs. In fact, while not significantly different, P recovery efficiency in wheat grain was numerically greater at higher struvite application rates, further supporting our conclusion that wheat P accumulation at 40P had not approached a maximum.

Apparent recovery efficiency provides an indication of the fraction of the applied nutrient that is taken up by the crop, assuming no effect of added nutrients on the efficiency of nutrient extraction by crop roots from soil reserves. The calculated values of P recovery efficiency in this study indicate that only a small proportion (4–7%) of the applied struvite was recovered in wheat grain. While we did not include a soluble P fertilizer treatment for comparison in this experiment, results of other studies indicate that the P recovery efficiency values we obtained are not dramatically different from those reported for MAP. In a pot experiment using two alkaline Manitoba soils, P recovery efficiency in wheat biomass was 8.9% for struvite and 12.4% for MAP, but differences between the two fertilizers were not significant (Katanda et al. 2016). Similarly, P recovery efficiency in wheat biomass grown in a pot experiment using a soil of pH 6.0 was 11% for struvite and 13% for MAP, with no significant difference between the two fertilizers (Talboys et al. 2016). Apparent recovery efficiency in pot studies is likely to be greater than in field studies due to optimal soil moisture and temperature conditions for plant growth and more complete soil exploration by roots in pots than under field conditions. For wheat, the proportion of total above-ground tissue P contained in the straw is estimated to be 12% (Selles et al. 1995); therefore, including P recovered in wheat straw should increase total P recovery efficiency values in the present study by a small amount.

The small proportion of P recovered in the present study raises questions about the fate of the remaining >90% of P applied as struvite. Other studies have reported finding undissolved remnants of struvite granules in soil following incubation or plant growth experiments, constituting approximately 50–90% of struvite applied, whereas no remnants of soluble fertilizer granules were detectable (Talboys et al. 2016; Degryse et al. 2017; Rech et al. 2019). In the present study, we were able to identify remnants of struvite granules in field soil approximately one year after application, though we did not attempt to quantify them. Little is known about the multi-year dissolution patterns of granular struvite, though Degryse et al. (2017, p. 150) have estimated that "the time required to reach near-complete [struvite] dissolution may range from days to years depending on fertilizer, soil and plant properties." Residual biomass yield benefits from struvite have been observed in subsequent crops in pot studies (Katanda et al. 2016; Wollmann et al. 2018), but soluble P fertilizers are also known to contribute to residual effects over several years, as the reaction products of soluble P fertilizers with soil can continue to supply plant-available P over time (Bailey et al. 1977; Roberts and Johnston 2015). Low P recovery in the year of application is not unique to struvite but is also common with soluble P fertilizers and highlights the challenges of fertilizer P management in general. Whether the slow dissolution of struvite granules in soils contributes to better long-term P recovery efficiency relative to soluble fertilizers, and the nature of any potential mechanisms contributing to such a benefit, are questions that deserve further research.

2.4.3 Flax

2.4.3.1 Plant Population

Flax plant population was 4–10% lower than our target population of 700 plants m^{-2} but was still greater than the recommended target population of 400–600 plants m^{-2} for flax in Manitoba (Manitoba Agriculture, n.d.). Struvite application rate had a small positive effect on flax plant population (Table 2.4), for reasons that are unclear. However, the absence of plant population decline with increased struvite application rates supports the findings of Katanda et al. (2019), who showed that struvite caused less seedling injury to canola (*Brassica napus* L.) relative to MAP. The recommended maximum safe rate of seed-placed MAP application for flax and canola in Manitoba is 9 kg P ha^{-1} (Manitoba Agriculture 2007), well below the lowest rate used in the present study. Previous research has demonstrated that seed-placed MAP applied to flax at 15 kg P ha^{-1} or more can result in seedling injury (Bailey and Grant 1989). Thus, struvite seems to be a safe P source for in-furrow seed placement with flax.

Table 2.4 Flax plant population density, grain yield, P concentration, P accumulation, and apparent P recovery efficiency in grain as affected by struvite application rate across three experimental years

Struvite rate	**Plant population**	**Grain yield**	**Grain P conc.**	**Grain P accum.**	**P recovery efficiency**
	plants m^{-2}	Mg ha^{-1}	mg g^{-1}	kg ha^{-1}	%
0 kg P ha^{-1}	632 (53) [a] b [b]	1.23 (0.09)	3.8 (0.12) b	4.7 (0.47)	-
20 kg P ha^{-1}	648 (54) ab	1.24 (0.09)	4.0 (0.12) ab	5.0 (0.47)	1.6 (0.85)
30 kg P ha^{-1}	658 (55) ab	1.22 (0.09)	4.2 (0.12) a	5.0 (0.47)	1.2 (0.85)
40 kg P ha^{-1}	671 (56) a	1.28 (0.09)	4.1 (0.12) ab	5.3 (0.47)	1.6 (0.85)
			p-values		
ANOVA	0.01	0.50	0.04	0.12	0.74
Linear [c]	0.001	-	0.01	-	-
Quadratic [c]	0.72	-	0.34	-	-

[a] Means presented are estimated using the least squares method, with SEM shown in parentheses.

[b] Where results of ANOVA are significant, means within columns followed by the same letter are not significantly different according to Tukey's test (α = 0.05).

[c] Results of orthogonal polynomial contrasts, conducted only after identifying a significant effect of struvite rate according to ANOVA.

2.4.3.2 Grain Yield, P Concentration, and P Accumulation

Mean flax grain yield in individual treatments and years ranged from 1.06 to 1.52 Mg ha^{-1}. These yields are greater than average organic flax yields on commercial farms (Entz et al. 2001) and in a long-term organic experiment (Carkner et al. 2020) in the region and are similar to flax yields reported in some conventional field experiments (Bailey et al. 1977; Carkner et al. 2020). Mean flax grain P concentration ranged from 3.6 to 4.5 mg g^{-1}, intermediate between flax fertilized with P and nil-P controls in a pot study using a Manitoba soil with moderately low STP (11.4 mg kg^{-1}; Jiao et al. 2007). Mean flax grain P accumulation ranged from 3.9 to 6.4 kg ha^{-1}, somewhat less than in the wheat experiment, a pattern that is consistent with previous observations of wheat and flax P accumulation in Manitoba (Bailey et al. 1977).

Struvite application rate had a significant effect on flax grain P concentration across study years, but not on flax grain yield or P accumulation (Table 2.4). Regression analysis showed a positive linear relationship between struvite application rate and grain P concentration ($y = 3.81 + 0.0093x$; $p = 0.0062$; $r^2 = 0.15$). The increase in grain P concentration was small relative to that observed in pot-grown flax receiving a high rate of P fertilizer in a similar soil (Jiao et al. 2007) and the lack of effect on grain P accumulation shows that the overall increase in effective P supply to flax due to struvite application was negligible.

Past research has shown varying results on flax response to P fertilizers in low-STP soils, with some studies showing a significant grain yield increase due to application of soluble P fertilizers (Grant and Bailey 1993; Xie et al. 2016) and others showing little to no response (Racz et al. 1965; Strong and Soper 1974). The lack of yield response in the present study could be due to flax obtaining sufficient P from the soil, despite very low concentrations of STP; relatively high grain yields across treatments support this possibility. Other possible explanations that take into consideration the significant increase in grain P concentration include interactions with weeds or arbuscular mycorrhizal fungi due to P addition. For example, greater weed growth in fertilized plots in some years (Thiessen Martens, unpublished data, 2021) may have suppressed grain yield in fertilized treatments, negating a potential yield increase due to P addition. Alternatively, addition of P as struvite may have suppressed mycorrhizal colonization of flax roots, as is commonly reported with soluble P sources (Kahiluoto et al. 2001; Gao et al. 2011), possibly limiting flax yield due to loss of other benefits associated with the mycorrhizal symbiosis, such as zinc uptake (Thompson 1996; McGonigle et al. 2011). However, the mid-season flax root colonization with mycorrhizal fungi, measured in the 2018 experiment, was not affected by struvite application rate (Appendix I). Recent research has shown that mycorrhizal colonization in annual crop plants is maintained at higher levels when fertilized with slow-release rather than soluble fertilizers, though struvite was not included in that study (Mäkelä et al. 2020). The effect of struvite application on mycorrhizal colonization of crop plants, as well as the ability of mycorrhizal fungi to access P in struvite, are questions that require further investigation.

2.4.3.3 Phosphorus Recovery Efficiency

Struvite application rate had no significant effect on the P recovery efficiency of flax (Table 2.4). Phosphorus recovery efficiency values were very low, as grain P accumulation in fertilized plots was similar to those in the unfertilized treatments, and suggest that only 1–2% of applied struvite was recovered by the flax crop. While additional P was undoubtedly contained in flax straw, including this proportion of P accumulation in the P recovery efficiency calculations in the present study would be unlikely to generate substantially different results unless flax straw yield or P concentration increased considerably with application of struvite.

2.4.4 Alfalfa–Grass Forage

2.4.4.1 Forage Yield, P Concentration, and P Accumulation

Mean annual alfalfa–grass forage yield in individual struvite application treatments and years ranged from 1.72 to 6.91 Mg ha^{-1}, slightly higher than the range observed on commercial organic farms (Entz et al. 2001) and similar to a long-term organic study in the region (Entz, unpublished data, 2021). Mean forage P concentration ranged from 1.0 to 1.9 mg P g^{-1}, similar to values observed in a P fertilization study on alfalfa in alkaline soils in China (Fan et al. 2016) but somewhat lower than values observed on organic dairy farms in Ontario (Main et al. 2013; Schneider et al. 2017). Mean annual forage P accumulation was 1.6 to 13.0 kg P ha^{-1} in individual treatments and years. Cumulative forage P accumulation over the course of the 3-yr experiment ranged from 9.0 kg ha^{-1} for 0P to 32.6 kg ha^{-1} for 90P, with significant differences among treatments (data not shown; $p < 0.0001$).

There was a significant struvite application rate by year interaction for annual forage yield and annual forage P accumulation according to ANOVA (Table 2.5). In both cases, the response variables increased linearly with struvite rate in all years but had significant differences in regression slopes among years (Figure 2.2A, C). For annual forage yield, the slope of the linear regression relationship was greater in 2018 and 2019 than in 2017 (Figure 2.2A), indicating that the magnitude of the crop productivity response to struvite application increased after the first year. The forage yield of the 90P treatment was 1.6-, 2.4-, and 3.4-fold greater than the 0P treatment in 2017, 2018 and 2019, respectively. Differences in forage yield among struvite application rates were amplified over time by both an increase in productivity at higher application rates, particularly between 2017 and 2018, and a decline in productivity in the 0P treatment, particularly in 2019 compared to the previous years (Figure 2.2A). For forage P accumulation, the greatest slope was observed in 2018, the second year of the study (Figure 2.2C). The initial increase in slope for forage P accumulation from 2017 to 2018 mirrored that of forage yield, while the decline in forage P accumulation response from 2018 to 2019 reflected the influence of relatively low forage P concentration in 2019 as discussed below. Forage yield in the 30P treatment, which corresponds to the recommended P fertilizer rate, was significantly greater than 0P only in 2018 and 2019 whereas forage P accumulation in 30P exceeded 0P in all years (data not shown). Nonetheless, our results show that the 30P rate was not sufficient to meet crop demand.

Table 2.5 Alfalfa–grass forage yield, P concentration, P accumulation, and apparent P recovery efficiency as affected by struvite application rate and year in a 3-yr continuous experiment

Factor	Forage yield	Forage P conc.	Forage P accum.	P recovery efficiency	
				Annual	Cumulative
	Mg ha^{-1}	mg g^{-1}	kg ha^{-1}	%	
Struvite rate					
0 kg P ha^{-1}	2.74 (0.20) [a]	1.1 (0.050) c [b]	2.99 (0.24)	-	-
30 kg P ha^{-1}	4.21 (0.17)	1.3 (0.050) b	5.73 (0.25)	9.0 (0.57)	27.4 (0.70)
60 kg P ha^{-1}	5.29 (0.15)	1.5 (0.054) a	8.31 (0.18)	8.5 (0.63)	26.0 (0.91)
90 kg P ha^{-1}	6.31 (0.37)	1.7 (0.050) a	10.87 (0.66)	8.6 (0.56)	26.3 (0.68)
Year					
2017	4.51 (0.17)	1.5 (0.047) a	6.91 (0.23)	6.9 (0.55) b	-
2018	5.13 (0.17)	1.6 (0.045) a	8.66 (0.30)	12.0 (0.64) a	-
2019	4.28 (0.29)	1.2 (0.045) b	5.37 (0.43)	8.2 (0.55) b	-
ANOVA	*p*-values				
Rate (R)	0.0004	<0.0001	<0.0001	0.86	0.57
Year (Y)	0.02	<0.0001	0.004	0.0001	-
R × Y	0.01	0.41	0.044	0.64	-
Orthogonal Polynomial Contrasts [c]					
Linear (R)	-	<0.0001	-	-	-
Linear (R × Y)	0.03	-	0.09	-	-
Quadratic (R)	-	0.32	-	-	-
Quadratic (R × Y)	0.14	-	0.49	-	-

[a] Means presented are estimated using the least squares method, with SEM shown in parentheses.

[b] Where ANOVA indicates a significant main effect but no interaction, main effect means followed by the same letter within columns and factors are not significantly different according to Tukey's test ($\alpha = 0.05$). Significant interactions are explored further through regressions (Figure 2.2).

[c] Results of orthogonal polynomial contrasts are presented only when the effect of rate or rate by year was significant according to ANOVA.

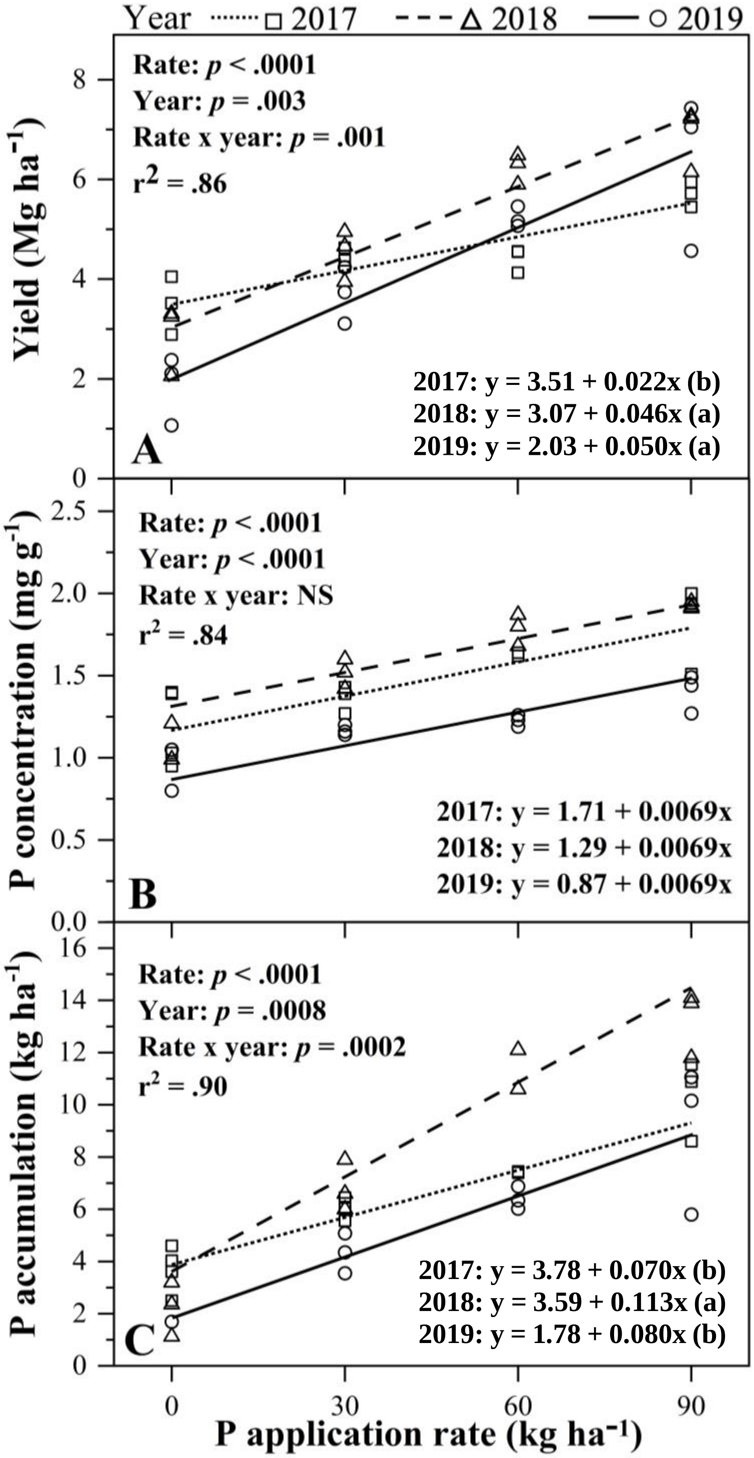

Figure 2.2 Regression relationships between P application rate as struvite and alfalfa-grass forage yield (A), forage P concentration (B), and forage P accumulation (C) in each of the three years of the study. Data points represent individual observations. Slopes of regression equations for forage yield and P accumulation followed by the same lower case letter are not significantly different based on pair-wise comparisons.

Forage P concentration was influenced by struvite application rate and year, but not their interaction, increasing linearly with struvite rate in all years but with lower values overall in 2019 than in 2017 and 2018 (Table 2.5; Figure 2.2B). Reduced forage P concentration in 2019 may have been caused by depletion of available P over time, or by dry growing conditions in this year, resulting in slow P diffusion in soil and reduced P availability. Forage P concentration was always below the critical threshold of 2.0–2.5 mg P g^{-1} thought to indicate P deficiency in pure stands of alfalfa at the early bloom stage (Kelling and Matocha 1990). However, the alfalfa–grass forage in this study was sampled at a relatively mature growth stage (alfalfa in full flower), which may have contributed to lower P concentrations, as alfalfa P concentration is known to decline with maturity (Kelling & Matocha, 1990). The presence of grasses in the forage mixture is not likely to affect the critical threshold, as thresholds for several cool-season grasses are similar to or slightly higher than that of alfalfa (2.4–3.0 mg P g^{-1}; Kelling & Matocha, 1990).

Overall, our results agree with the findings from a field study in Washington, USA, investigating the effect of application of low-purity struvite derived from dairy manure on alfalfa productivity and P accumulation on a low-STP alkaline soil (Hilt et al. 2016). That study also demonstrated greater yield, P concentration, and P accumulation in alfalfa fertilized with struvite relative to an unfertilized treatment (though not always significant), with the magnitude of treatment differences increasing over 3 yr. However, in that study, struvite was reapplied to fertilized plots in the second and third years; therefore, the larger effect in later years was not necessarily due to residual effects from the initial struvite application. Hilt et al. (2016) included MAP as a reference fertilizer in their study and found that alfalfa response to struvite was similar or inferior to response to MAP, depending on the year and fertilizer application rate.

The linear responses to struvite rate and lack of quadratic trend observed for forage yield in the present study (Table 2.5) indicate that the highest struvite rate may not have been sufficient to reach the critical P application rate required to maximize yield. A greater number and range of struvite application rates, along with additions of other nutrients to preclude other potential limitations to crop growth, would be required to clarify forage response to rates of struvite application.

The observed patterns provide strong evidence of a residual effect of struvite application beyond the year of application, which is particularly pertinent to management of perennial forage stands where farmers may choose to apply several years' worth of P in a single application every few years. This same approach may also be applicable to annual cropping systems, where the timing of struvite application within a crop sequence may be targeted to provide the greatest benefit to a range of crops with differing P requirements and acquisition strategies. Optimizing the timing of struvite application in a multi-year crop sequence will require greater understanding of struvite dissolution dynamics over time and under the influence of different crop types, as well as the identity and behavior of reaction products that form in soil as a result of struvite application.

2.4.4.2 Phosphorus Recovery Efficiency

The P recovery efficiency for struvite applied to the alfalfa–grass forage was not significantly affected by application rate but varied with year (Table 2.5). The greatest recovery efficiency was achieved in 2018, the second year of the study, and reflects the greater P accumulation in this year, supporting evidence for slow struvite dissolution patterns and residual effects on P availability to the forage crop. Total P recovery efficiency over the 3-yr duration of the experiment was 26–27%, with no significant difference among application rates (Table 2.5). The lack of a struvite rate effect on P recovery efficiency, alongside dramatic increases in forage yield and P accumulation, raises questions about the mechanism contributing to struvite availability in the soil at the study site. The fact that forage P recovery efficiency in the 30P treatment, which was clearly still P deficient, was not greater than P recovery efficiency in the 90P treatment suggests that the availability of P from struvite for plant uptake was governed largely by soil processes, along with crop demand. While considerably greater than the annual P recovery efficiency observed for wheat and flax, the cumulative P recovery achieved by alfalfa over 3 yr still leaves >70% of P applied in struvite unrecovered. As with wheat and flax, determining the long-term fate of struvite in soil, including study of dissolution kinetics and characterization of reaction products, will be necessary to guide recommendations for use of struvite in different soil types and cropping systems.

2.4.4.3 Forage Composition

The composition of the alfalfa–grass forage, measured in the third year of the experiment (2019), was quite heterogeneous within and among plots due to the presence of large, mature alfalfa and bunchgrass plants and patchy alfalfa winterkill that occurred in the winter of 2016–2017. Grass species were mainly orchardgrass (*Dactylis glomerata* L.) and meadow bromegrass (*Bromus biebersteinii* Roem & Schult.) and the few weeds that were present were mainly dandelion (*Taraxacum officinale* F. H. Wigg.).

Forage composition was affected by struvite rate, as revealed by significant effects among the biomass and proportions of alfalfa and grass components in several instances (Table 2.6). Alfalfa biomass increased significantly with increasing struvite rate, showing 3.5-, 5.4-, and 8.6-fold increases over the 0P treatment in the 30P, 60P, and 90P treatments, respectively. Grass and weed biomass yields did not vary with struvite rate.

Table 2.6 Biomass and proportion (prop.) of forage components in the third year after struvite application to an alfalfa–grass forage stand as affected by struvite application rate

Struvite rate	Alfalfa		Grass		Weeds	
	Biomass	Prop.	Biomass	Prop.	Biomass	Prop.
	kg ha^{-1}	%	kg ha^{-1}	%	kg ha^{-1}	%
0 kg P ha^{-1}	650 (210) [a] d [b]	34 (2.7) b	1110 (160)	60 (2.9) a	94 (43)	6.1 (2.7)
30 kg P ha^{-1}	2280 (210) c	61 (2.8) a	1320 (160)	36 (2.9) b	96 (43)	2.5 (1.5)
60 kg P ha^{-1}	3540 (210) b	68 (2.7) a	1600 (160)	31 (2.8) b	85 (43)	1.6 (1.1)
90 kg P ha^{-1}	5560 (250) a	76 (3.0) a	1540 (190)	21 (3.0) b	192 (49)	2.3 (1.5)
			p-values			
ANOVA	<0.0001	0.0008	0.19	0.002	0.27	0.25

[a] Means presented are estimated using the least squares method, with SEM shown in parentheses.

[b] Where results of ANOVA are significant, means within columns followed by the same letter are not significantly different according to Tukey's test ($\alpha = 0.05$).

When assessing the proportion of each forage component in the mix, we found that struvite application rate had a significant effect on the proportions of grass and alfalfa but no effect on the proportion of weeds (Table 2.6). All treatments receiving struvite were dominated by alfalfa (>60%), whereas the 0P treatment was dominated by grasses.

Visual observation indicated that the increase in alfalfa yield in the fertilized plots was due to larger plant size, rather than greater plant population, suggesting that the existing alfalfa plants were more responsive to addition of struvite P than were the grasses. Legumes are thought to employ a variety of mechanisms to enhance P acquisition from soil or fertilizers but are also known to be more sensitive to P deficiency than non-legumes (Li et al. 2011). In studies assessing the response of individual crop types to struvite addition, legume species such as soybean (*Glycine max* L.) and red clover (*Trifolium pratense* L.) have shown stronger responses to struvite application relative to cereal crops (Rech et al., 2019; Wollman et al., 2018), but the response of components in crop mixtures to struvite addition has received little attention. Recent research has shown that addition of P as superphosphate to several four-species legume–grass mixtures increased the competitive ability of alfalfa to a greater extent than the other forage species in the mix (Bi et al. 2019). Thus, we speculate that the disproportionate response of alfalfa to added struvite in the present study was due mainly to P addition to this very low-STP soil, rather than the form of P as struvite.

The proportion of legume in forage mixtures is important as it may impact forage quality and the biological N fixation capacity of the forage stand. Research in Alberta demonstrated that after the initial 2 yr of a forage stand, perennial forage mixtures with greater legume proportions, especially alfalfa, produced greater crude protein yield than mixtures with little to no legume, despite not producing greater biomass yield (Bork et al. 2017). Maximum biological N fixation in *Medicago* spp.–grass mixtures was estimated to occur with legume biomass around 72–75%, based on observations of mixtures ranging from 39 to 98% legume (Li et al. 2020). Nitrogen fixation is an especially important consideration for organic farmers relying on legume-based forages to supply N to future crops in a rotation. Our findings emphasize the importance of P nutrition in maintaining high-quality forage and supporting N fixation in organic and low-input cropping systems.

2.5 Conclusions

Our unique results demonstrate that struvite can be an effective P source for certain crops under organic management in a low-STP, calcareous soil in the Northern Great Plains, partially supporting our first hypothesis that crop yield, P concentration, and P accumulation would increase with struvite application rate for all crops. Spring wheat and alfalfa–grass forage showed a significant increase in yield, P concentration, and P accumulation when fertilized with increasing rates of struvite, but flax showed little to no response. We found no evidence of wheat or alfalfa–grass reaching maximum yield or P accumulation at relatively high rates of struvite application, indicating that the rates required to achieve optimum crop yields in the year of application may need to be substantially greater than recommendations for soluble P fertilizers based on soil tests. However, the increasing strength of the alfalfa–grass yield and P accumulation response in the later years of the study, which supports our second hypothesis regarding the residual effect of struvite application, provides strong evidence for the efficacy of struvite as a multi-year P source, perhaps reducing P application requirements in later years. Future experiments should include higher rates of struvite and a multi-year approach to establish crop response curves and identify application rates that optimize P supply to crops in the year of application as well as subsequent growing seasons in annual and perennial cropping systems.

The lack of relationship between struvite application rate and P recovery efficiency does not support our third hypothesis that P recovery efficiency would decline with increasing rates, as would be expected if higher rates of struvite addition fully met crop demand for P. This finding, along with the small proportion of P recovered by crops in this study, raises questions about the fate of unused struvite in the soil. Dissolution of applied struvite granules appeared to be slow, possibly causing long-term P supply to come directly from dissolving struvite rather than from the reaction products formed in the soil, as occurs with soluble fertilizers. Future studies should investigate the identity and solubility of P species in residual P fractions and the patterns of continued P release from struvite over time, particularly in the period between crop harvest and establishment of the following crop. Such research would provide additional insights into the long-term agronomic potential and P use efficiency of struvite as well as the risks of P loss to the environment.

2.6 References

Ackerman, J.N., Zvomuya, F., Cicek, N., and Flaten, D. 2013. Evaluation of manure-derived struvite as a phosphorus source for canola. Can. J. Plant Sci. **93**: 419–424. doi:10.4141/cjps2012-207.

Ahmed, N., Shim, S., Won, S., and Ra, C. 2018. Struvite recovered from various types of wastewaters: Characteristics, soil leaching behaviour, and plant growth. Land Degrad. Dev. **29**: 2864–2879. doi:10.1002/ldr.3010.

Bailey, L.D., and Grant, C.A. 1989. Fertilizer phosphorus placement studies on calcareous and non-calcareous chernozemic soils: Growth, P-uptake and yield of flax. Commun. Soil Sci. Plant Anal. **20**: 635–654. Taylor & Francis. doi:10.1080/00103628909368106.

Bailey, L.D., Spratt, E.D., Read, D.W.L., Warder, F.G., and Ferguson, W.S. 1977. Residual effects of phosphorus fertilizer II. For wheat and flax grown on chernozemic soils in Manitoba. Can. J. Soil Sci. **57**: 263–270. doi:10.4141/cjss77-032.

Benjannet, R., Nyiraneza, J., Khiari, L., Cambouris, A., Fuller, K., Hann, S., and Ziadi, N. 2020. Potato response to struvite compared with conventional phosphorus fertilizer in Eastern Canada. Agron. J. **112**: 1360–1376. doi:10.1002/agj2.20118.

Bi, Y., Zhou, P., Li, S., Wei, Y., Xiong, X., Shi, Y., Liu, N., and Zhang, Y. 2019. Interspecific interactions contribute to higher forage yield and are affected by phosphorus application in a fully-mixed perennial legume and grass intercropping system. Field Crops Res. **244**: 107636. doi:10.1016/j.fcr.2019.107636.

Bork, E.W., Gabruck, D.T., McLeod, E.M., and Hall, L.M. 2017. Five-year forage dynamics arising from four legume–grass seed mixes. Agron. J. **109**: 2789–2799. doi:10.2134/agronj2017.02.0069.

Canadian General Standards Board 2018. Organic production systems: General principles and management standards, CAN/CGSB-32.310-2015. Canadian General Standards Board.

Canadian General Standards Board 2021. Organic production systems: Permitted substances lists, CAN/CGSB-32.311-2020. Canadian General Standards Board. [Online] Available: http://publications.gc.ca/collections/collection_2020/ongc-cgsb/P29-32-311-2020-eng.pdf [2021 Apr. 8].

Carkner, M., Bamford, K., Thiessen Martens, J., Wilcott, S., Stainsby, A., Stanley, K., Dick, C., and Entz, M.H. 2020. Building capacity from Glenlea, Canada's oldest organic rotation study. Pages 103–122 *in* G.S. Bhullar and A. Riar, eds. Long-term farming systems research. Academic Press, London, UK. doi:10.1016/B978-0-12-818186-7.00007-2.

Chen, M., and Graedel, T.E. 2016. A half-century of global phosphorus flows, stocks, production, consumption, recycling, and environmental impacts. Glob. Environ. Change **36**: 139–152. doi:10.1016/j.gloenvcha.2015.12.005.

Cihacek, L.J., Yellajosula, G., Jacobson, K.A., and Swenson, L. 2015. Comparison of seven SO_4-S extraction methods for analysis by turbidimetry or ICP spectrometry. Commun. Soil Sci. Plant Anal. **46**: 2649–2659. doi:10.1080/00103624.2015.1089266.

Combs, S.M., and Nathan, M.V. 2015. Soil organic matter. Page 12.1-12.6 *in* M.V. Nathan and R.H. Gelderman, eds. Recommended chemical soil testing procedures for the North Central region. Missouri Agricultural Experiment Station SB 1001, Columbia, MO.

Cordell, D., and White, S. 2014. Life's bottleneck: Sustaining the world's phosphorus for a food secure future. Annu. Rev. Environ. Resour. **39**: 161–188. doi:10.1146/annurev-environ-010213-113300.

Degryse, F., Baird, R., Silva, R., and McLaughlin, M. 2017. Dissolution rate and agronomic effectiveness of struvite fertilizers - effect of soil pH, granulation and base excess. Plant Soil **410**: 139–152. doi:10.1007/s11104-016-2990-2.

Entz, M.H., Guilford, R., and Gulden, R. 2001. Crop yield and soil nutrient status on 14 organic farms in the eastern portion of the northern Great Plains. Can. J. Plant Sci. **81**: 351–354. doi:10.4141/p00-089.

Environment and Climate Change Canada 2021. Canadian climate normals. [Online] Available: https://climate.weather.gc.ca/climate_normals/ [2021 May 29].

Everaert, M., Degryse, F., McLaughlin, M.J., De Vos, D., and Smolders, E. 2017. Agronomic effectiveness of granulated and powdered P-exchanged Mg–Al LDH relative to struvite and MAP. J. Agric. Food Chem. **65**: 6736–6744. doi:10.1021/acs.jafc.7b01031.

Fan, J.-W., Du, Y.-L., Wang, B.-R., Turner, N.C., Wang, T., Abbott, L.K., Stefanova, K., Siddique, K.H.M., and Li, F.-M. 2016. Forage yield, soil water depletion, shoot nitrogen and phosphorus uptake and concentration, of young and old stands of alfalfa in response to nitrogen and phosphorus fertilisation in a semiarid environment. Field Crops Res. **198**: 247–257. doi:10.1016/j.fcr.2016.08.014.

Filippelli, G.M. 2018. Balancing the global distribution of phosphorus with a view toward sustainability and equity. Glob. Biogeochem. Cycles **32**: 904–908. doi:10.1029/2018GB005923.

Gao, X., Tenuta, M., Flaten, D.N., and Grant, C.A. 2011. Cadmium concentration in flax colonized by mycorrhizal fungi depends on soil phosphorus and cadmium concentrations. Commun. Soil Sci. Plant Anal. **42**: 1882–1897. doi:10.1080/00103624.2011.587572.

Gee, G.W., and Or, D. 2002. Particle-size analysis. Pages 255–293 *in* Methods of soil analysis. John Wiley & Sons, Ltd. doi:10.2136/sssabookser5.4.c12.

Gelderman, R.H., and Beegle, D. 2015. Nitrate-nitrogen. Page 5.1-5.4 *in* M.V. Nathan and R.H. Gelderman, eds. Recommended chemical soil testing procedures for the North Central region. Missouri Agricultural Experiment Station SB 1001, Columbia, MO.

Grant, C.A., and Bailey, L.D. 1993. Interactions of zinc with banded and broadcast phosphorus fertilizer on the dry matter and seed yield of oilseed flax. Can. J. Plant Sci. **73**: 7–16. doi:10.4141/cjps93-003.

Havlin, J.L., and Soltanpour, P.N. 1980. A nitric acid plant tissue digest method for use with inductively coupled plasma spectrometry. Commun. Soil Sci. Plant Anal. **11**: 969–980. Taylor & Francis. doi:10.1080/00103628009367096.

Hertzberger, A.J., Cusick, R.D., and Margenot, A.J. 2020. A review and meta-analysis of the agricultural potential of struvite as a phosphorus fertilizer. Soil Sci. Soc. Am. J. **84**: 653–671. doi:10.1002/saj2.20065.

Hilt, K., Harrison, J., Bowers, K., Stevens, R., Bary, A., and Harrison, K. 2016. Agronomic response of crops fertilized with struvite derived from dairy manure. Water. Air. Soil Pollut. **227**: 388. doi:10.1007/s11270-016-3093-7.

Huygens, D., and Saveyn, H.G.M. 2018. Agronomic efficiency of selected phosphorus fertilisers derived from secondary raw materials for European agriculture. A meta-analysis. Agron. Sustain. Dev. **38**: 52. doi:10.1007/s13593-018-0527-1.

Ibrahim, K., Wang, Q., Wang, L., Zhang, W., Peng, C., and Zhang, S. 2021. Determining the optimum level of soil Olsen phosphorus and phosphorus fertilizer application for high phosphorus-use efficiency in *Zea mays* L. in black soil. Sustainability **13**: 5983. doi:10.3390/su13115983.

IFOAM n.d. The four principles of organic agriculture. [Online] Available: https://www.ifoam.bio/en/organic-landmarks/principles-organic-agriculture [2022 Mar. 28].

Jiao, Y., Grant, C.A., and Bailey, L.D. 2007. Growth and nutrient response of flax and durum wheat to phosphorus and zinc fertilizers. Can. J. Plant Sci. **87**: 461–470. doi:10.4141/P05-212.

Kahiluoto, H., Ketoja, E., Vestberg, M., and Saarela, I. 2001. Promotion of AM utilization through reduced P fertilization 2. Field studies. Plant Soil **231**: 65–79.

Katanda, Y., Zvomuya, F., Flaten, D., and Cicek, N. 2016. Hog-manure-recovered struvite: Effects on canola and wheat biomass yield and phosphorus use efficiencies. Soil Sci. Soc. Am. J. **80**: 135–146. doi:10.2136/sssaj2015.07.0280.

Katanda, Y., Zvomuya, F., Flaten, D., Cicek, N., and Amarakoon, I. 2019. Effects of seed-placed hog manure-recovered struvite on canola seedling emergence. Agron. J. **111**: 390–396. doi:10.2134/agronj2018.04.0270.

Kelling, K.A., and Matocha, J.E. 1990. Plant analysis as an aid in fertilizing forage crops. Pages 603–643 *in* Soil testing and plant analysis. John Wiley & Sons, Ltd. doi:10.2136/sssabookser3.3ed.c23.

Knight, J.D., Buhler, R., Leeson, J.Y., and Shirtliffe, S. 2010. Classification and fertility status of organically managed fields across Saskatchewan, Canada. Can. J. Soil Sci. **90**: 667–678. doi:10.4141/cjss09082.

Li, Q., Zhang, H., Huang, Y., and Zhou, D. 2020. Forage nitrogen yield and soil nitrogen in artificial grasslands with varied Medicago seedling proportion. Arch. Agron. Soil Sci. **66**: 110–125. Taylor & Francis. doi:10.1080/03650340.2019.1600675.

Li, S.-X., Wang, Z.-H., and Stewart, B.A. 2011. Differences of some leguminous and nonleguminous crops in utilization of soil phosphorus and responses to phosphate fertilizers. Adv. Agron. **110**: 125–249. doi:10.1016/B978-0-12-385531-2.00003-7.

Main, M.H., Lynch, D.H., Voroney, R.P., and Juurlink, S. 2013. Soil phosphorus effects on forage harvested and nitrogen fixation on Canadian organic dairy farms. Agron. J. **105**: 827–835. doi:10.2134/agronj2012.0500.

Mäkelä, P.S.A., Wasonga, D.O., Solano Hernandez, A., and Santanen, A. 2020. Seedling growth and phosphorus uptake in response to different phosphorus sources. Agronomy **10**: 1089. doi:10.3390/agronomy10081089.

Manitoba Agriculture 2007. Manitoba soil fertility guide. Province of Manitoba. [Online] Available: https://www.gov.mb.ca/agriculture/crops/soil-fertility/soil-fertility-guide/pubs/soil_fertility_guide.pdf [2021 Apr. 11].

Manitoba Agriculture 2021. Daily report. [Online] Available: https://web43.gov.mb.ca/climate/DailyReport.aspx [2021 May 29].

Manitoba Agriculture n.d. Mitigating the risks of delayed seeding. [Online] Available: https://www.gov.mb.ca/agriculture/crops/crop-management/mitigating-risks-delayed-seeding.html [2021 Sep. 8].

Martin, R.C., Lynch, D.H., Frick, B., and Straaten, P. van 2007. Phosphorus status on Canadian organic farms. J. Sci. Food Agric. **87**: 2737–2740. doi:10.1002/jsfa.3077.

McGonigle, T.P., Hutton, M., Greenley, A., and Karamanos, R. 2011. Role of mycorrhiza in a wheat–flax versus canola–flax rotation: A case study. Commun. Soil Sci. Plant Anal. **42**: 2134–2142. doi:10.1080/00103624.2011.596242.

Meyer, G., Frossard, E., Mäder, P., Nanzer, S., Randall, D.G., Udert, K.M., and Oberson, A. 2018. Water soluble phosphate fertilizers for crops grown in calcareous soils – an outdated paradigm for recycled phosphorus fertilizers? Plant Soil **424**: 367–388. doi:10.1007/s11104-017-3545-x.

Möller, K., Oberson, A., Bünemann, E.K., Cooper, J., Friedel, J.K., Glæsner, N., Hörtenhuber, S., Løes, A.-K., Mäder, P., Meyer, G., Müller, T., Symanczik, S., Weissengruber, L., Wollmann, I., and Magid, J. 2018. Improved phosphorus recycling in organic farming: Navigating between constraints. Adv. Agron. **147**: 159–237. doi:10.1016/bs.agron.2017.10.004.

Murphy, J., and Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta **27**: 31–36. doi:10.1016/S0003-2670(00)88444-5.

Nicksy, J., and Entz, M.H. 2021. Recycled nutrients as a phosphorus source for Canadian organic agriculture: a perspective. Can. J. Soil Sci. **101**: 571–580. doi:10.1139/cjss-2021-0014.

Olsen, S.R., Cole, C.V., F. S. Watanabe, and Dean, L.A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture, Washington, DC. [Online] Available: http://hdl.handle.net/2027/uiug.30112019277042.

Peters, J.B., Nathan, M.V., and Laboski, C.A.M. 2015. pH and lime requirement. Page 4.1-4.7 *in* M.V. Nathan and R.H. Gelderman, eds. Recommended chemical soil testing procedures for the North Central region. Missouri Agricultural Experiment Station SB 1001, Columbia, MO.

PlantMaps (n.d.). Canada Köppen-Geiger climate classification map. [Online] Available: https://plantmaps.com/koppen-climate-classification-map-canada.php [2021 Apr. 8].

Racz, G.J., Webber, M.D., Soper, R.J., and Hedlin, R.A. 1965. Phosphorus and nitrogen utilization by rape, flax, and wheat. Agron. J. **57**: 335–337. doi:10.2134/agronj1965.00021962005700040007x.

Rech, I., Withers, P.J.A., Jones, D.L., and Pavinato, P.S. 2019. Solubility, diffusion and crop uptake of phosphorus in three different struvites. Sustainability **11**: 134. doi:10.3390/su11010134.

Reid, K., Schneider, K., and Joosse, P. 2019. Addressing imbalances in phosphorus accumulation in Canadian agricultural soils. J. Environ. Qual. **48**: 1156–1166. doi:10.2134/jeq2019.05.0205.

Roberts, T.L., and Johnston, A.E. 2015. Phosphorus use efficiency and management in agriculture. Resour. Conserv. Recycl. **105**: 275–281. doi:10.1016/j.resconrec.2015.09.013.

Schneider, K.D., Lynch, D.H., Bünemann, E.K., and Voroney, R.P. 2017. Vegetative composition, arbuscular mycorrhizal fungi root colonization, and biological nitrogen fixation distinguish organic and conventional perennial forage systems. Agron. J. **109**: 1697–1706. doi:10.2134/agronj2016.12.0700.

Schneider, K.D., Thiessen Martens, J.R., Zvomuya, F., Reid, D.K., Fraser, T.D., Lynch, D.H., O'Halloran, I.P., and Wilson, H.F. 2019. Options for improved phosphorus cycling and use in agriculture at the field and regional scales. J. Environ. Qual. **48**: 1247–1264. doi:10.2134/jeq2019.02.0070.

Selles, F., Campbell, C.A., and Zentner, R.P. 1995. Effect of cropping and fertilization on plant and soil phosphorus. Soil Sci. Soc. Am. J. **59**: 140–144. doi:10.2136/sssaj1995.03615995005900010022x.

Strong, W.M., and Soper, R.J. 1974. Phosphorus utilization by flax, wheat, rape, and buckwheat from a band or pellet-like application. I. Reaction zone root proliferation. Agron. J. **66**: 597–601. doi:10.2134/agronj1974.00021962006600050001x.

Syers, J.K., Johnston, A.E., and Curtin, D. 2008. Efficiency of soil and fertilizer phosphorus use: Reconciling changing concepts of soil phosphorus behaviour with agronomic information. Food and Agriculture Organization of the United Nations, Rome.

Talboys, P.J., Heppell, J., Roose, T., Healey, J.R., Jones, D.L., and Withers, P.J.A. 2016. Struvite: a slow-release fertiliser for sustainable phosphorus management? Plant Soil **401**: 109–123. doi:10.1007/s11104-015-2747-3.

Thompson, J.P. 1996. Correction of dual phosphorus and zinc deficiencies of linseed (*Linum usitatissimum* L.) with cultures of vesicular-arbuscular mycorrhizal fungi. Soil Biol. Biochem. **28**: 941–951. doi:10.1016/0038-0717(95)00185-9.

Turmel, M.-S., Entz, M.H., Bamford, K., and Thiessen Martens, J.R. 2009. The influence of crop rotation on the mineral nutrient content of organic vs. conventionally produced wheat grain: Preliminary results from a long-term field study. Can. J. Plant Sci. **89**: 915–919. doi:10.4141/CJPS09006.

Vaisman, I., Entz, M.H., Flaten, D.N., and Gulden, R.H. 2011. Blade roller–green manure interactions on nitrogen dynamics, weeds, and organic wheat. Agron. J. **103**: 879–889. doi:10.2134/agronj2010.0437.

Warncke, D., and Brown, J.R. 2015. Potassium and other basic cations. Page 7.1-7.3 *in* M.V. Nathan and R.H. Gelderman, eds. Recommended chemical soil testing procedures for the North Central region. Missouri Agricultural Experiment Station SB 1001, Columbia, MO.

Weissengruber, L., Möller, K., Puschenreiter, M., and Friedel, J.K. 2018. Long-term soil accumulation of potentially toxic elements and selected organic pollutants through application of recycled phosphorus fertilizers for organic farming conditions. Nutr. Cycl. Agroecosystems **110**: 427–449. doi:10.1007/s10705-018-9907-9.

Welsh, C., Tenuta, M., Flaten, D.N., Thiessen-Martens, J.R., and Entz, M.H. 2009. High yielding organic crop management decreases plant-available but not recalcitrant soil phosphorus. Agron. J. **101**: 1027–1035. doi:10.2134/agronj2009.0043.

Withers, P.J.A. 2019. Closing the phosphorus cycle. Nat. Sustain. **2**: 1001–1002. doi:10.1038/s41893-019-0428-6.

Withers, P.J.A., Neal, C., Jarvie, H.P., and Doody, D.G. 2014. Agriculture and eutrophication: Where do we go from here? Sustainability **6**: 5853–5875. doi:10.3390/su6095853.

Wollmann, I., Gauro, A., Müller, T., and Möller, K. 2018. Phosphorus bioavailability of sewage sludge-based recycled fertilizers. J. Plant Nutr. Soil Sci. **181**: 158–166. doi:10.1002/jpln.201700111.

Xie, Y., Niu, X., and Niu, J. 2016. Effect of phosphorus fertilizer on growth, phosphorus uptake, seed yield, yield components, and phosphorus use efficiency of oilseed flax. Agron. J. **108**: 1257–1266. doi:10.2134/agronj2015.0537.

3. EFFECT OF STRUVITE APPLICATION ON LABILE PHOSPHORUS POOLS IN CONTRASTING MANITOBA SOILS

3.1 Abstract

Optimal management of the sparingly soluble fertilizer struvite requires a greater understanding of its behaviour in differing soil types and its effect on phosphorus (P) pools extracted in agronomic and environmental soil tests. The objective of this study was to quantify changes in labile soil P pools over time in soils amended with different rates (100 and 200 mg P kg^{-1}) of granular (0.9 mm) struvite or monoammonium phosphate (MAP) and incubated for 112 d without plants. Water-extractable P (WEP) and Olsen-P concentrations were initially greater in soils amended with struvite rather than MAP, contrary to expectations. The unexpectedly high WEP and Olsen-P concentrations in struvite-amended soils after only 4 or 7 d of incubation suggest that sample grinding may have increased the extractability of P in undissolved struvite granules. For both fertilizers, WEP declined over time and converged to similar concentrations by Day 112. Olsen-P concentrations in soils amended with the high rate of struvite declined below those for the same rate of MAP by Day 70. Olsen-P concentrations were lower in a neutral-pH sandy clay loam than in an alkaline clay soil, whereas WEP concentrations were greater in the sandy clay loam, but only at the high application rate and in the initial days of incubation, suggesting that a high rate of fertilizer application temporarily overwhelmed P retention processes in the sandy clay loam. The relationship between WEP and Olsen-P varied with soil and fertilizer types and changed with duration of incubation. These findings demonstrate the complexity of P dynamics in P-amended soils, emphasizing the need to clarify the roles of specific soil properties, soil sample preparation, and P extraction methods in determining the effects of struvite application on soil P pools.

3.2 Introduction

Recovery of phosphorus (P) from waste streams for use as a fertilizer has recently gained attention as part of the solution to the twin problems of declining phosphate rock reserves and environmental degradation associated with P lost to the environment, in the face of an ongoing need for sufficient P to support agricultural production (Sharpley et al. 2018; Withers 2019). More than 100 P-recovery systems are now in operation in wastewater treatment plants around the world, most of which produce struvite ($NH_4MgPO_4 \cdot 6H_2O$) as the recovered P product (Kabbe 2021). Among recovered P products, struvite in particular shows good potential as a fertilizer due to its low level of contamination and relatively high bioavailability (Schürmann et al. 2012; Meyer et al. 2018; Hall et al. 2020). Struvite has been shown to perform relatively well as a plant fertilizer (Huygens and Saveyn 2018; Hertzberger et al. 2020) and to potentially reduce the risk of P loss in runoff (Everaert et al. 2018). However, the variability in crop response to struvite (Hertzberger et al. 2020) highlights the importance of soil and crop properties in governing its dissolution dynamics.

Struvite has low solubility in water but its solubility in acidic extracts is similar to that of conventional P fertilizers (Duboc et al. 2017; Kratz et al. 2019). Struvite dissolution in soil is typically slower than that of soluble fertilizers but is influenced by soil properties such as pH, texture, and P retention capacity, as well as struvite properties such as granule size (Degryse et al. 2017; Hertzberger et al. 2020; Anderson et al. 2020). The roles of these and other factors in determining P release from struvite require clarification to guide the development of struvite fertilizer management practices that optimize P supply to crops without creating undue risk of loss to the environment.

Standard extractions performed on sparingly soluble fertilizers directly do not adequately predict their agronomic efficacy (Meyer et al. 2018; Kratz et al. 2019) and are likely poor predictors of the potential for P loss to the environment as well. Performing standard agronomic soil extractions on incubated soil–fertilizer mixtures may provide a better indication of the agronomic effectiveness of struvite and other recovered fertilizers than the solubility of the fertilizers themselves by accounting for the effects of soil–fertilizer interactions (Kratz et al. 2019). This approach has been used, for example, to compare the agronomic efficacy of various recycled fertilizers (Duboc et al. 2017) and to evaluate the risk of P runoff loss in soils amended with soluble fertilizer or manure (Kumaragamage et al. 2011). Repeated sampling of incubated soil can also provide insights into the processes that contribute to the fate of added P as it is detected in different pools over time. This approach has been used to demonstrate differences in the behaviour of manure relative to soluble fertilizer (Griffin et al. 2003) and to assess the effect of soil type on struvite dissolution (Anderson et al. 2020).

Characterizing labile soil P through chemical extractions is not straightforward, due to the many species of P in soil and the processes by which P may be brought into soluble forms. Many different tests have been developed to assess labile soil P for the purposes of agronomic and environmental management, with different extractions used in different soil types and for different purposes. Water-extractable P (WEP) measures P intensity, the pool of P that is in solution or readily brought into solution, and is immediately available for plant uptake (Beegle 2005). This test is considered a poor indicator of plant-available P, but can be a good indicator of the risk of loss to the environment through runoff (Wang et al. 2012). For the neutral to alkaline soils characteristic of the Northern Great Plains, the sodium bicarbonate extraction developed by Olsen et al. (1954) (Olsen-P) has become the standard agronomic soil test predicting plant response to added P fertilizer and has also been shown to be a better indicator than WEP of the risk of loss to the environment in Manitoba soils (Kumaragamage et al. 2011). The P extracted in the Olsen test likely includes soil solution P, weakly adsorbed inorganic P, slightly soluble Ca-P, and readily hydrolyzed organic P (Schoenau and O'Halloran 2008; Kratz et al. 2019). An additional test that attempts to account for the role of low-molecular-weight organic anions from plant roots in P acquisition is a weak citrate extraction, which has been used to explore the role of root exudation of organic anions in calcareous soils (Ström et al. 2005) and is part of a suite of tests used to characterize P availability in soils across the UK (DeLuca et al. 2015). Due to the presumed importance of organic anions in the ability of plants to access struvite P (Talboys et al. 2016), a citrate extraction may provide insights into struvite dissolution dynamics and plant-available P concentration in struvite-amended soils.

The objective of this study was to quantify changes in labile soil P pools, measured as WEP and Olsen-P, over time in contrasting low soil test P (STP) soils amended with different rates of struvite or MAP. We also included a preliminary analysis of citrate-extractable P (CEP) in these soils. We hypothesized that (1) WEP and Olsen-P pools would initially be smaller in soils amended with struvite than with MAP, especially in an alkaline soil, but differences between fertilizers would diminish with time, (2) the CEP pool would initially be greater in soils amended with struvite than those amended with MAP, and (3) the relationships between P extractions targeting different labile P pools would differ among fertilizer types, soil types, and duration of incubation, due to differing effects of soil properties on the dissolution and transformation processes of P derived from struvite and MAP.

3.3 Materials and Methods

3.3.1 Experimental and Treatment Designs

This experiment tested the main and interactive effects of soil type, P fertilizer type, P application rate, and incubation duration on labile soil P concentrations and P recovery using a completely randomized design with sampling date (incubation duration) as a repeated measure.

The two soil types were a Dencross clay with alkaline pH, classified as a Gleyed Rego Black Chernozem, with a history of forage and annual crop production under organic management, and a Wampum sandy clay loam with neutral pH, classified as a Gleyed Gray Luvisol, with a history of annual crop production under conventional management. The two fertilizers were the commercial struvite fertilizer Crystal Green® ($N-P_2O_5-K_2O$ analysis 5-28-0 plus 10% Mg, equivalent to 122 mg P g^{-1}; Ostara Nutrient Recovery Technologies Inc., Vancouver, BC) and commercial MAP ($N-P_2O_5-K_2O$ analysis 11-52-0, equivalent to 227 mg P g^{-1}; CropKing Inc., Lodi, OH). MAP was chosen as the reference fertilizer due to its similar N:P ratio to struvite and because it is widely used in Manitoba. Both fertilizers were applied in granular form, using SGN90 (0.9 mm diameter) granules of struvite and <2 mm diameter granules of MAP isolated by sieving. Fertilizers were applied in granular form rather than finely ground, as granular struvite has been observed to behave differently in soil than ground struvite (Degryse et al. 2017; Anderson et al. 2020). However, small granules were used to achieve a more uniform distribution of the fertilizers throughout the soil than would be possible with large granules. The fertilizer application rates were 100 and 200 mg P (kg soil)$^{-1}$, equivalent to 150 and 300 kg P ha^{-1} based on a soil bulk density of 1.0 g cm^{-3} and soil depth of 15 cm. The low rate was equal to the highest application rate used in a companion plant growth experiment (Chapter 4), approximately four times the recommended P application rate for legume forages in a low-P soil in Manitoba (Manitoba Agriculture 2007).

Within each soil type, fertilizer type and application rate treatments were assigned in a factorial-plus-one-control design, resulting in five fertilizer treatments: 100 mg P kg^{-1} as struvite (S-100), 200 mg P kg^{-1} as struvite (S-200), 100 mg P kg^{-1} as MAP (M-100), 200 mg P kg^{-1} as MAP (M-200), and a nil-P control. Each soil–fertilizer treatment combination was replicated four times, for a total of 40 experimental units. Each experimental unit was comprised of six sub-units, including one unamended baseline sample and five incubated portions assigned randomly to one of five sampling dates (Days 4, 7, 28, 70 and 112 of incubation). Groups of sub-units within each experimental unit were kept together during the incubation.

3.3.2 Soil Preparation and Experiment Set-up

Soils were collected in autumn 2018 from the upper 15-cm layer of annual crop fields near Libau (50.241258, -96.728878; Dencross clay) and Stead (50.414696, -96.489661; Wampum sandy clay loam) in east-central Manitoba. Field-moist soils were hand-sieved to pass a 10 mm screen to remove crop residue and stones and air-dried for at least 48 h at ~30 °C, stored for 7 mo, and then homogenized prior to experiment set-up. Duplicate samples of each soil type were analyzed for chemical and physical properties (Table 3.1). Additional samples (~50 g, n = 3) were oven-dried at 105 °C until reaching constant weight to determine the moisture content of the air-dried soil. The water-holding capacity of each soil type was determined by packing air-dry soil into transparent columns, applying differing quantities of water from the top, and selecting as the container capacity the quantity of water that wet the entire column without draining excess water. Target moisture contents for the Dencross and Wampum soils were 0.44 and 0.29 g g^{-1}, respectively (equivalent to 67 and 61% water-filled pore space).

Table 3.1 Selected chemical properties and particle size analysis of experimental soils used in the incubation study

Soil property	**Dencross**	**Wampum**
Olsen-P, mg kg^{-1}	6.3 (0.3) [a]	7.8 (1.0)
Water-extractable P (WEP), mg kg^{-1}	2.3 (0.4)	1.5 (0.8)
Citrate-extractable P (CEP), mg kg^{-1}	4.4 (1.7)	7.0 (2.0)
Total P, mg kg^{-1}	665 (2)	308 (6)
pH (in water)	8.1 (0.1)	7.0 (0.1)
Nitrate-N, mg kg^{-1}	9 (1)	7 (1)
Total N, % (combustion analyzer method)	0.30 (0.02)	0.17 (0.01)
Exchangeable K, mg kg^{-1}	383 (13)	235 (1)
Sulphate-S, mg kg^{-1}	8 (1)	10 (6)
Exchangeable Ca, mg kg^{-1}	6700 (140)	2130 (20)
Exchangeable Mg, mg kg^{-1}	976 (28)	328 (4)
CEC, meq/100g (summation of cations)	42.8 (0.9)	14.0 (0.1)
Organic C, % (combustion minus inorganic C)	4.2 (0.1)	1.9 (0.1))
Calcium carbonate, CCE % (pressure method)	5.6 (0.2)	0.9 (0.1)
Sand, %	26 (1)	67 (3)
Silt, %	28 (0)	13 (1)
Clay, %	46 (1)	20 (1)
Texture class	Clay	Sandy clay loam

[a] Values are means with standard deviation in parentheses (*n* = 2, except for Olsen-P, water-extractable P, and citrate-extractable P, where *n* = 20).

Air-dry soil for individual experimental units was mixed thoroughly and then separated into five 300-g (oven-dry equivalent) sub-units plus a 100-g baseline sample. Pre-weighed granular formulations of struvite and MAP fertilizers were mixed with individual sub-units of dry soil and placed into 10 cm diameter plastic plant pots lined with transparent plastic bags. Reverse osmosis (RO) water was added to each sub-unit to bring the soil to container capacity. The tops of the bags were tied loosely and bags were perforated in several places above the soil surface to allow for air exchange. Pots were incubated at 20±2 °C in the dark in a walk-in incubation chamber and were returned to their target moisture content (based on weight) at least once every two weeks. The position of experimental units within the incubator was randomized at each of the first two sampling dates and every two weeks for the remainder of the experiment. One experimental unit (Wampum soil, S-100 treatment) was removed from the experiment due to an error during set-up. An incubator malfunction at Day 26 of the incubation caused several pots to be flooded with tap water. Three pots, all assigned to the Day 70 sampling date, were removed from the experiment because of excessive wetness. Several others were wetted to moisture contents above the target (up to 0.52 and 0.38 g g^{-1} for the Dencross and Wampum soils, respectively) but were allowed to dry to the target moisture by opening the plastic bags and were retained in the experiment; none of these pots were identified as outliers during the statistical analysis.

3.3.3 Soil Sampling, Analyses, and Calculations

On each of the five sampling dates, sub-units (pots) assigned to that date were sampled destructively by thoroughly mixing the soil and separating a sample of about 80–90 g dry soil equivalent. These samples were air-dried for about 48 h at ~30 °C, ground to pass a 2-mm screen, and later ground to a fine powder using a Christy & Norris Lab Mill (Christy Turner Ltd., Ipswich, UK). Results from a preliminary test indicated that variability among analytical replicates was reduced by grinding soil to a fine powder, perhaps due to the presence of remnants of struvite granules in unground soil samples.

Soil samples from all sampling dates, including baseline samples, were analyzed for labile P concentration according to three parallel extractions—WEP, Olsen-P, and CEP—using the extraction procedures outlined in Table 3.2. WEP and CEP concentrations were determined using ICP and thus include organic P, whereas the Olsen-P extraction, with colorimetric determination, includes only molybdate-reactive P. However, for Manitoba soils, the difference between ICP and colorimetric determination of Olsen extracts is minimal (Adesanwo et al. 2013). For the CEP analysis, only the data from the first and the last sampling dates were used. The WEP concentration data from Day 70 were excluded from analysis due to the presence of several outliers in data from this sampling date.

Table 3.2 P extraction methods used to characterize water-extractable (WEP), bicarbonate-extractable (Olsen-P) and citrate-extractable (CEP) P pools in incubated soils

	Extraction		
	WEP	**Olsen-P**	**CEP**
Soil mass	2.5 g	2.5 g	2.5 g
Extractant volume	25 ml	50 ml	50 ml
Soil: solution ratio (g: ml)	1:10	1:20	1:20
Extractant	Ultrapure water with resistance of 18.2 mΩ	0.5M sodium bicarbonate, adjusted to pH 8.5	25 mM citric acid in ultrapure water, adjusted to pH 7.5
Extraction time	30 min	30 min	180 min
Filtration	Whatman #42 filter paper (2.5 µm)	Advantec #1 filter paper (6 µm)	Whatman #42 filter paper (2.5 µm)
Determination	ICP-OES, 213.617 nm (Optima 5300DV, Perkin Elmer, Waltham, MA)	Colorimetric, molybdate blue (San^{SERIES}, Skalar, Breda, The Netherlands)	ICP-OES, 213.617 nm (Optima 5300DV, Perkin Elmer, Waltham, MA)
Reference	Self-Davis et al. (2009)	Olsen et al. (1954), Schoenau and O’Halloran (2008)	Ström et al. (2005)

The proportion of added P recovered by each extraction was calculated for each soil sample using the following equation:

$$\text{P recovery} = (P_f - P_{uf})/(P_{app}) \qquad (1)$$

where P_f is the P concentration in the fertilized treatment, P_{uf} is the P concentration in the unfertilized control in the same soil type at the same sampling date (mean of four replicates), and P_{app} is the P applied, all in mg kg^{-1}.

A sample of each fertilizer was digested in HNO_3 in a MARS 6 microwave digester (CEM, Matthews, NC) and analyzed for total P by ICP–OES to verify consistency with the P analysis stated by manufacturers. The analyzed P concentrations were 122 and 226 mg P g^{-1} for struvite and MAP, respectively, corresponding closely to the stated P analysis.

3.3.4 Statistical Analyses

An analysis of variance was conducted using PROC GLIMMIX of SAS® OnDemand for Academics (version 9.4, SAS Institute Inc., Cary, NC) to determine the effects of soil type, fertilizer type, application rate, and duration of incubation, as well as their interactions, on P concentration and recovery in each of the extractions. All factors were considered fixed effects, with sampling date modeled as a repeated measurement using the spatial power covariance structure to account for the unequal spacing between dates and the Kenward–Roger denominator degrees of freedom method. Final P concentration and recovery data (after 112 d) were also analyzed separately to allow for more meaningful comparisons to a companion pot experiment (Chapter 4), with results shown in Appendix II. Recovery of P from soil was analyzed as a beta distribution (with means and standard errors back-transformed using the ILINK function in the LSMEANS statement) and P concentration was analyzed as a Gaussian distribution. Homogeneity of variance for P concentration data was assessed by visual inspection of residual plots and tests of covariance among treatment groups, adjusting for unequal variances using the RANDOM _RESIDUAL_ / GROUP statement in GLIMMIX where necessary. Normal distribution of residuals was confirmed by the Shapiro–Wilk test (W-statistic > 0.9). Post-hoc means comparison was conducted using the Tukey multiple comparisons method. Effects were considered significant at $p < 0.05$. Due to the factorial-plus-control design of fertilizer type and application rate treatments, *p*-values from ANOVA reflect effects of the fertilizer treatments excluding the nil-P control, but the control is included in the means comparison.

The relationships between soil P pools were explored with ordinary least squares regression analysis, using analysis of covariance (ANCOVA, with PROC MIXED of SAS software) to test for differences in slopes between soil types, fertilizer types, and application rates, and among sampling dates. Models were constructed to test each categorical factor individually and in combination as interactive effects. The assumptions of homogeneity of variance and normal distribution were assessed as above.

3.4 Results

3.4.1 Soil Properties

Both soils were initially low in labile P pools but differed in other properties that could be expected to affect struvite dissolution and P adsorption and/or precipitation processes (hereafter referred to collectively as retention processes) in soils (Table 3.1). In particular, the Dencross soil had higher pH and higher concentrations of organic matter, clay, Ca, Mg, and carbonate than the Wampum soil.

3.4.2 General Patterns in Extractable Labile P

The P concentrations in all three extractions were influenced by fertilizer type, application rate, soil type, and time of incubation in various interactions (Table 3.3), as described in more detail below. The soil WEP concentration ranged from 3.7–67 mg kg^{-1} in fertilized treatments and 0.4–2.4 mg kg^{-1} in unfertilized controls. The Olsen-P concentration was 26–138 mg kg^{-1} in fertilized treatments and 7–10 mg kg^{-1} in unfertilized controls. The CEP concentration was 19–117 mg kg^{-1}

in fertilized treatments and 4–10 mg kg^{-1} in unfertilized controls, very similar to Olsen-P. The Olsen-P concentration in fertilized treatments exceeded the unfertilized controls in all treatments and sampling dates, whereas WEP and CEP concentrations at the low P application rate were statistically similar to the controls at some dates. Significant differences in WEP and Olsen-P occurred between soil types in the unfertilized control in some cases, but differences were small compared to the increases resulting from fertilizer addition (Figure 3.1); CEP did not differ between soils in the unfertilized control (mean 6.2 mg kg^{-1} across all dates). In general, P concentrations and recovery declined over time, indicating that P was gradually transformed from the labile P pools to more recalcitrant forms, but with differing patterns among combinations of the fertilizers, application rates, and soil types.

3.4.3 Interaction Between Fertilizer and Soil Type

Averaged across application rates and sampling dates, the overall effects of the two fertilizer types on WEP and Olsen-P were modulated by soil type, though the patterns differed for the two P extractions. In both soils, WEP concentration was substantially greater in struvite-amended soils than in MAP-amended soils, but with a greater difference between fertilizers in the Wampum soil than in the Dencross soil (Figure 3.1a). However, the means across sampling dates were strongly influenced by the high WEP concentrations in the early sampling dates. The Olsen-P concentration was also greater for struvite than MAP in the Wampum soil but did not differ between fertilizers in the Dencross soil and was overall greater in the Dencross soil than the Wampum soil (Figure 3.1c). The same patterns among fertilizer and soil types were also evident for WEP and Olsen-P recovery (Figure 3.1b, d) because of the similarly low background extractable P concentrations in the control treatments of both soils.

Table 3.3 Effect of soil type, fertilizer type, application rate, and duration of incubation (time) on labile P concentration and percent fertilizer-P recovery in incubated soil samples

	WEP		**Olsen P**		**CEP**	
Factor	**Conc.**	**Recovery**	**Conc.**	**Recovery**	**Conc.**	**Recovery**
	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	%
Soil						
Dencross	10.0 (0.6) [a]	8.7 (0.4)	42.8 (0.6)	46 (0.8)	27.2 (1.6)	24.0 (0.8)
Wampum	10.8 (0.6)	10.1 (0.4)	32.2 (0.6)	29 (0.7)	30.2 (1.6)	31.5 (0.9)
Fertilizer						
Control	1.5 (0.8)	-	8.5 (0.1)	-	6.2 (2.0)	
Struvite	25.2 (0.6)	12.0 (0.5)	68.0 (1.3)	38 (0.8)	53.7 (1.5)	28.0 (0.9)
MAP	13.2 (0.6)	7.2 (0.3)	65.0 (0.9)	37 (0.8)	48.7 (1.4)	27.3 (0.8)
Rate						
0	1.5 (0.8)	-	8.5 (0.1)	-	6.2 (2.0)	-
100	11.1 (0.6)	8.0 (0.4)	45.9 (0.9)	36 (0.8)	30.6 (1.5)	23.6 (0.8)
200	27.3 (0.6)	10.9 (0.4)	87.1 (1.3)	38 (0.8)	71.8 (1.4)	32.1 (0.8)
Time						
Day 4	14.7 (1.0)	15.2 (0.7)	45.9 (1.1)	49 (1.3)	35.5 (1.6)	34.8 (0.9)
Day 7	12.8 (1.1)	13.1 (0.6)	42.6 (1.0)	45 (1.3)	-	-
Day 28	9.2 (0.8)	9.0 (0.5)	40.4 (1.0)	41 (1.2)	-	-
Day 70	-	-	31.2 (0.7)	29 (1.2)	-	-
Day 112	4.7 (0.3)	4.1 (0.4)	27.4 (0.5)	24 (1.1)	21.8 (1.6)	21.4 (0.7)
ANOVA	*p*-values					
Soil (S)	0.03	0.01	<0.0001	<0.0001	0.01	<0.0001
Fertilizer (F)	<0.0001	<0.0001	0.07	0.21	0.01	0.57
Rate (R)	<0.0001	<0.0001	<0.0001	0.06	<0.0001	<0.0001
Time (T)	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
S × F	0.002	0.04	0.0003	0.001	0.06	0.006
S × R	0.0001	0.01	<0.0001	0.25	0.02	0.95
S × T	0.047	0.047	<0.0001	0.03	0.99	0.44
F × R	<0.0001	0.20	0.53	0.94	0.67	0.54
F × T	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
R × T	<0.0001	0.74	<0.0001	0.75	<0.0001	0.13
S × F × R	0.18	0.82	0.16	0.99	0.02	0.59
S × F × T	0.08	0.14	0.64	0.61	0.54	0.78
S × R × T	0.45	0.48	0.04	0.46	0.58	0.18
F × R × T	<0.0001	0.28	0.02	0.86	0.002	0.04
S × F × R × T	0.74	0.93	0.60	0.53	0.004	0.09

[a] Values are means with standard error of the mean in parentheses. The highest-order interactions that are significant are plotted in Figures 3.1–3.6 and 3.9.

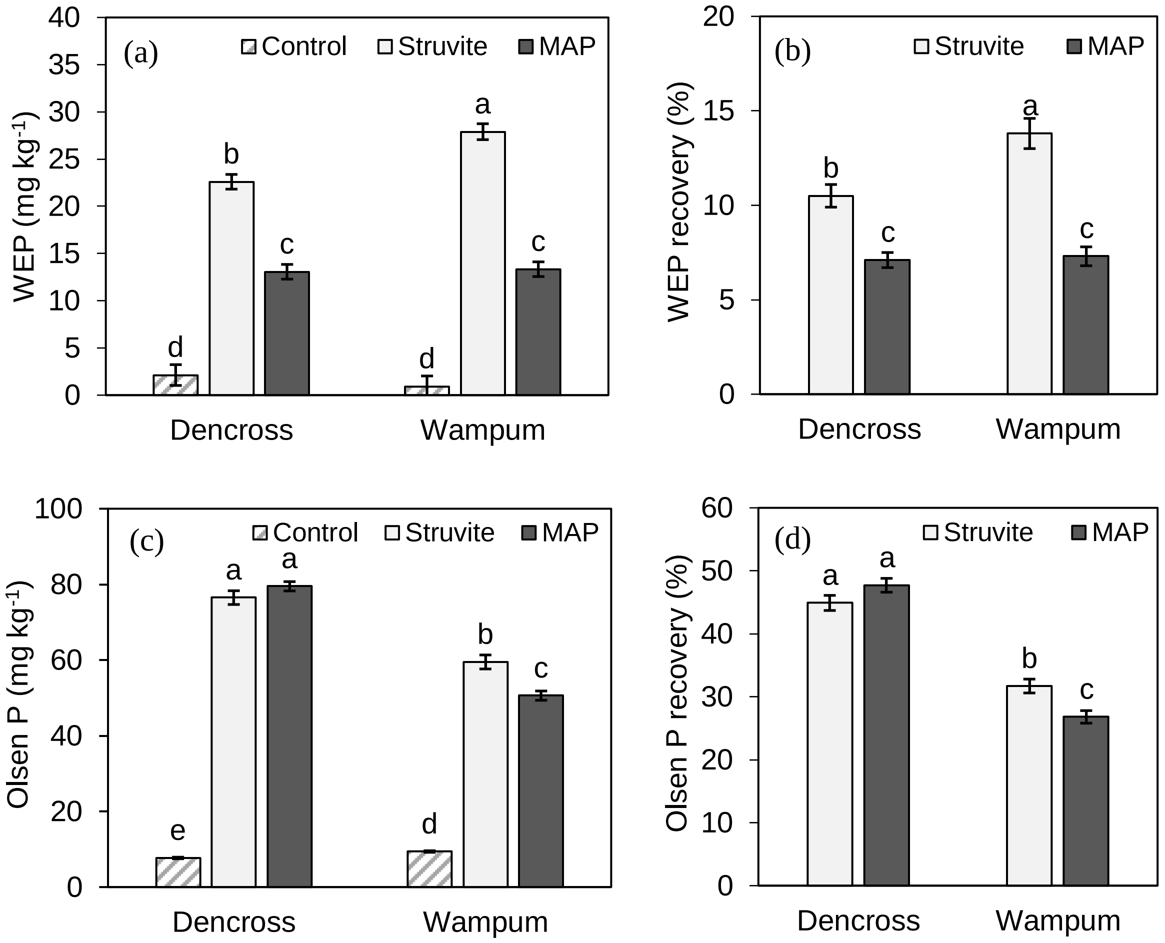

Figure 3.1 Interactive effect of soil type (Dencross clay, Wampum sandy clay loam) and fertilizer type (struvite, MAP) on water-extractable P (WEP) concentration (a) and percent fertilizer-P recovery (b) and Olsen-P concentration (c) and percent fertilizer-P recovery (d) averaged across P application rates and all sampling dates in soils incubated 112 d without plants. Bar height and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across soil and fertilizer treatments within each panel according to Tukey's test.

3.4.4 Temporal Effects of Fertilizer Type on Labile P Pools

Both WEP and Olsen-P concentrations were affected by the interaction of fertilizer type, application rate, and duration of incubation (Table 3.3). However, the P concentration and recovery among fertilizer types and rates followed somewhat different patterns for the two extractions.

Averaged across soil types, the WEP concentration in the S-200 treatment was more than double any other treatment on the first sampling date but converged with other fertilizer treatments by the final sampling date (Figure 3.2a). For both fertilizers, the increase in WEP concentration over the control in the high rate was 2.2–3.5 times that in the low rate throughout the incubation period; proportional differences between application rates tended to be greater for struvite than for MAP, except at Day 7.

Due to the high initial values in the struvite-amended soils, the decline in WEP concentration over time was more pronounced for struvite than for MAP, especially in the early sampling dates at the high application rate (Figure 3.2a). The WEP concentration was significantly greater with struvite than MAP in the first 28 d for the high rate and the first 7 d for the low rate. However, at 112 d, there were no differences between the two fertilizers within each application rate. The WEP concentration in the low MAP rate did not change over time and was statistically similar to the unfertilized control throughout the incubation period, despite being 4–8 mg kg^{-1} greater. Similar patterns were displayed in the proportion of added P recovered as WEP, with struvite exceeding MAP at all sampling dates except Day 112, but with no additional interaction with application rate (Figure 3.2b).

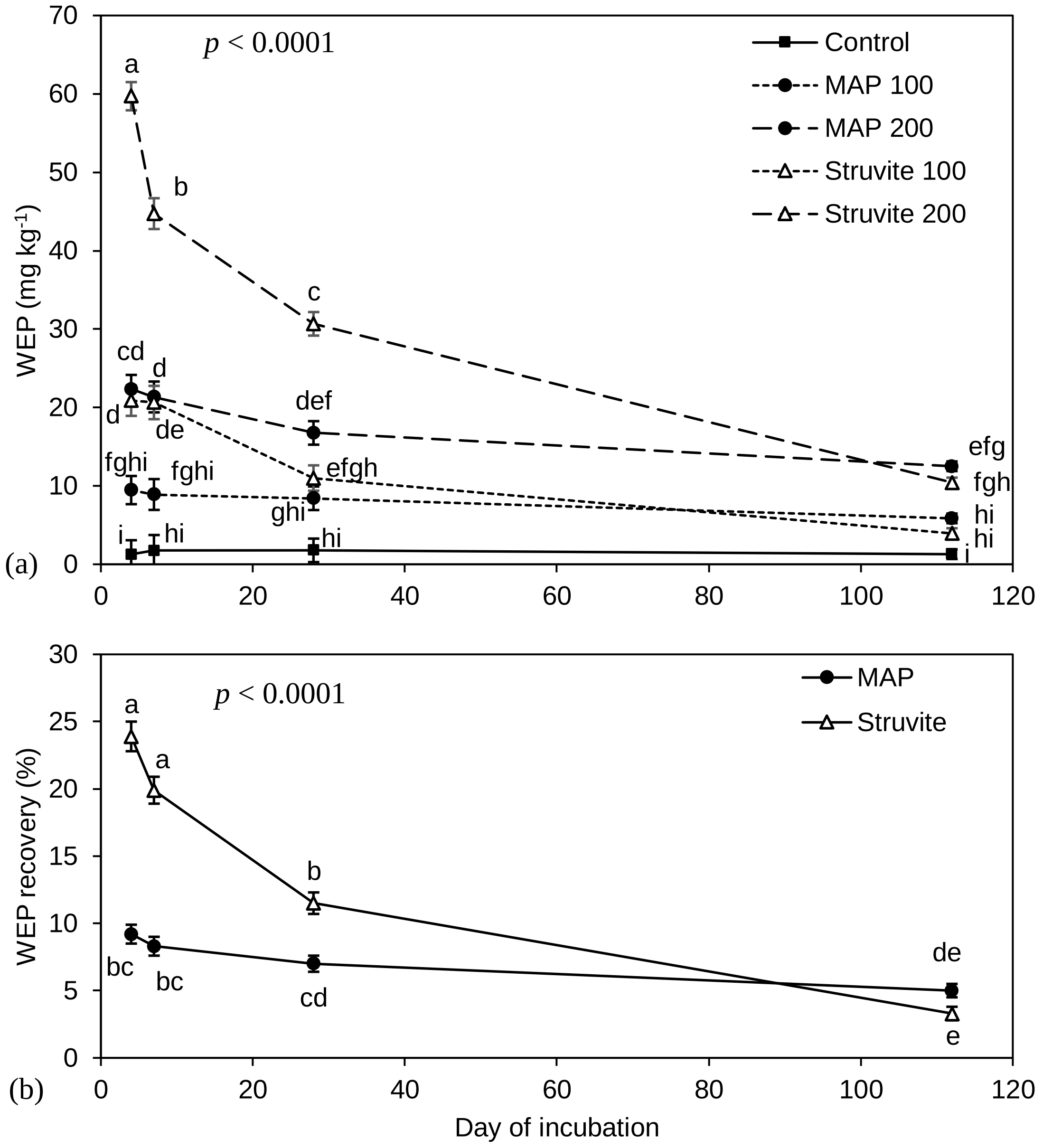

Figure 3.2 Temporal trends in water-extractable P (WEP) concentration (a) and percent fertilizer-P recovery (b) as a function of fertilizer type (MAP, struvite) and P application rate (100 or 200 mg kg^{-1}; for concentration only), averaged across soil types, in soils incubated 112 d without plants. Data points and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across all treatments and sampling dates within each panel, according to Tukey's test.

On average, the soil Olsen-P concentration in the two fertilizer treatments also differed with rate and duration of incubation, generally declining with time but following a different pattern than the WEP concentration. At the high application rate, the Olsen-P concentration in struvite-amended soil was greater than that in soil fertilized with MAP only at the first sampling date and was less than MAP at Day 70 and 112 (Figure 3.3a). At the low application rate, the two fertilizer treatments followed a similar crossover pattern part-way through the incubation period but differences between fertilizers were not significant at any date. Like WEP, the Olsen-P concentrations in all fertilizer and rate combinations except M-100 declined significantly over time, but Olsen-P concentrations for all fertilized treatments were always greater than the unfertilized control. Olsen-P recovery followed a similar pattern as Olsen-P concentration, with P recovered in struvite treatments exceeding that in MAP treatments at the early sampling dates and declining below MAP at later sampling dates (Figure 3.3b). Fertilizer application rate did not affect Olsen-P recovery as a main effect or in interaction with any other factor (Table 3.3).

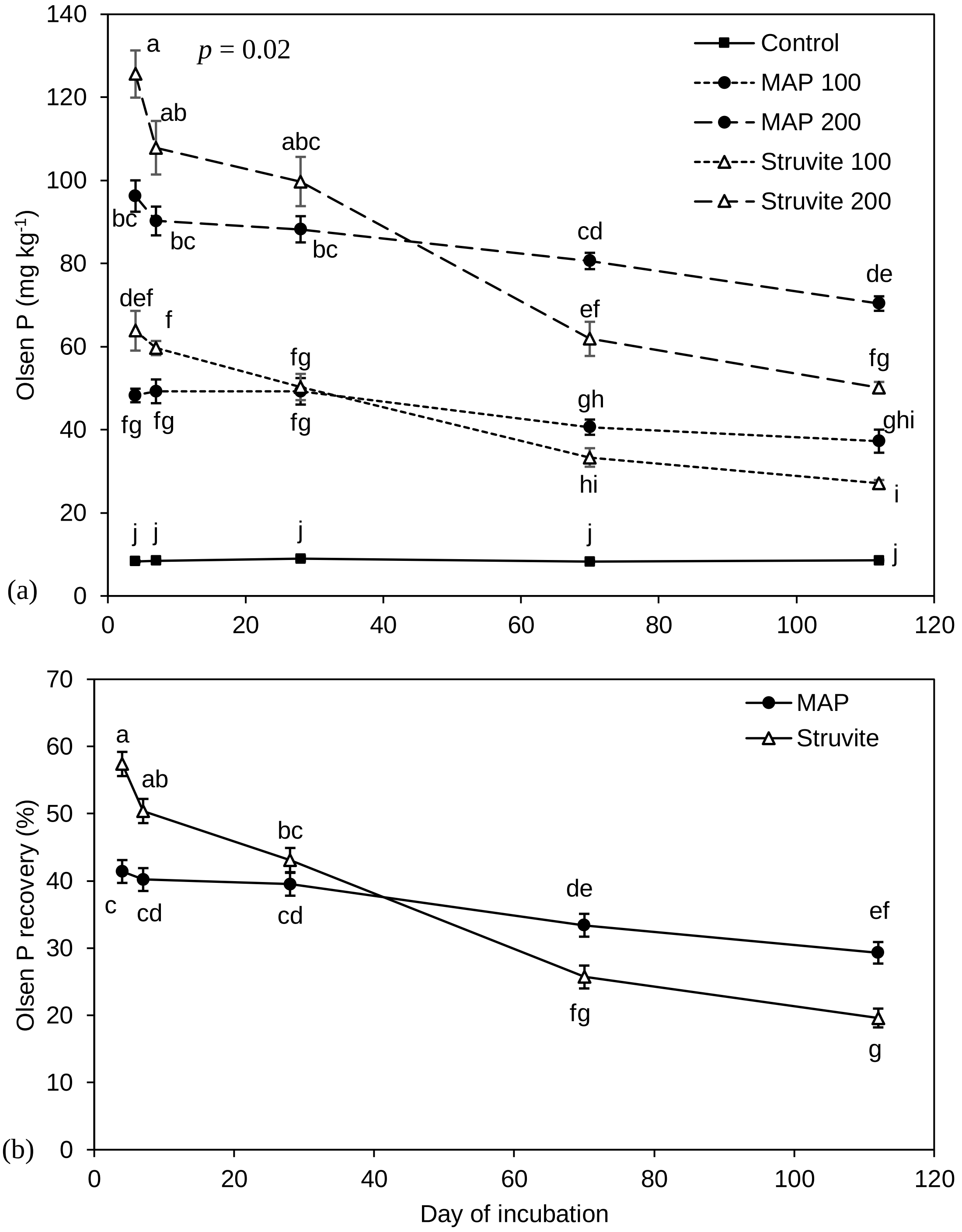

Figure 3.3 Temporal trends in Olsen-P concentration (a) and percent fertilizer-P recovery (b) as a function of fertilizer type (MAP, struvite) and P application rate (100 or 200 mg kg^{-1}; for concentration only), averaged across soil types, in soils incubated 112 d without plants. Data points and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across all treatments and sampling dates within each panel, according to Tukey's test.

3.4.5 Temporal Effects of Soil Type on Labile P Pools

Soil type also influenced the patterns of both WEP and Olsen-P concentrations and recovery of added P in these pools through an interaction effect with application rate and time (Table 3.3). The difference in P concentration between the two soils was greater overall for the Olsen fraction than the water-extractable fraction. Averaged across fertilizer types and application rates, WEP concentration and recovery showed a greater decline in the Wampum soil than the Dencross soil between Days 7 and 28, converging to similar values at Day 28 and thereafter (Figure 3.4). However, the difference in WEP concentration between the two soils, averaged across fertilizers and sampling dates, occurred only at the high application rate (Figure 3.5a). The interaction of soil type and application rate was also evident in the significantly greater proportion of added P recovered as WEP at the high rate in the Wampum soil relative to other treatments (Figure 3.5b).

Olsen-P concentration declined with incubation duration for both soils and P application rates, though differences among dates for the low rate in the Wampum soil were not significant (Figure 3.6a). Unlike the Olsen-P patterns observed between fertilizer types, the ranking of the two soils remained consistent over time. The Olsen-P concentration was higher in the Dencross soil than the Wampum soil, following the opposite pattern to WEP, and differences in Olsen-P between the two soils diminished over time for both application rates. The proportion of added P recovered as Olsen-P in the Dencross soil followed the same pattern, exceeding that in the Wampum soil throughout the incubation though the magnitude of differences diminished with time (Figure 3.6b).

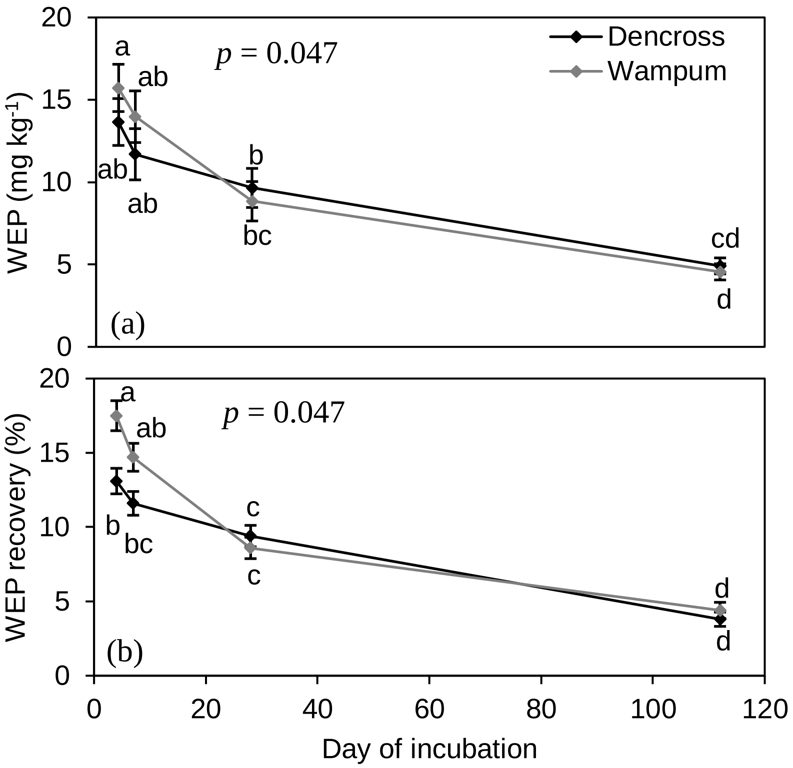

Figure 3.4 Temporal trends in water-extractable P (WEP) concentration (a) and percent fertilizer-P recovery (b) as a function of soil type (Dencross clay, Wampum sandy clay loam), averaged across fertilizer types, in soils incubated 112 d without plants. Data points and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across soils and sampling dates according to Tukey's test.

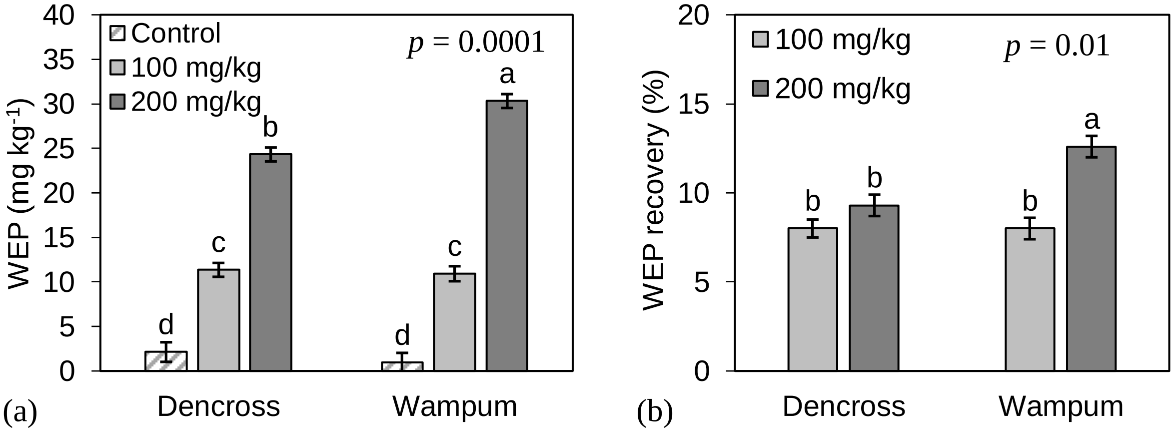

Figure 3.5 Interactive effect of soil type (Dencross clay, Wampum sandy clay loam) and fertilizer application rate (100 or 200 mg P kg^{-1}) on water-extractable P (WEP) concentration (a) and percent fertilizer-P recovery (b), averaged across fertilizer types and sampling dates, in soil incubated 112 d without plants. Bar height and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across soils and fertilizer application rates according to Tukey's test.

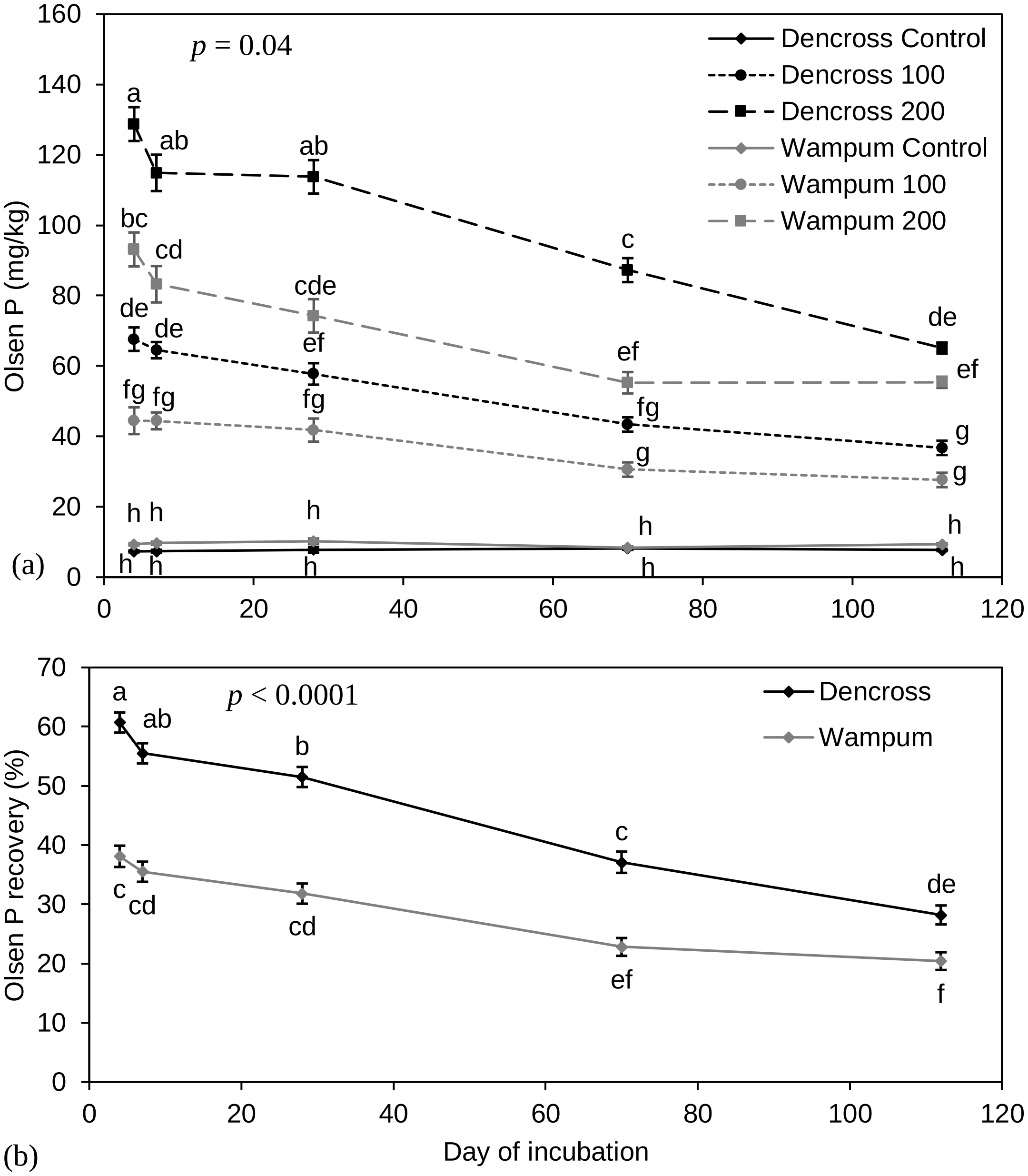

Figure 3.6 Temporal trends in Olsen-P concentration (a) and percent fertilizer-P recovery (b) as a function of soil type (Dencross clay, Wampum sandy clay loam) and fertilizer application rate (100 or 200 mg P kg^{-1}; for concentration data only), averaged across fertilizer types, in soils amended with struvite and MAP and incubated for 112 d. Data points and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across all treatments and sampling dates according to Tukey's test.

3.4.6 Effect of P Application Rate

The concentrations of WEP and Olsen-P were affected by fertilizer application rate, as expected, but always in interaction with duration of incubation, soil type, and/or fertilizer type (Table 3.3). In these interactions, the effect of application rate was evident in the magnitude, not the direction, of differences among treatments. Olsen-P concentrations at the high rate were approximately double those for the low rate for both fertilizers (Figure 3.3a) and both soil types (Figure 3.6a), indicating a proportional response to the added P. However, WEP concentrations were more than double in the high rate than in the low rate for both fertilizers at the early sampling dates (Figure 3.2a) and in the Wampum soil averaged across dates (Figure 3.5a). The disproportionate increase in WEP at the high rate in the Wampum soil was also reflected in the WEP recovery data (Figure 3.5b).

3.4.7 Relationship between Olsen-P and WEP

Olsen-P concentration in fertilizer-amended soils showed a strong positive linear correlation with WEP, but the relationship differed among soil–fertilizer treatment groups, as shown by a significant interaction between soil type and fertilizer type ($p < 0.0001$) in ANCOVA. Slopes were significantly greater for MAP than for struvite and were greater in the Dencross soil than the Wampum soil overall, but the magnitude of the difference in slope between the fertilizer types was greater in the Dencross soil than the Wampum soil (Figure 3.7).

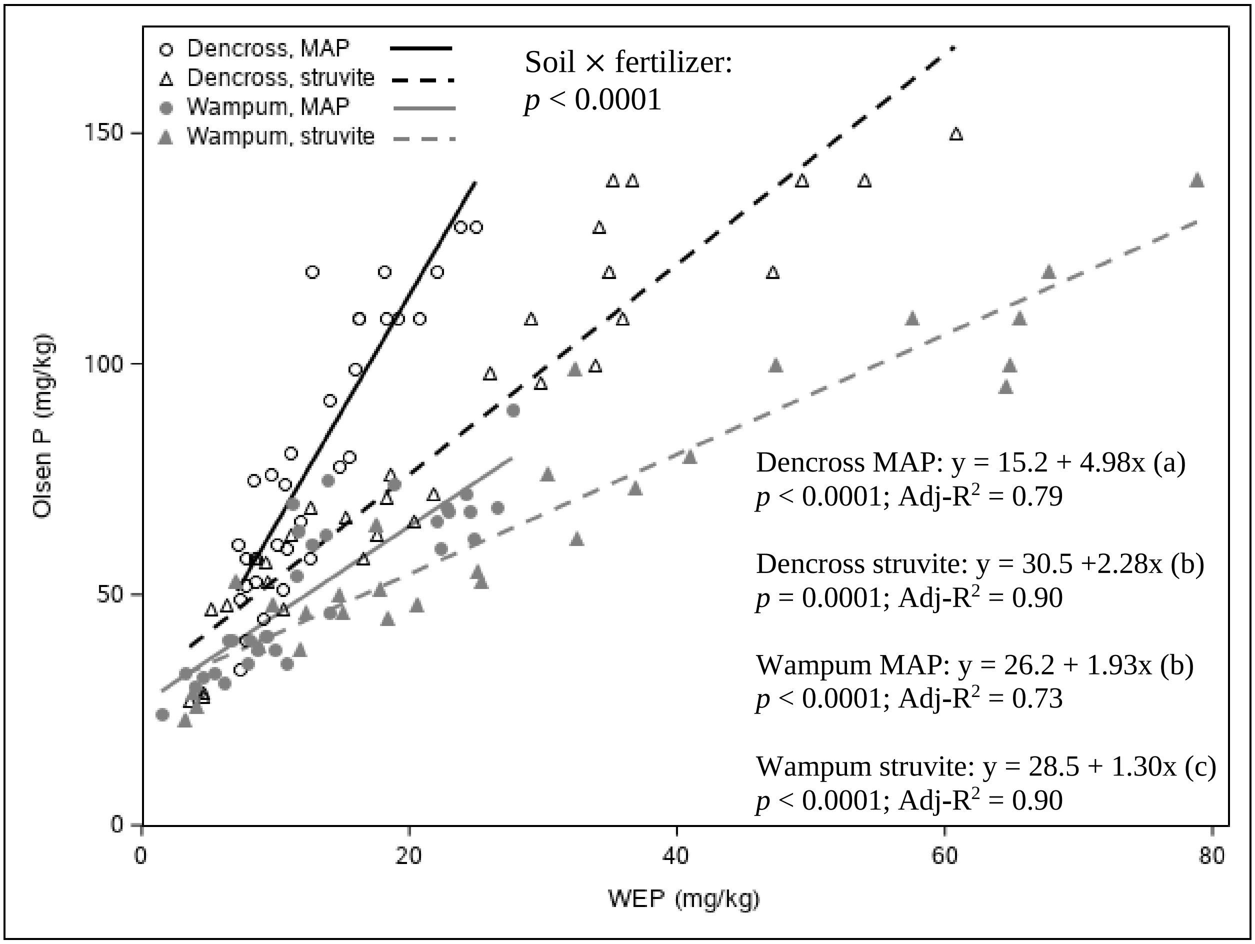

Figure 3.7 Regression relationships between water-extractable P (WEP) and Olsen-P in fertilized samples grouped by soil–fertilizer treatment. Lower case letters in parentheses following regression equations indicate significant differences in slope, according to pairwise comparisons.

A significant effect of sampling date on the regression of Olsen-P on WEP was also evident across treatments ($p = 0.04$), with slopes becoming steeper with increasing duration of incubation (Figure 3.8). This pattern reflects the greater decline in WEP concentration over time relative to Olsen-P concentration. Fertilizer application rate did not affect the relationship between WEP and Olsen-P ($p = 0.13$).

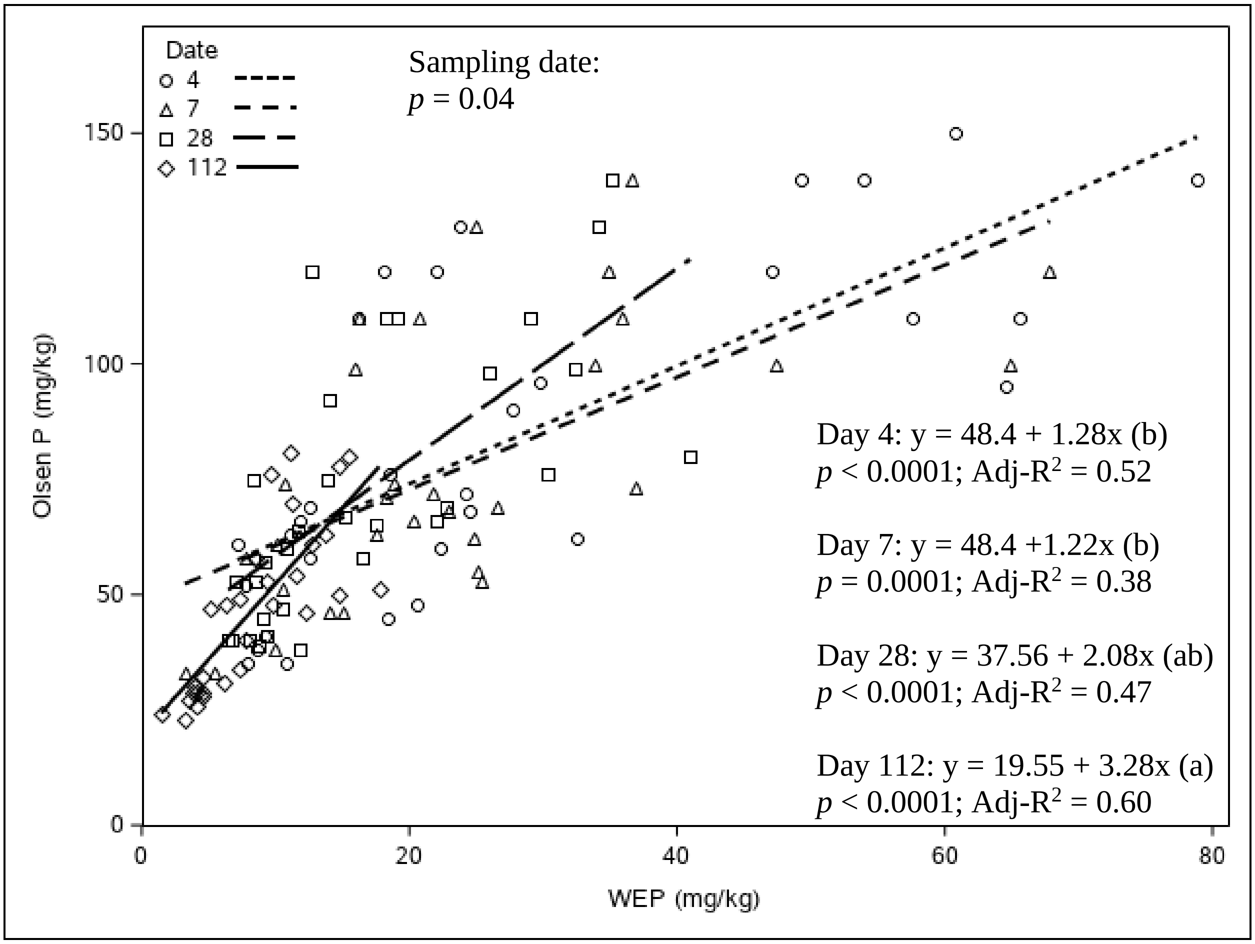

Figure 3.8 Regression relationships between water-extractable P (WEP) and Olsen-P in fertilized samples grouped by sampling date (4, 7, 28, 112). Lower case letters in parentheses following regression equations indicate significant differences in slope, according to pairwise comparisons.

3.4.8 Patterns in Citrate-extractable P

Citrate-extractable P concentration was affected by a significant interaction of all factors (Table 3.3; Figure 3.9) but the patterns among treatments and sampling dates were similar to those for WEP and Olsen-P, indicating that the citrate extraction did not differ substantially from other extractions in its ability to detect undissolved struvite in soil. The only cases where CEP differed between fertilizers, within the same soil and application rate, were in the Wampum soil at the high application rate at Day 4, where CEP concentrations for struvite exceeded those for MAP, and in the Dencross soil at the low application rate at Day 112, where the opposite occurred. In the Dencross soil, CEP concentrations declined from Day 4 to Day 112 for both struvite rates and the high MAP rate, whereas in the Wampum soil the only case in which the sampling dates differed was with the high rate of struvite. The recovery of fertilizer P as CEP was affected by a soil by fertilizer interaction, in which CEP recovery from struvite was greater in the Wampum soil than the Dencross soil but recovery from MAP did not differ between the soils (data not shown). An interactive effect of fertilizer type, application rate, and sampling date on CEP recovery reflected the patterns in CEP concentrations, with a greater decline in recovery from the high rate of struvite than in the other treatments (data not shown).

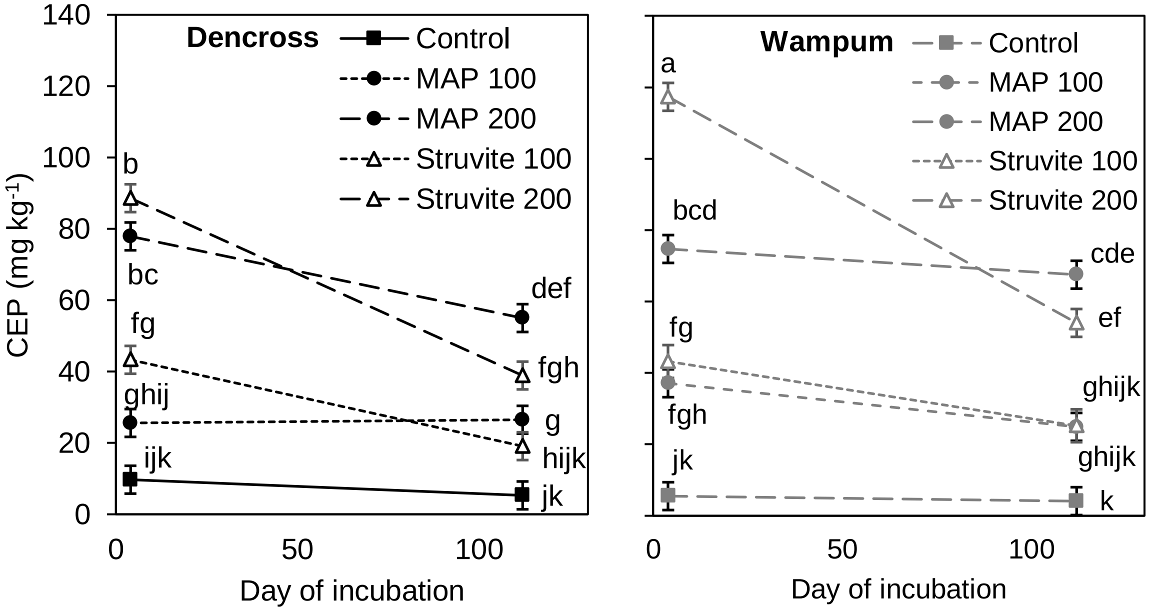

Figure 3.9 Temporal trends in citrate-extractable P (CEP) concentration as a function of soil type (Dencross, Wampum), fertilizer type (MAP, struvite) and fertilizer P application rate (100 or 200 mg kg^{-1}) in soils incubated 112 d without plants. Data points and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across both panels, according to Tukey's test.

3.5 Discussion

The many interactions among various combinations of soil type, fertilizer type, application rate, and time of incubation for all P extractions demonstrate the complexity of P dynamics in soils fertilized with different P amendments. The observed differences between fertilizers and especially the interactions between fertilizer and soil type emphasize the need to clarify the dissolution dynamics of struvite and the subsequent reactions that govern the fate of struvite-P in soil and the environment. Due to the preliminary nature of the citrate extraction, the following discussion focuses primarily on the WEP and Olsen-P concentrations and recovery.

3.5.1 Extractability of P from Undissolved Struvite in Soil

The WEP concentrations and recovery observed with struvite at the first two sampling dates (Figure 3.2) were much higher than expected based on the presence of visually distinguishable (i.e., virtually intact) struvite granules in soil. Previous research has found high WEP concentrations in struvite-amended soil after one day of incubation in an acidic soil (Vogel et al. 2017) but dissolution is expected to be much slower in neutral- to alkaline-pH soils, especially with granular struvite (Degryse et al. 2017). A possible explanation is that finely grinding the soil samples (including applied struvite granules) before analysis increased the extractability of struvite-P in water. Other incubation studies (28–100 d) using a range of soils have found concentrations and recovery of P from granular struvite similar to or greater than from MAP in soluble P pools (WEP, $CaCl_2$-extractable P, or isotopically exchangeable P) when partially dissolved struvite granules were included in the analyzed sample, citing sample preparation (i.e., grinding) and wide solid-to-solution ratios in extractions (promoting dissolution) as likely contributors (Degryse et al. 2017; Everaert et al. 2017). In an incubation of slightly acidic Arkansas soils amended with granular (2.4 mm) or finely ground struvite, Anderson et al. (2020) reported an increase in WEP of 180 mg kg^{-1} over baseline concentrations in a soil amended with ground struvite at the first sampling date (0.5 months) when only 135 mg P kg^{-1} had been added, attributing this excessively high value to inclusion of applied struvite in the analyzed sample. Increases in WEP with granular struvite at the same sampling date in that study were about 15–25 mg kg^{-1} in all soils; whether granules were still distinguishable in soil at that time was not indicated. Thus, the high soil WEP concentrations in the struvite-amended soils of the present study could be an artefact of sample preparation techniques and extraction procedures. If this is the case, the observed WEP concentrations likely reflect artificially elevated solubility of struvite due to grinding rather than the dissolution of struvite granules in soil, especially at the early sampling dates.

The high Olsen-P concentrations and recovery (Figure 3.3) at the first two sampling dates in struvite-amended soils raise similar questions about the extractability of P directly from struvite. In an experiment testing the extractability of granular struvite (2.0–2.8 mm diameter) in standard soil tests when added either to washed quartz sand or soil, about 17–20% of P in struvite was recovered with the Olsen test, with no differences between sand and soil treatments (Gu et al. 2021). These findings demonstrate that a portion of the P in struvite is dissolved with the alkaline Olsen extractant, likely due to interactions between bicarbonate ions and exposed Mg-OH on struvite crystal surfaces (Gu et al. 2021). The P recovery values in that study are substantially lower than the recovery of P from struvite after 4 d of incubation in the present study; however, unlike the present study, the samples analyzed by Gu et al. (2021) were not incubated and were not finely ground before extraction. Thus, soil–fertilizer interactions occurring within the first few days after struvite application may contribute to P extractability from struvite. In a neutral-pH soil amended with finely ground struvite at 100 mg P kg^{-1} and incubated 5 d, soil Olsen-P was 83 mg kg^{-1} (corresponding to about 70% recovery over the unfertilized control), effectively predicting ($R^2 = 0.88$) tissue P accumulation by cereal rye (Duboc et al. 2017). However, the P extracted via standard soil tests conducted on soils amended with struvite and incubated for short periods is likely greater for ground than for granular struvite, as demonstrated by Anderson et al. (2020), so the results of Duboc et al. (2017) may not accurately reflect plant-availability of P from granular struvite. Further research is required to distinguish the effects of soil–fertilizer interactions and artefacts of sampling and extraction procedures for struvite-amended soils.

These findings raise questions about the relevance of standard soil extractions for environmental and agronomic management. Soil P concentrations in labile pools typically correlate well with P losses, as reported for manure-amended soils in Saskatchewan and Manitoba (Ashjaei et al. 2010; Kumaragamage et al. 2011) and a range of soils in Manitoba (Liu et al. 2021). However, the combined results of the previously cited soil incubation study (Everaert et al. 2017) and a companion rainfall simulation study using one of the same soils (Everaert et al. 2018) demonstrate a breakdown in the expected relationship between labile soil P concentration and P loss in struvite-amended soils; in these studies, soil solution P in a incubated neutral-pH soil was greater when amended with granular struvite rather than MAP, but P loss from struvite was dramatically lower than that from MAP in the rainfall simulation. Together, these studies suggest that including remnants of struvite granules in extracted samples overestimates the risk of P loss from struvite-amended soils. However, removing granules from soil before analysis is not practical and will underestimate extractable P concentrations (Gu et al. 2021). Additional research is needed to clarify the roles of soil sampling and extraction procedures on the extractability of P from struvite-amended soils and determine whether results from such extractions are appropriate as a basis for management decisions.

The overall similarity in patterns between the citrate extraction and the WEP and Olsen-P extractions (Table 3.3) was unexpected. However, we predicted low initial WEP and Olsen-P concentrations relative to MAP, so it was WEP and Olsen-P concentrations that diverged from the hypothesized pattern for struvite, while CEP followed the predicted pattern of high initial concentration followed by a decline over time. It is possible that the Olsen-P and citrate extractions have a similar mode of action in soil, with bicarbonate or citrate ions displacing orthophosphate on adsorption sites and/or precipitating with calcium. If this is the case, it is noteworthy that the citrate extraction yielded concentrations similar to those for Olsen-P despite being a much weaker extractant (25 mM, compared to 0.5 M for Olsen-P). Although the preliminary investigation of the citrate extraction in the present study did not provide unique insights into the P dynamics associated with struvite, further study of this extraction method is recommended.

3.5.2 Effects of Fertilizer Type

The observed general pattern of high initial labile P concentrations that decline over time indicates that added P from both fertilizers was gradually being transformed to more recalcitrant pools through P retention processes. This pattern is commonly observed in incubations of P-amended soils (Griffin et al. 2003; Kashem et al. 2004; Mackey et al. 2021), many of which demonstrate differing patterns among soil types and/or P amendments over time. For example, in two alkaline soils from Manitoba amended with MAP, manure, or biosolids, the decline in WEP and Olsen-P over time was greater and occurred more rapidly in a clay soil than in a silty clay loam and also differed among P amendments (Kashem et al. 2004).

The recovery of P from MAP in extractable pools was lower than expected at Day 4 (9% and 41% for WEP and Olsen-P, respectively; Figures 3.2, 3.3), based on the results of Kashem et al. (2004), who reported 40–50% and 54–74% recovery of MAP-P in WEP and Olsen-P extractions, respectively, after one week of incubation in alkaline Manitoba soils with moderate to fine texture. These findings suggest that MAP dissolved very rapidly in the present study and that considerable retention of P from MAP occurred within the first three days of the incubation, with labile P pools already approaching stable concentrations by Day 4, especially in the low application rate. In soils with high P retention capacity, nearly all of the P released from soluble fertilizers can be adsorbed or precipitated within the first week after application (Griffin et al. 2003). Sampling during the first days of incubation may have shown higher concentrations of extractable P in MAP-amended soil, similar to the concentrations observed for struvite-amended soils.

If WEP and Olsen-P in MAP treatments did indeed reach high concentrations and decline rapidly before Day 4, it appears that the availability of labile P in struvite treatments followed the same general pattern as MAP but that changes proceeded more slowly in struvite-amended soils. This finding is contrary to expectations based on evidence of slow dissolution of granular struvite in soil. For example, Anderson et al. (2020) found that WEP and Mehlich-3-P concentrations in soils amended with 2.4-mm struvite granules were stable or increased over time, but declined over time from high initial concentrations when finely ground struvite was applied. While there is some evidence for greater extractable P in soils amended with small struvite granules, relative to larger ones (Hertzberger et al. 2021), it is difficult to differentiate the effect of granule size from the effect of sample grinding in the present study.

Based on the sampling dates at which extractable P concentrations converged to statistically similar values for the two fertilizer treatments, the delay in P transformations for struvite-amended soils relative to those amended with MAP was approximately 7–28 d for Olsen-P (Figure 3.3) and 28–112 d for WEP (Figure 3.2); convergence occurred at the shorter end of these ranges at the low P application rate. In addition, Olsen-P appeared to stabilize at a lower concentration in soils amended with struvite rather than MAP. A study investigating the change in Olsen-P concentration over time with struvite or MAP (<2 mm particle size for both fertilizers) applied to an alkaline soil reported no difference in Olsen-P between struvite and MAP at 0 and 4 d of incubation, but then a pattern similar to the present study, with a period (7–14 d) in which struvite exceeded MAP, followed by a period (21–63 d) in which MAP exceeded struvite (Mackey et al. 2021). The extended period of high extractable P concentrations in struvite-amended soils in the present study suggests that, contrary to expectations, struvite may provide a greater supply of plant-available P than MAP, as well as a greater risk of P loss in runoff, in the days and weeks after fertilizer application.

Whether the P extracted from struvite- and MAP-amended soils was derived from the same P forms cannot be determined from the Olsen-P extraction. However, the presence of visually distinguishable granules of struvite at early sampling dates suggests that struvite had not yet undergone much transformation, whereas Olsen-P in MAP treatments likely included P that had already passed through the water-soluble pool and was weakly retained in soil. Additional research is required to clarify whether the P species extracted in the Olsen-P pool differ between MAP- and struvite-amended soils and, if so, whether the Olsen test serves as an adequate indicator of the plant-available P pool in struvite-amended soils. Gu et al. (2021) estimate that when residual struvite is included in analyzed soil samples, the Olsen test overestimates plant-available P by about 20%, but further study is needed to confirm these findings.

The lower Olsen-P concentrations in soils amended with struvite compared to those amended with MAP at the end of the incubation (Figure 3.3) are similar to the results of Mackey et al. (2021), who also used a small struvite particle size. However, others using larger granules have reported higher labile P concentrations with struvite than MAP following incubation, possibly due to the rapid retention of MAP-P in soil (Everaert et al. 2017; do Nascimento et al. 2018). As Olsen-P concentrations had not completely stabilized over the last two sampling dates in the present study, it is not clear whether the difference between struvite and MAP would persist over a longer period of time or whether values for the two fertilizers would eventually converge, as they did with WEP. Concentrations of WEP and Olsen-P would be expected to follow a nonlinear relationship (exponential decay, or similar) over time after P application, declining toward an asymptote representing a relatively stable relationship among soil P pools, as shown by Griffin et al. (2003). Some treatments showed signs of levelling off at later dates in the present study (Figures 3.2, 3.3, 3.4, 3.6); additional incubation time and sampling dates would have been required to identify the WEP and Olsen-P concentrations and the points in time at which WEP and Olsen-P stabilized.

3.5.3 Effects of Soil Type

The greater WEP concentrations obtained with struvite in the neutral-pH Wampum soil than in the alkaline Dencross soil when averaged across sampling dates (Figure 3.1a) provides evidence that struvite solubility was affected by soil pH, as expected. Averaged across fertilizer types, the effect of soil type on WEP trends over time was smaller and shorter-lived than the effects of fertilizer type but followed a similar pattern, with concentrations converging over time (compare Figures 3.2 and 3.4). In contrast, the effect of soil type on Olsen-P over time was distinctly different from the fertilizer effect (compare Figures 3.3 and 3.6), with consistently higher Olsen-P concentrations and recovery in the Dencross soil than the Wampum soil but differing patterns in the decline over time between soils.

Temporal changes in labile P after P amendment in contrasting soil types are often attributed to differing P retention capacity (Kashem et al. 2004; Kumaragamage et al. 2011; do Nascimento et al. 2018; Anderson et al. 2020), which is associated with soil pH, the degree of P saturation, organic matter content, clay content, and soil mineralogy, especially the concentrations of Al- and Fe-oxides in acidic soils and Ca and Mg in alkaline soils (Ige et al. 2011; Antoniadis et al. 2016; Duminda et al. 2017). In the soils of Manitoba, Mehlich-extractable Ca and Mg and oxalate-extractable Al are thought to have the most direct effects on soil P retention capacity, with soil texture producing an indirect effect associated with Ca content in calcareous soils (Ige et al. 2007). Although we did not measure P retention capacity or any of the most direct contributors thereto, the Dencross soil is expected to have a greater P retention capacity than the Wampum soil, based on its higher clay, exchangeable Ca, and exchangeable Mg concentrations (Table 3.1). The assumed lower P retention capacity in the Wampum soil could explain the higher WEP concentration and recovery in this soil at the high but not low P application rate (Figure 3.5) and at the early sampling dates of the incubation (Figure 3.4), at which an apparently smaller proportion of the applied P was transformed from water-soluble to more recalcitrant forms. The higher Olsen-P concentrations and recovery in the Dencross soil than in the Wampum soil at the high rate and early sampling dates, followed by convergence over time (Figure 3.6), indicate that net transformation of added P to more recalcitrant forms (i.e., those not detected by the Olsen test) was delayed in the Dencross soil, but perhaps did not differ between soils over the long term.

3.5.4 Effects of P Application Rate

The disproportionately high WEP concentration and fertilizer-P recovery in the Wampum soil at the high application rate (Figure 3.5) suggests that the P retention mechanisms transforming WEP to less soluble forms may have been overwhelmed at the high P rate. However, the rapid decline in WEP over time indicates that this effect was temporary. Thus, adsorption capacity on clay surfaces may have been rapidly exhausted in the Wampum sandy clay loam, with further transformation of WEP to less-soluble pools occurring through the slower precipitation process, likely as calcium phosphates (Sample et al. 1980). How these processes may differ between struvite and MAP is not clear, but the absence of a significant effect of application rate on WEP recovery from struvite- and MAP-amended soils (Table 3.3) suggests that the differences in WEP concentrations between fertilizer treatments at the first few sampling dates were not due to exhaustion of adsorption capacity.

The fertilizer application rates used in this study are much higher than recommended P rates for crop production in Manitoba, where the highest recommended rate for any crop, application method, and background soil P status is 55 kg P ha^{-1} (Manitoba Agriculture 2007). This corresponds to about 37 mg P kg^{-1} based on a bulk density of 1 g cm^{-3} and soil depth of 15 cm—only about one-third of our low application rate. Farmers may choose to apply struvite at very high application rates to provide a multi-year P supply, as shown to be effective in a field study (Chapter 2); thus, it is important to investigate the effects of high application rates. The differences between fertilizers and soils were consistently smaller in the lower application rate used in this study, and thus would likely be even smaller at the recommended P application rates for an annual application approach. However, even a small increase in Olsen-P (for example from 5 to 10 mg kg^{-1}) will have important effects on soil P supply to a crop. Whether the disproportionate effects of the high vs. low application rate on WEP and the proportionate effects on Olsen-P would also occur with lower rates requires further testing.

3.5.5 Relationship Between WEP and Olsen-P

The strong positive linear relationship between WEP and Olsen-P is consistent with previous research in various soil types and with a range of P amendments and land uses (Kumaragamage et al. 2007; Kristoffersen et al. 2020; Reid and Schneider 2021).

The significant interaction between soil type and fertilizer type on the relationship between WEP and Olsen-P demonstrates how soil properties may differentially affect the dissolution of struvite and MAP and subsequent P retention processes. The slopes for struvite treatments were strongly influenced by the very high WEP concentrations observed in the first two sampling dates, and thus may largely reflect the effects of grinding the samples rather than processes occurring within the soil. The steeper slopes for the Dencross soil than the Wampum soil for both fertilizers (Figure 3.7) may reflect greater P retention capacity in the Dencross soil. This is consistent with Reid and Schneider (2021) who found a similar effect of soil texture on the relationship between WEP and Olsen-P in Manitoba soils. Duminda et al. (2017) reported an extreme case of this pattern in a high-clay soil with high P retention capacity, in which dissolved reactive P content did not increase with P addition, resulting in a non-significant regression between dissolved reactive P and Olsen-P. That study also showed distinct relationships between these two P pools in several other soil types, demonstrating the importance of soil properties on this relationship. In contrast, Kashem et al. (2004) found no difference in the regression between WEP and Olsen-P for a clay soil and a silty clay loam soil from Manitoba amended with differing rates of MAP, manure, or biosolids; in that case, the very high P application rates used (up to 800 mg kg^{-1}) may have masked the effect of soil properties.

The difference in regression slopes among sampling dates (Figure 3.8) indicates that P transformation processes were continuing to act on WEP and Olsen-P pools throughout the incubation period, reducing the WEP pool more rapidly than the Olsen-P pool, as seen in the temporal trends discussed above.

The patterns in the WEP–Olsen-P relationship for the fertilized soils, especially the y-intercept values of 15–30 mg kg^{-1} Olsen-P (Figure 3.7), suggest that the added P preferentially accumulated (or perhaps remained, in the case of struvite) in the Olsen-P pool rather than the WEP pool up to a threshold, after which point WEP also increased. At lower Olsen-P concentrations than those in the fertilized treatments, the relationship between WEP and Olsen-P would likely diverge from the linear pattern, however. Though the unfertilized treatments were not included in the regression, the lowest WEP concentrations in the fertilized treatments, especially in later sampling dates and the low MAP rate, were similar to those in the unfertilized controls, whereas the Olsen-P concentrations in fertilized treatments were always greater than in the controls, as shown in the analysis of P extractions over time (Figures 3.2, 3.3, 3.6). Other studies have shown a change point in the relationship between $CaCl_2$-extractable P or dissolved reactive P and Olsen-P, with values for soluble P remaining very low and relatively stable at Olsen-P < 20–25 mg kg^{-1} and increasing thereafter in a linear or curvilinear pattern (Duminda et al. 2017; Kristoffersen et al. 2020). While struvite treatments appeared to adhere to this pattern, clarifying the processes that transform struvite-P from insoluble to soluble pools and then to retained forms, under differing soil conditions, will allow for a better understanding of the relationships among soil P pools of differing solubility and contribute to more effective management of struvite fertilizers.

3.6 Conclusions

The patterns of P extractability as WEP and Olsen-P associated with struvite did not follow expectations based on the low K_{sp} of struvite in water and previously observed slow release of P from struvite granules. The labile P pools associated with struvite application followed a pattern that was similar to MAP, though over a longer period, with WEP and Olsen-P pools declining over time to similar concentrations for the two fertilizers, except for Olsen-P at the high application rate, where MAP exceeded struvite. The weak citrate extraction followed the expected pattern but, due to its overall similarity to WEP and Olsen-P, did not provide additional insights on P dynamics associated to struvite.

Based on comparison to other studies, the small struvite granules used in this study seem to have behaved more like finely ground struvite than large granules in terms of P extractability as both WEP and Olsen-P early in the incubation, as well as in the trends over time. However, grinding the incubated samples prior to analysis is likely at least partially responsible for the observed pattern. Additional testing is needed to clarify the effect of sample preparation and extraction procedures on the extractability of struvite-P in various labile pools to more meaningfully interpret the counterintuitive results obtained, especially for WEP. If the P extractability in such tests reflects dissolution of struvite during extraction rather than in soil and does not correspond well with its behaviour in soil or the broader environment, alternative indicators of agronomic P-supplying power and environmental risk associated with struvite will be required. However, if our results accurately reflect the extractability of P from soils amended with struvite fertilizers, the P supply to plants and the risk of runoff losses of P from soils amended with small granules of struvite may be equal to or greater than those of soils amended with MAP during the first weeks after fertilizer application. The use of beneficial fertilizer management practices such as sub-surface banding of struvite at the time of planting will help to mitigate these risks. The differences in labile P pools due to application of struvite and MAP in the two contrasting soil types studied, along with the differences in relationships between the WEP and

Olsen-P pools among soils and fertilizers, also highlight the need to investigate the effects of specific soil properties on struvite dissolution and transformation dynamics. Additional studies on the agronomic response of crops and the losses of P in runoff under field conditions, in relation to struvite dissolution dynamics, are also needed.

3.7 References

Adesanwo, O.O., Ige, D.V., Thibault, L., Flaten, D., and Akinremi, W. 2013. Comparison of colorimetric and ICP methods of phosphorus determination in soil extracts. Commun. Soil Sci. Plant Anal. **44**: 3061–3075. doi:10.1080/00103624.2013.832771.

Anderson, R., Brye, K.R., Greenlee, L., and Gbur, E. 2020. Chemically precipitated struvite dissolution dynamics over time in various soil textures. Agric. Sci. **11**: 567–591. doi:10.4236/as.2020.116036.

Antoniadis, V., Koliniati, R., Efstratiou, E., Golia, E., and Petropoulos, S. 2016. Effect of soils with varying degree of weathering and pH values on phosphorus sorption. Catena **139**: 214–219. doi:10.1016/j.catena.2016.01.008.

Ashjaei, S., Tiessen, H., and Schoenau, J.J. 2010. Correlations between phosphorus fractions and total leachate phosphorus from cattle manure– and swine manure–amended soil. Commun. Soil Sci. Plant Anal. **41**: 1338–1349. doi:10.1080/00103621003759346.

Beegle, D. 2005. Assessing soil shosphorus for crop production by soil testing. Pages 123–143 *in* J. Thomas Sims and A.N. Sharpley, eds. Phosphorus: Agriculture and the Environment. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America, Madison, WI, USA. doi:10.2134/agronmonogr46.c5.

Degryse, F., Baird, R., Silva, R., and McLaughlin, M. 2017. Dissolution rate and agronomic effectiveness of struvite fertilizers - effect of soil pH, granulation and base excess. Plant Soil **410**: 139–152. doi:10.1007/s11104-016-2990-2.

DeLuca, T.H., Glanville, H.C., Harris, M., Emmett, B.A., Pingree, M.R.A., de Sosa, L.L., Cerdá-Moreno, C., and Jones, D.L. 2015. A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. Soil Biol. Biochem. **88**: 110–119. doi:10.1016/j.soilbio.2015.05.016.

Duboc, O., Santner, J., Golestani Fard, A., Zehetner, F., Tacconi, J., and Wenzel, W.W. 2017. Predicting phosphorus availability from chemically diverse conventional and recycling fertilizers. Sci. Total Environ. **599–600**: 1160–1170. doi:10.1016/j.scitotenv.2017.05.054.

Duminda, D.M.S., Kumaragamage, D., Indraratne, S.P., and Flaten, D. 2017. Fertilizer-induced phosphorus dynamics in alkaline-calcareous soils as influenced by soil chemical properties. Can. J. Soil Sci. **97**: 159–170. doi:10.1139/cjss-2016-0055.

Everaert, M., Degryse, F., McLaughlin, M.J., De Vos, D., and Smolders, E. 2017. Agronomic effectiveness of granulated and powdered P-exchanged Mg–Al LDH relative to struvite and MAP. J. Agric. Food Chem. **65**: 6736–6744. doi:10.1021/acs.jafc.7b01031.

Everaert, M., da Silva, R.C., Degryse, F., McLaughlin, M.J., and Smolders, E. 2018. Limited dissolved phosphorus runoff losses from layered double hydroxide and struvite fertilizers in a rainfall simulation study. J. Environ. Qual. **47**: 371–377. doi:10.2134/jeq2017.07.0282.

Griffin, T.S., Honeycutt, C.W., and He, Z. 2003. Changes in soil phosphorus from manure application. Soil Sci. Soc. Am. J. **67**: 645–653. doi:10.2136/sssaj2003.6450.

Gu, C., Zhou, Q., Cusick, R.D., and Margenot, A.J. 2021. Evaluating agronomic soil phosphorus tests for soils amended with struvite. Geoderma **399**: 115093. doi:10.1016/j.geoderma.2021.115093.

Hall, R.L., Boisen Staal, L., Macintosh, K.A., McGrath, J.W., Bailey, J., Black, L., Gro Nielsen, U., Reitzel, K., and Williams, P.N. 2020. Phosphorus speciation and fertiliser performance characteristics: A comparison of waste recovered struvites from global sources. Geoderma **362**: 114096. doi:10.1016/j.geoderma.2019.114096.

Hertzberger, A.J., Cusick, R.D., and Margenot, A.J. 2020. A review and meta-analysis of the agricultural potential of struvite as a phosphorus fertilizer. Soil Sci. Soc. Am. J. **84**: 653–671. doi:10.1002/saj2.20065.

Huygens, D., and Saveyn, H.G.M. 2018. Agronomic efficiency of selected phosphorus fertilisers derived from secondary raw materials for European agriculture. A meta-analysis. Agron. Sustain. Dev. **38**: 52. doi:10.1007/s13593-018-0527-1.

Ige, D.V., Akinremi, O.O., and Flaten, D.N. 2007. Direct and indirect effects of soil properties on phosphorus retention capacity. Soil Sci. Soc. Am. J. **71**: 95–100. doi:10.2136/sssaj2005.0324.

Ige, D.V., Akinremi, O.O., Flaten, D.N., Ajiboye, B., and Kashem, M.A. 2011. Phosphorus sorption capacity of alkaline Manitoba soils and its relationship to soil properties. Can. J. Soil Sci. doi:10.4141/S04-064.

Kabbe, C. 2021. Inventory of phosphorus "recovery and/or recycling" facilities operating or under construction at or downstream of wastewater treatment installations, v.10. P-Rex Environment. [Online] Available: https://www.phosphorusplatform.eu/images/download/Kabbe_P-recorecy_tech_implementation%20Table_2021_10_31.pdf [2022 Apr. 2].

Kashem, Md.A., Akinremi, O.O., and Racz, G.J. 2004. Extractable phosphorus in alkaline soils amended with high rates of organic and inorganic phosphorus. Can. J. Soil Sci. **84**: 459–467. doi:10.4141/S03-085.

Kratz, S., Vogel, C., and Adam, C. 2019. Agronomic performance of P recycling fertilizers and methods to predict it: A review. Nutr. Cycl. Agroecosystems **115**: 1–39. doi:10.1007/s10705-019-10010-7.

Kristoffersen, A.Ø., Krogstad, T., and Øgaard, A.F. 2020. Prediction of available phosphorus in soil: Combined use for crop production and water quality protection. J. Environ. Qual. **49**: 1575–1584. doi:https://doi.org/10.1002/jeq2.20165.

Kumaragamage, D., Akinremi, O.O., Flaten, D., and Heard, J. 2007. Agronomic and environmental soil test phosphorus in manured and non-manured Manitoba soils. Can. J. Soil Sci. **87**: 73–83. doi:10.4141/S06-030.

Kumaragamage, D., Flaten, D., Akinremi, O.O., Sawka, C., and Zvomuya, F. 2011. Soil test phosphorus changes and phosphorus runoff losses in incubated soils treated with livestock manures and synthetic fertilizer. Can. J. Soil Sci. **91**: 375–384. doi:10.4141/cjss10004.

Le Corre, K.S., Valsami-Jones, E., Hobbs, P., and Parsons, S.A. 2009. Phosphorus recovery from wastewater by struvite crystallization: A review. Crit. Rev. Environ. Sci. Technol. **39**: 433–477. doi:10.1080/10643380701640573.

Liu, J., Elliott, J.A., Wilson, H.F., Macrae, M.L., Baulch, H.M., and Lobb, D.A. 2021. Phosphorus runoff from Canadian agricultural land: A cross-region synthesis of edge-of-field results. Agricultural Water Management **255**: 107030. doi:10.1016/j.agwat.2021.107030.

Mackey, E., Harrison, J.H., Tao, H., Norberg, S., Adams-Progar, A., and Whitefield, E. 2021. Effect of application season on release of available phosphorus from soil receiving mono-ammonium phosphate or struvite. Water. Air. Soil Pollut. **232**: 467. doi:10.1007/s11270-021-05396-6.

Manitoba Agriculture 2007. Manitoba soil fertility guide. Province of Manitoba. [Online] Available: https://www.gov.mb.ca/agriculture/crops/soil-fertility/soil-fertility-guide/pubs/soil_fertility_guide.pdf [2021 Apr. 11].

Meyer, G., Frossard, E., Mäder, P., Nanzer, S., Randall, D.G., Udert, K.M., and Oberson, A. 2018. Water soluble phosphate fertilizers for crops grown in calcareous soils – an outdated paradigm for recycled phosphorus fertilizers? Plant Soil **424**: 367–388. doi:10.1007/s11104-017-3545-x.

do Nascimento, C.A.C., Pagliari, P.H., Faria, L. de A., and Vitti, G.C. 2018. Phosphorus mobility and behavior in soils treated with calcium, ammonium, and magnesium phosphates. Soil Sci. Soc. Am. J. **82**: 622. doi:10.2136/sssaj2017.06.0211.

Olsen, S.R., Cole, C.V., F. S. Watanabe, and Dean, L.A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture, Washington, DC. [Online] Available: http://hdl.handle.net/2027/uiug.30112019277042.

Reid, K., and Schneider, K. 2021. Modelling dissolved phosphorus losses from accumulated soil phosphorus and applied fertilizer and manure for a national risk indicator. Can. J. Soil Sci.: 1–14. doi:10.1139/cjss-2021-0049.

Sample, E.C., Soper, R.J., and Racz, G.J. 1980. Reactions of phosphate fertilizers in soils. Pages 263–310 *in* F.E. Khasawneg, E.C. Sample, and E.J. Kamprath, eds. The Role of Phosphorus in Agriculture. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, WI. doi:10.2134/1980.roleofphosphorus.c12.

Schoenau, J.J., and O’Halloran, I.P. 2008. Sodium bicarbonate-extractable phosphorus. Pages 89–94 *in* M.R. Carter and E.G. Gregorich, eds. Soil Sampling and Methods of Analysis, 2nd edition. CRC Press, Boca Raton, FL, USA.

Schürmann, B., Everding, W., Montag, D., and Pinnekamp, J. 2012. Fate of pharmaceuticals and bacteria in stored urine during precipitation and drying of struvite. Water Sci. Technol. **65**: 1774–1780. doi:10.2166/wst.2012.041.

Self-Davis, M.L., Moore, Jr., P.A., and Joern, B.C. 2009. Water- or dilute salt-extractable phosphorus in soil. Pages 22–24 *in* J.L. Kovar and G.M. Pierzynski, eds. Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters, 2nd edition. Southern Extension/Research Activity - Information Exchange Group 17, Blacksburg, VA.

Sharpley, A., Jarvie, H., Flaten, D., and Kleinman, P. 2018. Celebrating the 350th anniversary of phosphorus discovery: A conundrum of deficiency and excess. J. Environ. Qual. **47**: 774–777. doi:10.2134/jeq2018.05.0170.

Ström, L., Owen, A.G., Godbold, D.L., and Jones, D.L. 2005. Organic acid behaviour in a calcareous soil: implications for rhizosphere nutrient cycling. Soil Biol. Biochem. **37**: 2046–2054. doi:10.1016/j.soilbio.2005.03.009.

Talboys, P.J., Heppell, J., Roose, T., Healey, J.R., Jones, D.L., and Withers, P.J.A. 2016. Struvite: a slow-release fertiliser for sustainable phosphorus management? Plant Soil **401**: 109–123. doi:10.1007/s11104-015-2747-3.

Vogel, T., Kruse, J., Siebers, N., Nelles, M., and Eichler-Löbermann, B. 2017. Recycled products from municipal wastewater: Composition and effects on phosphorus mobility in a sandy soil. J. Environ. Qual. **46**: 443–451. doi:10.2134/jeq2016.10.0392.

Wang, Y.T., Zhang, T.Q., O'Halloran, I.P., Tan, C.S., Hu, Q.C., and Reid, D.K. 2012. Soil tests as risk indicators for leaching of dissolved phosphorus from agricultural soils in Ontario. Soil Sci. Soc. Am. J. **76**: 220–229. doi:10.2136/sssaj2011.0175.

Withers, P.J.A. 2019. Closing the phosphorus cycle. Nat. Sustain. **2**: 1001–1002. doi:10.1038/s41893-019-0428-6.

4. THE POTENTIAL OF STRUVITE FERTILIZER TO OPTIMIZE PLANT GROWTH, LABILE SOIL PHOSPHORUS, AND MYCORRHIZAL FUNGI IN ALFALFA

4.1 Abstract

Recycled phosphorus (P) fertilizers such as struvite ($NH_4MgPO_4 \cdot 6H_2O$) are an essential part of the circular economy for nutrients. Despite struvite's low solubility in water, it shows promise as an effective P fertilizer. Optimal management of struvite fertilizer requires consideration of effects on plant growth and P uptake as well as on soil P status and arbuscular mycorrhizal fungi (AMF), which can be inhibited by high soil test P or application of soluble fertilizers. The objective of this study was to assess the effects of applying struvite or MAP at differing rates (25–100 mg P kg^{-1}) to differing soil types on the agronomic response of alfalfa (*Medicago sativa* L.), the labile soil P pools following crop growth, and AMF root colonization. Alfalfa biomass and P uptake increased over the unfertilized control with the highest application rate in a neutral-pH, coarse-textured soil but showed no response to P application in an alkaline clay soil. Alfalfa response did not differ between struvite and MAP after 130 d of plant growth. Concentrations of water-extractable, Olsen, and citrate-extractable P increased with higher P application rates for both fertilizers and were affected by soil type in some cases. Root colonization by AMF declined with increasing P application rate, but the degree of suppression at the highest application rate was smaller with struvite than with MAP. These findings provide evidence that the slow-release properties of struvite may help to optimize P supply to crops, soil P status, and root colonization with AMF, but that trade-offs among outcomes still occur.

4.2 Introduction

Recovering phosphorus (P) from waste streams for use as fertilizer is essential for sustainable management of regional and global nutrient flows, as it reduces the P load to aquatic ecosystems receiving municipal wastewater and can replace at least a portion of the fertilizer derived from mined phosphate rock (Schneider et al. 2019; Withers 2019; Nicksy and Entz 2021). Struvite ($NH_4MgPO_4{\cdot}6H_2O$) precipitated from municipal wastewater or other nutrient-rich waste waters shows promise as a recycled P fertilizer as it tends to be relatively pure and promotes good crop response, compared to other recovered P products (Weissengruber et al. 2018; Wollmann et al. 2018; Huygens and Saveyn 2018). The bioavailability of struvite appears to be greater than its low solubility in water would predict (Meyer et al. 2018), implicating soil or plant processes, or both, in struvite dissolution and plant uptake. Overall, struvite fertilizer shows good agronomic potential relative to soluble P fertilizers in acidic to neutral-pH soils, but generally somewhat reduced crop response under alkaline soil conditions (Hertzberger et al. 2020).

Developing appropriate management guidelines to optimize the use of struvite fertilizer in cropping systems requires not only evidence of good crop response, but also an understanding of the effects of struvite application on concentrations of labile soil P (P in solution or readily brought into solution) and soil health parameters in response to agronomically relevant application rates in various soil types. The goal of such an approach is not to maximize crop growth at all costs, but to achieve adequate crop growth and nutrition while minimizing the risk of P loss from soil to the environment and reducing negative effects on the soil microbial community, including arbuscular mycorrhizal fungi (AMF). The relatively good crop response to struvite despite its low solubility suggests that its use may allow for a balance between providing an adequate P supply for crop growth and limiting adverse effects on AMF and the environment. Most studies on struvite have considered only one application rate and are thus unable to provide insights on the role of application rate in optimizing multiple outcomes.

Soil properties as well as properties of the struvite itself have been shown to affect the dissolution of this slow-release fertilizer. Although struvite dissolution is thought to be promoted by root activity, in particular via the production of organic acids (Talboys et al. 2016), soil incubations without plants have also shown increases in labile soil P pools (resin-, water-, calcium chloride-, or bicarbonate-extractable P) due to struvite addition (Duboc et al. 2017; Meyer et al. 2018; Anderson et al. 2020). Struvite dissolution is typically slower in alkaline soils than in neutral to acidic soils, especially when applied in granular rather than powdered form (Degryse et al. 2017; Everaert et al. 2017; do Nascimento et al. 2018). The effect of struvite application on soil P concentration in various pools has also been investigated in plant growth studies (Vogel et al. 2015, 2017; Szymańska et al. 2019; Hall et al. 2020), generally reporting that struvite addition increased labile soil P after plant growth, despite plant P offtake. However, most plant growth studies have been conducted in acidic to neutral-pH soils and often with powdered struvite, which favour dissolution.

Plant root colonization by AMF is sensitive to concentrations of labile soil P, tending to be lower in soils with higher soil test P (Mäder et al. 2000; Hamel and Strullu 2006; Schneider et al. 2017). Root colonization can also be sensitive to the addition of soluble P fertilizers, as demonstrated in a British Columbia study in which the addition of only 7 kg P ha^{-1} as triple superphosphate fertilizer placed in the seed row reduced AMF root colonization in corn, while the same fertilizer banded 5 cm from the seed row at 30 kg P ha^{-1} had no effect (Bittman et al. 2006). In a highly controlled experiment, an ample P supply very early in the life cycle of barrel medic suppressed mycorrhizal colonization, but the same concentration of P applied after colonization had been established did not inhibit the mycorrhizal symbiosis (Balzergue et al. 2013). Thus, struvite may have a smaller inhibitory effect on AMF than soluble fertilizers due to its slow-release properties, especially if P availability from struvite is slightly restricted early in plant development. Mycorrhizal fungi typically access the same P pools as plants and may be able to access the P in struvite, as reported in a study on tomato (Di Tomassi et al. 2021). In contrast, a study on cereal rye showed a negative effect of AMF colonization on P uptake from struvite (Schwalb et al. 2021).

Soil P extractions targeting various labile P pools can be used to predict both the quantity of P available for plant uptake and the risk of P loss to the environment. Thus, the effect of any soil amendment on labile P pools is important for optimizing its management. Water-extractable P (WEP) is often used as an indicator of the risk of P losses to the environment (Pote et al. 1996; Wang et al. 2010). Sodium bicarbonate extraction, specifically using the Olsen method (Olsen et al. 1954), is routinely used to predict crop response to P fertilizer in the neutral to alkaline soils of the Northern Great Plains region and may also be a better indicator than WEP of the risk of P loss from these soils (Kumaragamage et al. 2011). However, such extractions may not adequately characterize the soil P dynamics associated with sparingly soluble P fertilizers such as struvite (Meyer et al. 2018; Kratz et al. 2019; Gu et al. 2021). Other soil tests aim to quantify P pools accessed through the activity of organic acids/anions in root exudates using weak solutions of organic acids alone or in mixtures (Ström et al. 2005; DeLuca et al. 2015; Haney et al. 2016), and could potentially be used as an indicator of P supply to crops in struvite-amended soils. Applying P fertilizers in excess of crop demand leads to accumulation of P in soil (though not always in labile pools) but if excess P remains in soil as undissolved struvite, its contribution to labile P pools may be small relative to that of soluble fertilizers. Depending on how this undissolved struvite interacts with differing extractants, it may be undetected in water and Olsen extractions but may be detected in an extraction using an organic anion such as citrate.

To begin to address questions regarding optimal struvite management, we conducted a pot study in which we applied struvite or MAP at differing rates to alfalfa (*Medicago sativa* L.) grown in two contrasting soils with low Olsen-P. Alfalfa is an economically important crop in the prairie region of Canada and globally, and has been shown to respond well to struvite fertilizers in field experiments (Hilt et al. 2016; Chapter 2). Our objective was to assess the effects of fertilizer type (struvite vs. monoammonium phosphate (MAP)), P application rate, and soil type on the agronomic response of alfalfa, the labile soil P pools following crop growth, and AMF root colonization. We hypothesized that (1) increasing rates of P application would increase alfalfa productivity and P uptake, but to a lesser degree with struvite than with MAP, especially in an alkaline soil; (2) concentrations of water-extractable, bicarbonate-extractable, and citrate-extractable P would increase with increasing rates of P addition, but with differing patterns associated with struvite than with MAP; and (3) alfalfa root colonization with AMF would be unaffected by the rate of struvite application, but would decline with increasing rates of MAP application in both soils.

4.3 Materials and Methods

4.3.1 Soils

Soil was collected from the surface layer (0-15 cm) of farm fields near Libau (50.241258, -96.728878) and Stead (50.414696, -96.489661), MB. Soils were sieved to pass a 10-mm screen, air-dried, homogenized, and analyzed for chemical and physical properties (Table 4.1). The soil from Libau was a Gleyed Rego Black Chernozem of the Dencross series, with alkaline pH and clay texture. The soil from Stead was a Gleyed Gray Luvisol of the Wampum series, with neutral pH and sandy clay loam texture. Based on soil fertility recommendations for the region (Manitoba Agriculture 2007), both soils were low in Olsen-P, nitrate-N, and S and the Wampum soil was deficient in B, but other nutrients were considered adequate. The Dencross soil had a history of organically managed forage and annual crop production whereas the Wampum soil had a history of conventional production of annual crops.

The moisture content of air-dried soils was determined by comparing the weights of three ~50 g samples of each soil type before and after drying at 105 °C. Water-holding capacity (container capacity) of the two soils was determined by packing soil to the appropriate bulk density in a set of transparent, open-bottom columns and wetting soil from the top with differing quantities of water. The quantity of water that moistened the entire column without causing drainage from the bottom was selected as the container capacity, which was used as the maximum target soil moisture content throughout the experiment. Target gravimetric soil moisture content was 0.44 and 0.29 g g^{-1} for the Dencross and Wampum soils, respectively, corresponding to water-filled pore space of 67% and 61%. Bulk density of the soil after settling, determined by measuring the soil height in each pot at the end of the experiment, was 1.0 g cm^{-3} for the Dencross soil and 1.2 g cm^{-3} for the Wampum soil.

Table 4.1 Chemical properties and particle size analysis of the experimental soils

Soil property	**Dencross**	**Wampum**
Olsen-P, mg kg^{-1}	5.7 (0.5) [a]	8.2 (0.8)
Water-extractable P, mg kg^{-1}	2.12 (0.19)	1.88 (0.22)
Citrate-extractable P, mg kg^{-1}	4.2 (0.6)	6.0 (0.6)
Total P, mg kg^{-1}	665 (2)	308 (6)
pH (in water)	8.1 (0.1)	7.0 (0.1)
Nitrate-N, mg kg^{-1}	9 (1)	7 (1)
Total N, % (combustion analyzer method)	0.30 (0.02)	0.17 (0.01)
Exchangeable K, mg kg^{-1}	383 (13)	235 (1)
Sulphate-S, mg kg^{-1}	8 (1)	10 (6)
Exchangeable Ca, mg kg^{-1}	6700 (140)	2130 (20)
Exchangeable Mg, mg kg^{-1}	976 (28)	328 (4)
Boron, mg kg^{-1}	1.46 (0.02)	0.37 (0)
Chloride, mg kg^{-1}	2.8 (0.4)	2.8 (1.1)
Copper, mg kg^{-1} (DTPA-extractable)	1.0 (0.02)	0.55 (0.01)
Iron, mg kg^{-1} (DTPA-extractable)	21.2 (0)	57.6 (0.1)
Manganese, mg kg^{-1} (DTPA-extractable)	4.7 (0.02)	4.3 (0.1)
Na, mg kg^{-1}	25 (4)	15 (1)
Zinc, mg kg^{-1} (DTPA-extractable)	0.63 (0.01)	1.21 (0.01)
Soluble salts, mmhos/cm (1:1 soil:water)	0.54 (0.04)	0.19 (0.01)
Cation exchange capacity, meq/100g (sum of cations)	42.8 (0.9)	14.0 (0.1)
Calcium carbonate, % CCE (pressure method)	5.6 (0.2)	0.9 (0.1)
Organic C, % (combustion minus inorganic C)	4.2 (0.1)	1.9 (0.1)
Sand, %	26 (1)	67 (3)
Silt, %	28 (0)	13 (1)
Clay, %	46 (1)	20 (1)
Texture class	Clay	Sandy clay loam

[a] Values are means with standard deviation in parentheses (*n* = 2, except for Olsen-P, water-extractable P, and citrate-extractable P, where *n* = 27).

4.3.2 Fertilizers

The fertilizers used were struvite (Crystal Green®, N-P_2O_5-K_2O analysis 5-28-0, with 10% Mg, equivalent to 122 mg P g^{-1}; Ostara Nutrient Recovery Technologies Inc., Vancouver, BC) and MAP (N-P_2O_5-K_2O analysis 11-52-0, equivalent to 227 mg P g^{-1}; CropKing Inc., Lodi, OH). Granular formulations of both products were used. Struvite granules were ~0.9 mm in diameter (SGN 90) and granular MAP was sieved to isolate granules less than 2 mm in diameter, to limit the effect of granule size on P distribution through the soil.

4.3.3 Experimental Design

This experiment tested the main and interactive effects of soil type (Dencross vs. Wampum), fertilizer type (struvite vs. MAP), and P application rate on alfalfa aboveground biomass, P uptake, and P recovery efficiency, labile soil P pools, and AMF root colonization in a completely randomized design with four replications per treatment. The experimental unit was the pot, each containing two plants (equivalent to 110 plants m^{-2}). The P application rates were 25, 50 and 100 mg P kg^{-1} soil (62, 124 and 248 mg P pot^{-1}, designated as P62, P124, and P248), equivalent to 34, 68 and 136 kg P ha^{-1}, respectively, calculated based on the soil surface area of the pots. These rates are equivalent to approximately one, two, and four times the recommended rate for alfalfa in soils with very low Olsen-P in Manitoba (Manitoba Agriculture 2007). An unfertilized control treatment (P0) was also included for each soil type, in a factorial-plus-one-control design with fertilizer type and application rate. Hereafter, treatment combinations are designated by combinations of the P rate, fertilizer type, and soil type, as appropriate; for example, P62–MAP–Dencross denotes the lowest rate of MAP addition in the Dencross soil and P248–Struvite denotes the highest rate of struvite addition across both soils.

4.3.4 Experiment Set-up and Management

Pots 15.2 cm in internal diameter and 30 cm in height made from PVC pipe, with sealed bottoms to prevent drainage, were filled with the air-dry equivalent of 5 kg of oven-dry soil, in two layers. Unamended dry soil (2.5 kg) was added to the bottom of all pots to provide adequate rooting depth for alfalfa plants and watered to 80% of container capacity with reverse osmosis (RO) water. This layer was followed by an upper layer (2.5 kg; approx. 15 and 12 cm deep for the Dencross and Wampum soils, respectively) of soil with nutrient amendment treatments applied, also watered to 80% of container capacity after placing it in the pots. Granular struvite and MAP were added at the designated rates and mixed thoroughly with dry soil for the upper layer prior to placing soil in the pot. Supplemental S was added to all pots as powdered gypsum at 91 mg S pot^{-1} (equivalent to 50 kg S ha^{-1}) and mixed thoroughly with upper-layer soil during this step. Supplemental boron was added to the Wampum soil at 1.8 mg B pot^{-1} (equivalent to 1 kg B ha^{-1}) as sodium tetraborate decahydrate dissolved in the irrigation water for the upper soil layer. To create growing conditions as similar as possible to field production systems and reduce interference with plant mechanisms associated with P uptake and AMF symbiosis, no supplemental nitrogen (N) was added to pots beyond what was contained in struvite and MAP and alfalfa seeds were inoculated with *Rhizobium* bacteria to promote biological N fixation. However, to provide insights on potential N limitation on alfalfa biomass production and nutrient uptake, an additional treatment was included alongside the main experiment in which the lowest (P62) rate of struvite was supplemented with a non-limiting rate of N (see Appendix III). Pots were then placed in a temperature-controlled, naturally lit greenhouse to equilibrate overnight.

The following day, the remaining 20% of RO water required to bring pots to container capacity was added, based on the weight of the pots. Alfalfa (cv. 4020 MF) seeds were surfacesterilized using diluted household bleach (1% v/v), rinsed with RO water, and coated with *Rhizobium* inoculant for alfalfa (Nitragin Gold; Novozymes, Saskatoon, SK). Twelve seeds were planted in each pot at a depth of approximately 10 mm. Plants were thinned to two plants per pot at the seedling stage.

Mean daily greenhouse temperature was maintained between 19.8 and 24.3 °C throughout the 130-d experiment. Natural light was supplemented with artificial lighting for 16 h d^{-1}. Pots were watered from the top using RO water every 2-4 d. At least once per week, pots were watered to their target soil moisture content based on the weight of individual pots. When not watered by weight individually, five randomly selected pots from each soil type were weighed and water was added to all pots of that soil type based on the smallest water requirement of the selected pots. The position of the pots on the greenhouse bench was re-randomized every week. Pesticides were applied as needed to control insect pests (thrips, aphids) and diseases (powdery mildew). Additional supplementary S was applied as potassium sulphate (55 mg S pot^{-1}, equivalent to 30 kg ha^{-1}) dissolved in irrigation water at Day 109 due to early signs of S deficiency in a few plants.

4.3.5 Plant Sample Collection and Analyses

Alfalfa above-ground biomass production was measured by repeatedly harvesting plant shoots 1–2 cm above the soil surface on three dates (55, 90, and 130 d after planting), when plants reached the bud or early flowering stage. Harvested plant material was oven-dried at ~60°C for at least 48 h until reaching constant weight before weighing. Entire samples from each sampling date were ground with a ball mill (Spex 8000 Mixer/Mill; Spex Industries, Metuchen, NJ). Individual samples were analyzed for total P concentration using inductively coupled plasma–optical emission spectroscopy (ICP–OES; Optima 5300DV, Perkin Elmer, Waltham, MA) after digesting

0.5 g of plant tissue in HNO_3 in a MARS 6 microwave digester (CEM, Matthews, NC). Total N concentration was determined by dry combustion followed by analysis with an Elementar rapid N cube (Langenselbold, Germany).

Shoot P uptake per pot at each harvest date was calculated as the product of shoot dry matter weight and plant P concentration. Cumulative shoot biomass production and P uptake were calculated by summing these parameters across the three harvest dates. The overall P concentration in cumulative biomass (referred to as "cumulative P concentration" in tables) was calculated by dividing cumulative P uptake by cumulative biomass production. Apparent P recovery efficiency in cumulative shoot biomass from applied fertilizers was calculated for each fertilized pot using the difference method (Syers et al. 2008) with the following equation:

$$PR_{biomass} = \frac{PU_f - PU_{uf}}{P_{app}} \qquad (1)$$

where PU_f and PU_{uf} are the P uptake in shoot biomass in the fertilized (individual pot) and unfertilized (mean of 4 replicates within each soil type) treatments, respectively, and P_{app} is the quantity of P applied, all in mg pot^{-1}.

4.3.6 Soil Sample Collection and Analyses

During the experimental set-up, a baseline soil sample was collected from the unamended dry soil for each pot immediately before adding amendments and filling pots. After the final plant harvest, soil samples were collected from each pot by extracting four 1.8-cm diameter cores approximately 1–2 cm from both the base of a plant and the pot wall, using the tip from a standard soil probe. Sampling depth was 45% of the total soil depth (i.e., 90% of the estimated amended soil depth and approximately 11-14 cm), calculated using the final soil depth after settling. Sampling to a depth slightly less than the amended depth reduced the risk of inadvertently including unamended soil from the lower soil layer in the sample. Soil samples were air-dried and hand-sieved to pass a 2-mm screen to remove pebbles and organic materials. Samples were then ground to a fine powder using a soil mill (8” Christy & Norris Lab Mill, Christy Turner Ltd., Ipswich, UK) to ensure homogenous distribution of any residual struvite granules in soil.

All baseline and final soil samples were analyzed for WEP, Olsen-P, and CEP pools, using the methods described in Chapter 3 (Table 3.2). Briefly, Olsen-P was determined by extracting 2.5 g of soil in 50 mL of 0.5 M sodium bicarbonate adjusted to pH 8.5, followed by colorimetric analysis to determine molybdate-reactive P (Olsen et al. 1954; Murphy and Riley 1962; Schoenau and O’Halloran 2008). For WEP, 2.5 g soil was extracted in 25 ml of ultrapure water with a 30-min shake time (modified from Self-Davis et al. 2009) and total P concentration determined by ICP–OES (Optima 5300DV; Perkin Elmer). For CEP, 2.5 g of soil was extracted with 50 ml of 25 mM citric acid solution adjusted to pH 7.5 (Ström et al. 2005) and total P concentration determined by ICP–OES.

The proportion of applied fertilizer P recovered from soil in each extractant was calculated for each pot with the following equation:

$$PR_{soil} = \frac{P_f - P_{uf}}{P_{app}} \qquad (2)$$

where P_f is the soil P concentration of the fertilized treatment, P_{uf} is the soil P concentration of the unfertilized control in the corresponding soil type (mean of four replications), and P_{app} is the rate of P applied, all in mg P (kg soil)$^{-1}$.

4.3.7 Root Sample Collection and Analysis of Mycorrhizal Arbuscular Abundance

After collecting final soil samples, plant root samples were collected by extracting the root systems and any adhering soil from each pot. Root systems were cut to 45% of the total soil depth, hand-washed, and examined for the presence of nodules. Fine roots (<~3 mm diameter) were collected and stored in 70% (v/v) ethyl alcohol solution at ~4°C. Root samples were cleared and stained with Scheaffer black ink (catalogue number SHF94231; Scheaffer Pen & Art Supply Co., Shelton, CT), following the methods of Vierheilig et al. (1998). In brief, roots were cleared by boiling in 10% KOH for 20 min, stained in a 5% (v/v) solution of ink and household vinegar, and then stored in acidified water at ~4 °C. Stained roots were cut to 1-2 cm length and were mounted in glycerol on microscope slides. Root colonization with AMF was assessed by examining samples under a compound microscope using the magnified intersections method (McGonigle et al., 1990), which determines the proportion of intersection points between roots and microscope crosshairs in which AMF structures are present; only the data for arbuscules are presented, as they are the mycorrhizal structure in which P exchange occurs, but presence of fungal vesicles and hyphae was also noted. An average of 200 and minimum of 135 intersection points were assessed for each pot.

4.3.8 Statistical Analyses

An analysis of variance (ANOVA) was conducted to determine the effects of fertilizer type, P application rate, and soil type on each dependent variable using the GLIMMIX procedure of SAS® software (Version 9.4, SAS OnDemand for Academics; SAS Institute, Cary, NC), designating all factors as fixed effects, with fertilizer type and P rate in a factorial-plus-one-control treatment design. For plant variables, analysis focused on the cumulative biomass, P uptake, and P concentration over the entire duration of the experiment. Data from the first harvest (Cut 1) were also analyzed separately to determine the importance of variable plant regrowth patterns following cutting and to assess the early crop response to added P.

The assumption of homogeneity of variance was verified by visual examination of residual plots and tests of covariance among treatment groups using the GLIMMIX procedure. Where heterogeneous variance was found among treatment groups, the RANDOM _RESIDUAL_ / GROUP statement and the Satterthwaite degrees of freedom approximation method were used to adjust for unequal variances. Apparent P recovery efficiency in plant biomass was analyzed as a Gaussian distribution because the presence of negative values for some experimental units precluded designation as a beta distribution. Recovery of fertilizer P from soil was analyzed as a beta distribution and occurrence of arbuscules (hereafter referred to as AMF colonization rate) was analyzed as a binomial distribution; the resulting means and standard errors were back-transformed using the ILINK function in the LSMEANS statement. For other variables, normal distribution of residuals was confirmed by the Shapiro-Wilk test with a W-statistic > 0.9 and data were transformed where necessary (e.g., for soil P concentrations in some cases) by specifying a log-normal (base e) distribution in the model statement and then back-transformed to the original scale for presentation. Post-hoc means comparison was conducted using the Tukey multiple comparisons method. All effects were considered significant at $p < 0.05$. For variables that include the nil-P control treatment in the factorial-plus-control design with fertilizer type and application rate, ANOVA *p*-values represent the significance of tests among fertilizer treatments only, but the means comparison includes the control treatment.

Pearson correlation coefficients were calculated among soil P pools for all samples as well as soil and fertilizer type treatment groups. Spearman's rank correlation coefficients were used to characterize the relationships between AMF root colonization and each soil P pool in final samples as well as the P application rate; this correlation method was used due to non-normal distribution of root colonization data. All correlations were conducted using the CORR procedure of SAS software and were considered significant at $p < 0.05$.

4.4 Results and Discussion

4.4.1 Plant Establishment and Growth Patterns

Alfalfa seedlings established successfully in all but two pots; these were reseeded but then excluded from analysis due to delayed plant growth and development. The quantity of biomass harvested declined slightly with each of the three harvests (data not shown). After the first harvest, the regrowth pattern of individual plants varied but these differences did not appear to be associated with specific treatments. One plant failed to regrow after cutting and this pot was excluded from all analyses except plant parameters measured at Cut 1. Insect pests (thrips, aphids) were difficult to control and affected the health and productivity of all plants to some degree.

Applying supplemental N with the P62 struvite rate had no significant effect on plant biomass, P or N concentration, or P or N uptake (Appendix III). Thus, alfalfa was apparently sufficient in N in the main experiment even though supplemental N was not applied.

During experiment tear-down, plant roots were observed throughout the entire soil depth, including the unamended bottom layer, but the absence of a root mat on the bottom or sides of the pots indicated that plant root growth was not overly restricted. Based on visual observation during root collection and experiment tear-down, most root branching (and thus probably nutrient uptake) occurred in the upper soil layer, with mainly the primary tap root extending into the unamended lower soil layer.

4.4.2 Plant Biomass and P Uptake

Mean cumulative alfalfa shoot biomass in individual treatments ranged from 7.1 g pot^{-1} in P0–Wampum to 15.8 g pot^{-1} in P264–MAP–Wampum, corresponding to about 3–7 Mg ha^{-1}. These values are very similar to those observed in a field study assessing similar struvite application rates to alfalfa–grass forage at Libau, MB, the site where the Dencross soil was collected (Chapter 2).

Fertilizer type (struvite vs. MAP) had no effect on cumulative above-ground plant biomass production, P uptake, tissue P concentration, or P recovery efficiency as a main effect or in interaction with any other factor (Table 4.2). These results show that the solubility of the two fertilizers was not a major factor in determining the total P availability to alfalfa plants when considering the entire growth period. However, P uptake at Cut 1 was significantly lower for struvite than for MAP (Table 4.2), indicating that plants were initially P-deficient in the struvite treatments. Because the fertilizers were mixed throughout the upper half of the soil rather than banded in the rooting zone, the alfalfa seedlings may have been unable to obtain enough P from the small amount of slowly dissolving struvite in the root zone. The P diffusion distance from struvite is less than that from MAP, especially in alkaline soils (Degryse et al. 2017; do Nascimento et al. 2018). Thus, the effect of struvite placement in the soil relative to rooting zones is an important consideration for optimizing struvite application practices. Previous research has reported a similar or slightly lower response of field-grown alfalfa to struvite relative to MAP (Hilt et al. 2016), but did not evaluate crop response over short time scales.

Table 4.2 Effects of soil type, fertilizer type, and P application rate on alfalfa shoot biomass, tissue P concentration, P uptake, and P recovery efficiency in shoot biomass over the entire experiment (Cumulative, 130 d after planting) and at first plant harvest (Cut 1, 55 d after planting)

	Cumulative				**Cut 1**			
Factor	**Shoot biomass**	**Tissue P conc.**	**Shoot P uptake**	**P recov. efficiency**	**Shoot biomass**	**Tissue P conc.**	**Shoot P uptake**	**P recov. efficiency**
	g pot^{-1}	mg g^{-1}	mg pot^{-1}	%	g pot^{-1}	mg g^{-1}	mg pot^{-1}	%
Soil								
Dencross	9.13 (0.35)	2.54 (0.04)	23.1 (1.0)	4.9 (1.0)	3.16 (0.19)	2.40 (0.06) b	7.6 (0.5)	1.3 (0.3)
Wampum	9.24 (0.38)	2.78 (0.05)	25.9 (1.1)	7.8 (1.0)	3.56 (0.19)	2.66 (0.06) a	9.4 (0.5)	2.0 (0.3)
Fertilizer								
Control	7.68 (0.38) [a]	2.63 (0.06)	20.1 (1.2)	-	2.90 (0.25)	2.50 (0.08)	7.2 (0.6) c [b]	-
Struvite	10.5 (0.43)	2.72 (0.03)	28.0 (1.2)	6.1 (1.0)	3.63 (0.14)	2.55 (0.05)	9.2 (0.3) b	1.2 (0.3) b
MAP	10.9 (0.54)	2.65 (0.03)	29.7 (1.2)	6.6 (1.0)	4.01 (0.15)	2.57 (0.05)	10.3 (0.4) a	2.1 (0.3) a
Rate								
0 mg pot^{-1}	7.68 (0.38)	2.63 (0.06)	20.1 (1.2)	-	2.90 (0.24)	2.50 (0.08)	7.2 (0.6)	-
62 mg pot^{-1}	9.39 (0.39)	2.65 (0.04)	24.8 (1.0)	7.6 (1.2)	3.02 (0.18)	2.62 (0.06)	7.9 (0.4)	1.1 (0.4)
124 mg pot^{-1}	10.4 (0.64)	2.62 (0.04)	27.1 (1.5)	5.6 (1.2)	3.88 (0.18)	2.46 (0.06)	9.5 (0.4)	1.9 (0.4)
248 mg pot^{-1}	12.3 (0.71)	2.78 (0.04)	34.7 (1.9)	5.9 (1.2)	4.57 (0.18)	2.59 (0.06)	11.9 (0.4)	1.9 (0.4)
ANOVA	*p*-value							
Soil (S)	0.17	<0.0001	0.003	0.05	0.001	0.01	<0.0001	0.16
Fertilizer (F)	0.61	0.14	0.34	0.70	0.07	0.78	0.03	0.046
Rate (R)	0.008	0.01	0.0007	0.46	<0.0001	0.12	<0.0001	0.28
S × F	0.85	0.054	0.44	0.19	0.86	0.14	0.55	0.37
S × R	0.008	<0.0001	0.0001	0.01	0.0004	0.12	<0.0001	0.002
F × R	0.21	0.34	0.28	0.55	0.02	0.28	0.07	0.10
S × F × R	0.80	0.72	0.77	0.45	0.67	0.82	0.82	0.49

[a] Values are means with SEM in parentheses.

[b] Means within factors and columns followed by the same letter are not significantly different according to Tukey's test, if not involved in an interactive effect. Significant interactions are plotted in Figures 4.1–4.3.

Cumulative alfalfa plant performance responded to P application rate only in the Wampum soil, as indicated by significant interactions between soil and P rate for biomass, P concentration, and P uptake (Table 4.2; Figure 4.1). In the Wampum soil, the P248 application rate produced greater biomass and P uptake than the other application rates, averaged across fertilizers, and was the only P rate to outperform the unfertilized control (Figure 4.1a, c). Cumulative tissue P concentration was greater for P248 than the lower application rates in the Wampum soil, but none of the fertilized treatments differed significantly from P0 (Figure 4.1b). Differences in tissue P concentration were relatively small, even when statistically significant, and so the patterns of P uptake were driven largely by plant biomass rather than tissue P concentration. In the Dencross soil, plant biomass, P uptake, and P concentration were not affected by the rate of P addition (Figure 4.1a, b, c). The apparent P recovery efficiency in cumulative shoot biomass was similar for the two fertilizers but varied with the interaction of soil and P rate (Table 4.2; Figure 4.1d); P recovery efficiency did not differ for the three application rates within each soil type, but was greater in the Wampum than in the Dencross soil at the highest application rate only. The only differences between soils in cumulative biomass, P uptake, P concentration, and P recovery occurred at the P248 application rate, where the alfalfa response was consistently greater in the Wampum soil than in the Dencross soil.

The general effects of P rate and soil type on cumulative plant biomass and P uptake were also observed at Cut 1 (Table 4.2; Figure 4.2), suggesting that the plant regrowth patterns after cutting did not have a major effect on the relative plant performance among treatments over all three harvests, except perhaps at the P124 rate (compare Figures 4.1 and 4.2). Tissue P concentration at Cut 1 was greater in the Wampum soil than in the Dencross soil and was not affected by P rate or fertilizer type (Table 4.2). Shoot biomass at Cut 1 was affected by an additional interaction between fertilizer type and application rate; at the P248 rate, shoot biomass for both fertilizers exceeded the control, but the two fertilizers followed different patterns at the lower rates, where struvite increased biomass above the control at P124, whereas MAP did not (Figure 4.3). Shoot P recovery efficiency at Cut 1 was lower for struvite than for MAP (Table 4.2) and varied with the interaction of soil and P rate, with higher efficiency at the highest application rate in the Wampum soil and the opposite pattern in the Dencross soil (Figure 4.2c).

The overall low recovery of fertilizer-P in shoot biomass suggests that plants utilized very little of the added P. However, calculating the incremental P recovery using the difference method assumes that fertilized and unfertilized plants used the same quantity of P from soil reserves, when in fact fertilized plants may have preferentially used fertilizer P rather than background soil P. Tracer studies could identify the source of P taken up by plants to clarify differences in P acquisition between fertilized and unfertilized plants.

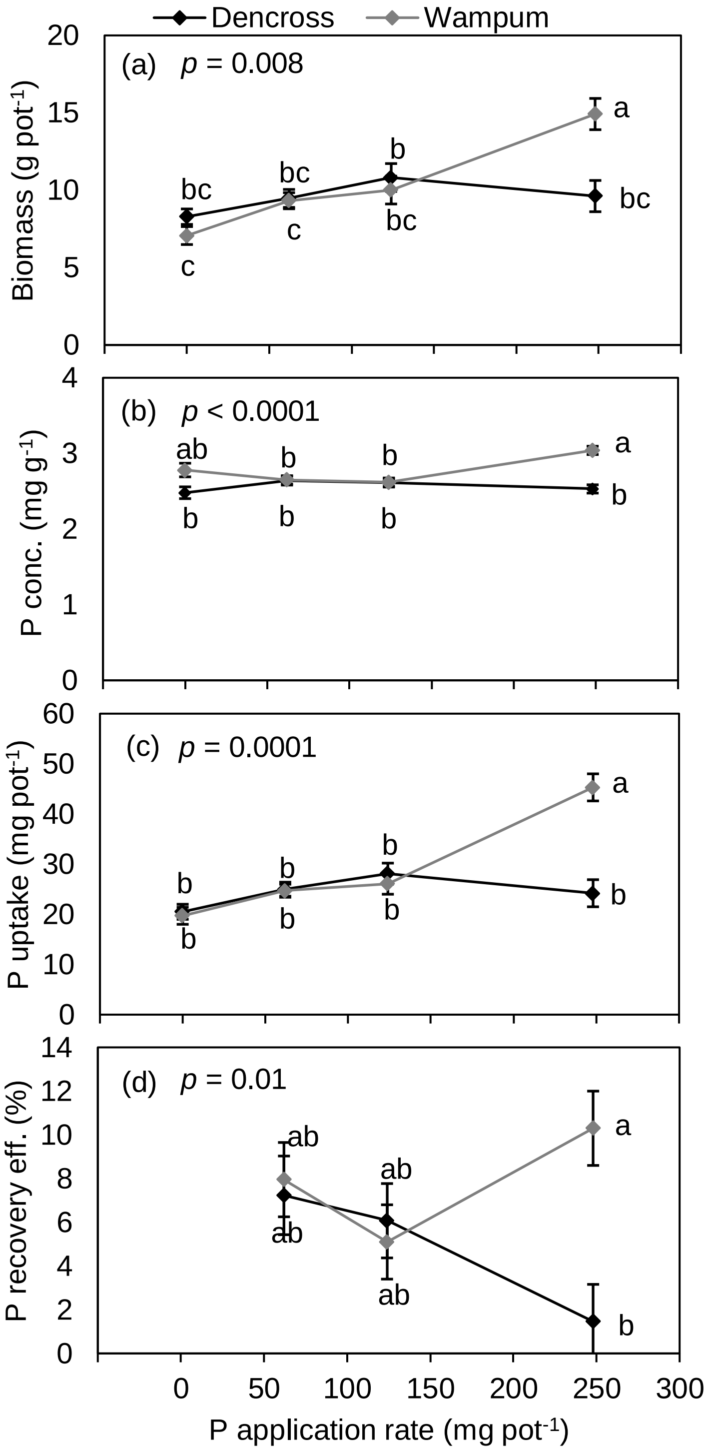

Figure 4.1 Interactive effects of soil type (Dencross clay, Wampum sandy clay loam) and P application rate (0, 62, 124, 148 mg P pot^{-1}) on cumulative alfalfa biomass production (a), tissue P concentration (b), P uptake (c), and P recovery efficiency in biomass (d) over 130 d of growth, averaged across fertilizer types (struvite, MAP). Data points and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across soils and P application rates according to Tukey's test.

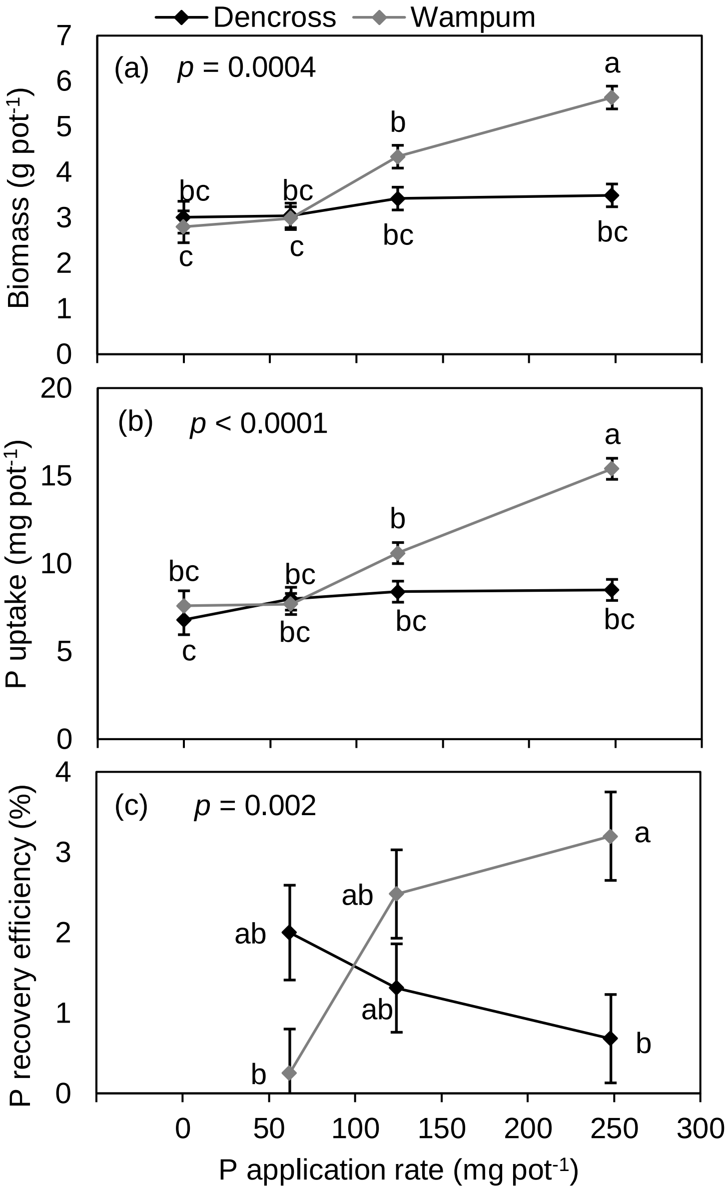

Figure 4.2 Interactive effects of soil type (Dencross clay, Wampum sandy clay loam) and P application rate (0, 62, 124, 148 mg P pot^{-1}) on alfalfa biomass production (a), P uptake (b), and P recovery efficiency in biomass (c) at Cut 1 (55 d), averaged across fertilizer types (struvite, MAP). Data points and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across soils and P application rates according to Tukey's test.

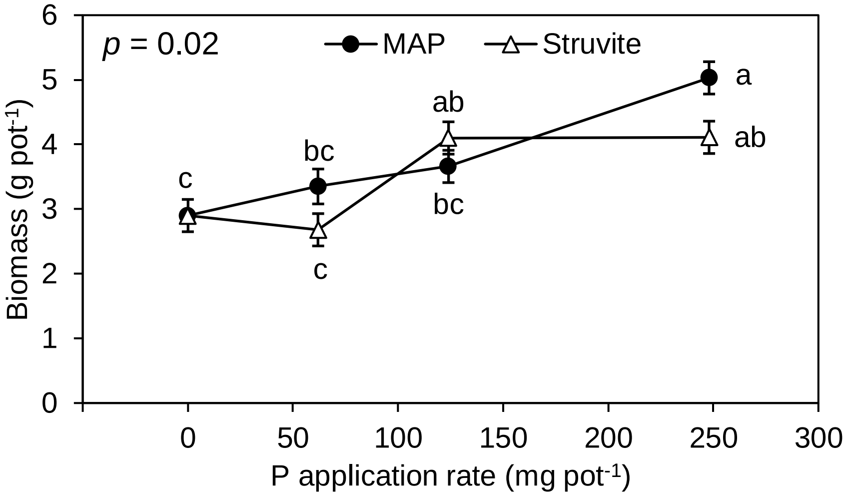

Figure 4.3 Interactive effect of fertilizer type (struvite, MAP) and fertilizer application rate (0, 62, 124, 248 mg pot^{-1}) on shoot biomass harvested at Cut 1 (55 d), averaged across soil types. Data points and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across soils and P application rates according to Tukey's test.

The reason for the lack of plant response to increasing application rates of either fertilizer in the Dencross soil is unclear. The alkaline pH of this soil may have limited plant response to struvite (Hertzberger et al. 2020) but this does not explain the lack of response to MAP. In a field study at the site from which the Dencross soil was collected (Chapter 2), alfalfa biomass increased linearly with struvite application rates similar to those used in the present study, so we expected a strong response to added P in a controlled environment as well. However, in the field study, granular struvite was banded into the root zone of a previously established forage stand, creating a concentrated zone of P addition within a well-developed root system, potentially creating conditions more favourable for plant P uptake. Based on the concentrations of Olsen-P in the Dencross soil at the end of the experiment in both struvite and MAP treatments, as discussed below, P should not have been a limiting factor for plant growth and so other factors must be considered. The absence of significant differences in plant response in the supplemental N comparison (Appendix III) indicates that any differences due to N supply should be negligible. Besides P and N, baseline soil tests showed that only S (both soils) and B (Wampum soil) were deficient. Supplementation of these nutrients was expected to correct these deficiencies but deficiencies of these or other nutrients cannot be definitively ruled out. Other differences between the two soils were related to physical properties, possibly causing differences in root growth in the differing soil textures and/or soil water dynamics, but no clear connection can be drawn between these conditions and the lack of plant response to added P in the Dencross soil. Damage from insect pests in the greenhouse study may have limited the ability of most plants to respond to added P.

4.4.3 Labile Soil P Pools

Initial Olsen-P concentration was low in both soils but was greater in the Wampum soil than the Dencross soil (Table 4.1; $p < 0.0001$). Initial WEP displayed a weak trend in the opposite direction ($p = 0.06$) and initial CEP did not differ between the soil types ($p = 0.37$).

At the end of the pot study, all three P pools were highly correlated to each other in the whole dataset, as well as in groupings by soil type and fertilizer type, though not in the unfertilized controls (Table 4.3). The relative size of the P pools detected in the final samples by the three extractants was Olsen-P > CEP > WEP (Table 4.4). The relative size of Olsen-P and WEP pools, as well as the significant correlations between the different P extractions in final samples, are consistent with past research describing the relationships between WEP and Olsen-P in soils from Manitoba and elsewhere (Leytem and Westermann 2005; Kumaragamage et al. 2007; Ige et al. 2011; Reid and Schneider 2021). Studies using CEP as part of a suite of P extractions to characterize differing soil types have also reported a strong correlation between Olsen-P and CEP (DeLuca et al. 2015; Jalali and Jalali 2016).

Table 4.3 Pearson correlations (*r*) and *p*-values among water-extractable P (WEP), Olsen-P, and citrate-extractable P (CEP) concentrations in soils following 130 d of alfalfa growth under controlled conditions

		WEP and Olsen-P		**Olsen-P and CEP**		**WEP and CEP**	
Sample group	***n***	***r***	***p*-value**	***r***	***p*-value**	***r***	***p*-value**
All samples	54	0.86	<0.0001	0.85	<0.0001	0.81	<0.0001
Dencross soil	27	0.93	<0.0001	0.97	<0.0001	0.90	<0.0001
Wampum soil	27	0.84	<0.0001	0.97	<0.0001	0.82	<0.0001
Control	7	-0.30	0.51	-0.45	0.31	-0.10	0.83
Struvite	24	0.62	0.001	0.69	0.0002	0.78	<0.0001
MAP	23	0.96	<0.0001	0.87	<0.0001	0.81	<0.0001

Table 4.4 Effects of soil type, fertilizer type, and P application rate on the concentration and percent fertilizer-P recovery of water-extractable P (WEP), Olsen-P, and citrate-extractable P (CEP) in soil following 130 d of alfalfa growth under controlled conditions

	WEP		**Olsen-P**		**CEP**	
Factor	**Conc.**	**Recov.**	**Conc.**	**Recov.**	**Conc.**	**Recov.**
	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	%
Soil						
Dencross	3.6 (0.3)[a]	4.6 (0.6)	18.6 (0.6) a [b]	40 (3.3)	12.3 (0.9)	21 (1.8)
Wampum	2.0 (0.3)	5.0 (0.6)	16.3 (0.4) b	22 (2.6)	14.0 (1.7)	25 (1.9)
Fertilizer						
Control	1.8 (0.3)	-	11.6 (0.2)	-	6.1 (1.6)	-
Struvite	4.2 (0.3)	5.0 (0.6)	24.7 (0.7)	26 (2.8)	18.6 (0.9)	22 (1.8)
MAP	4.0 (0.3)	4.6 (0.6)	27.8 (1.7)	34 (3.2)	21.9 (1.9)	24 (1.9)
P Application Rate						
0 mg pot^{-1}	1.8 (0.3)	-	11.6 (0.2) c	-	6.1 (1.6)	-
62 mg pot^{-1}	2.3 (0.2)	3.2 b (0.6)	17.1 (0.6) c	23 b (3.3)	11.6 (0.6)	21 (2.2)
124 mg pot^{-1}	4.1 (0.4)	5.4 ab (0.7)	25.7 (1.5) b	31 ab (3.5)	17.3 (1.0)	22 (2.2)
248 mg pot^{-1}	7.3 (0.6)	6.2 a (0.8)	41.1 (3.0) a	37 a (4.0)	31.7 (2.9)	26 (2.3)
ANOVA			*p*-value			
Soil (S)	<0.0001	0.67	0.001	0.0002	0.21	0.11
Fertilizer (F)	0.71	0.63	0.09	0.08	0.15	0.51
Rate (R)	<0.0001	0.02	<0.0001	0.04	<0.0001	0.36
S × F	0.005	0.01	0.15	0.03	0.37	0.21
S × R	0.01	0.41	0.37	0.34	0.04	0.02
F × R	0.81	0.76	0.32	0.11	0.35	0.25
S × F × R	0.47	1.00	0.53	0.24	0.70	0.43

[a] Values presented are least-squares means with SEM in parentheses.

[b] Means within factors and columns followed by similar letters and not involved in an interactive effect are not significantly different according to Tukey's test. Significant interactions are plotted in Figures 4.4 and 4.5.

The effect of fertilizer type on soil P pools was only significant in interaction with other factors. Final soil WEP concentration and recovery, as well as Olsen-P recovery, were affected by significant soil by fertilizer interactions (Table 4.4; Figure 4.4). These interactions demonstrated differing direction of WEP response for the two fertilizers in the two soils, although differences among means for fertilized treatments were not significant (Figure 4.4a, b). In the Dencross soil, only MAP increased WEP concentration above that of the unfertilized control, whereas in the Wampum soil, both fertilizers increased WEP over the control. Water-extractable P concentrations were low in all treatments relative to many observations in Manitoba soils incubated with added P amendments (Kashem et al. 2004; Kumaragamage et al. 2007, 2011). Final WEP concentrations in this pot study followed a very similar pattern to those on the final sampling date of the companion incubation study (Appendix II, Figure II-1), but were slightly lower in the pot study, perhaps due to the overall lower P application rates used here or because of plant P uptake from the soil solution.

Olsen-P concentration was similar for the two fertilizers (Table 4.4). However, the fertilizer-P recovery as Olsen-P was greater for MAP than for struvite in the Dencross soil but not the Wampum soil (Figure 4.4c), similar to the pattern observed for Olsen-P concentration and recovery at the final sampling date of the companion incubation study (Appendix II, Figure II-2). Citrate-extractable P concentration and recovery were not affected by fertilizer type as a main effect or in interaction with other factors (Table 4.4).

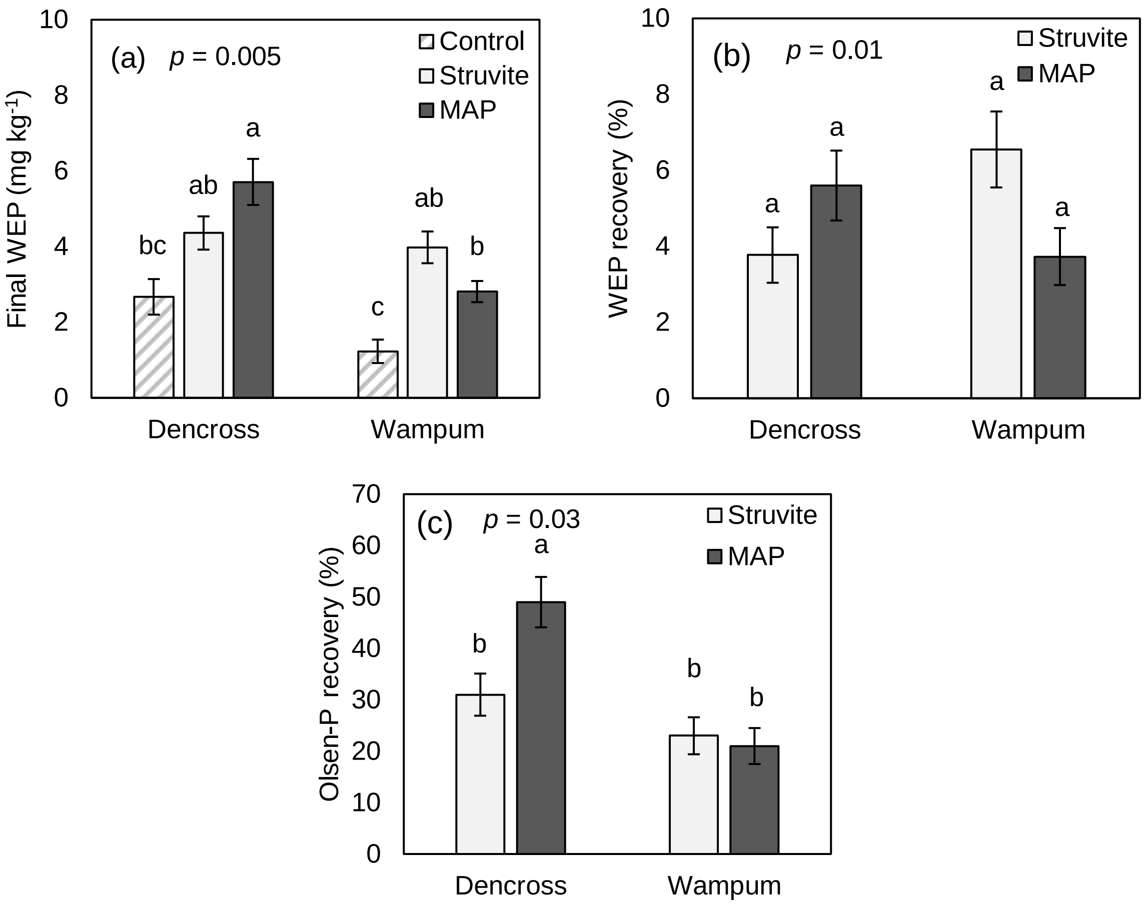

Figure 4.4 Interactive effects of soil (Dencross clay, Wampum sandy clay loam) and fertilizer type (struvite, MAP) on final WEP concentration (a), WEP recovery (b), and Olsen-P recovery (c), averaged across P application rates. Bar heights represent the least-squares means and error bars represent standard error of the mean. Lower-case letters indicate mean groupings across factors within each panel according to Tukey's test.

The similar effects of struvite and MAP addition on labile soil P pools in most cases indicate that the solubility properties of the two fertilizers were not a major factor in determining the net results of their behaviour in soil. In the companion incubation experiment, soil WEP and Olsen-P concentrations after 112 d of incubation were also similar between fertilizers when applied at 100 mg P kg^{-1} (Chapter 3), which is equivalent to the highest rate in the present study. The small struvite granule size (0.9 mm diameter) may have allowed for more rapid struvite dissolution relative to the large granules used in the field studies in Chapter 2, perhaps diminishing the differences between struvite and MAP dissolution expected in the present study. A recent plant growth experiment comparing 1.5- and 3.0-mm struvite granules reported greater soil test P with the smaller granules, likely due to more rapid dissolution (Hertzberger et al. 2021). However, past research has found that undissolved struvite in soil can contribute to the bicarbonate-extractable P pool and even soil solution P extracted in soil tests, possibly due to sample grinding and/or wide soil-to-solution ratios in extractions (Everaert et al. 2017; Meyer et al. 2018; Gu et al. 2021). Thus, although the size of the extracted P pools was similar for the two fertilizers in the present study, the P compounds present in soil in each fertilizer treatment may have differed. Remnants of struvite granules were not visible in soil at the end of the pot study, but small particles of undissolved struvite may have remained. Speciation of the P in soil samples is needed to clarify whether undissolved struvite remained in soil and contributed to the P in the soil extractions.

Soil type had a small but significant effect on Olsen-P concentration, with higher Olsen-P in the Dencross soil than in the Wampum soil (Table 4.4), even though the baseline samples showed the opposite pattern (Table 4.1; $p < 0.0001$). In the companion incubation study, a similar pattern between soil types was seen at early incubation dates (Chapter 3) but was evident only in the MAP-amended soils the end of the incubation period (Appendix II, Figure II-2). The reason for this effect is unclear, but could be due to differences in P buffering and retention processes and capacity. Another possible explanation for lower Olsen-P in the Wampum soil is simply that the greater plant P uptake in this soil reduced the size of the Olsen-P pool relative to the Dencross soil. However, plant P uptake only varied between soils at the highest application rate and there was no evidence of an interaction between soil type and P rate for final Olsen-P (Table 4.4).

All final soil P pools increased with increasing P application rate, but the effects of P rate on final WEP and CEP concentrations were modulated by soil type (Table 4.4; Figure 4.5). The lowest rate (P62) did not significantly increase final P concentration above the unfertilized control in any of the extractions except for CEP in the Dencross soil (Figure 4.5a), likely due to the combination of P retention in soil through adsorption and/or precipitation and plant P uptake. Further increases in P rate generally produced greater extractable P concentrations, though differences among individual means were not always significant (Table 4.4; Figure 4.5a, c). Final WEP was greater in the Dencross soil than the Wampum soil at the lowest application rate, but these differences diminished as WEP increased with higher application rates (Figure 4.5c). Though the soil by rate interaction for final CEP was significant, means for soils did not differ significantly at any single application rate or show any meaningful pattern (Figure 4.5a). Citrate-extractable P recovery was greater in the Wampum soil than in the Dencross soil only at the P124 rate (Figure 4.5b).

The Dencross soil was expected to have higher P retention capacity than the Wampum soil based on its relatively high exchangeable Ca, exchangeable Mg, and clay contents (Table 4.1), properties that are highly correlated with P retention capacity in calcareous soils (Ige et al. 2011). In a soil with low retention capacity, added P would tend to remain in the water-extractable pool, as observed in soil incubation studies (Kumaragamage et al. 2007; Duminda et al. 2017). The low WEP concentration in the Wampum soil relative to the Dencross soil at low application rates in the present study does not fit this expected pattern, but crop P uptake may have depleted the excess WEP that was not retained in soil. A better understanding of the P release and transformation processes associated with struvite in differing soil types, especially in the presence of plant roots, is needed to clarify effects of struvite application on various soil P pools and implications for crop P supply and the risk of P loss in runoff.

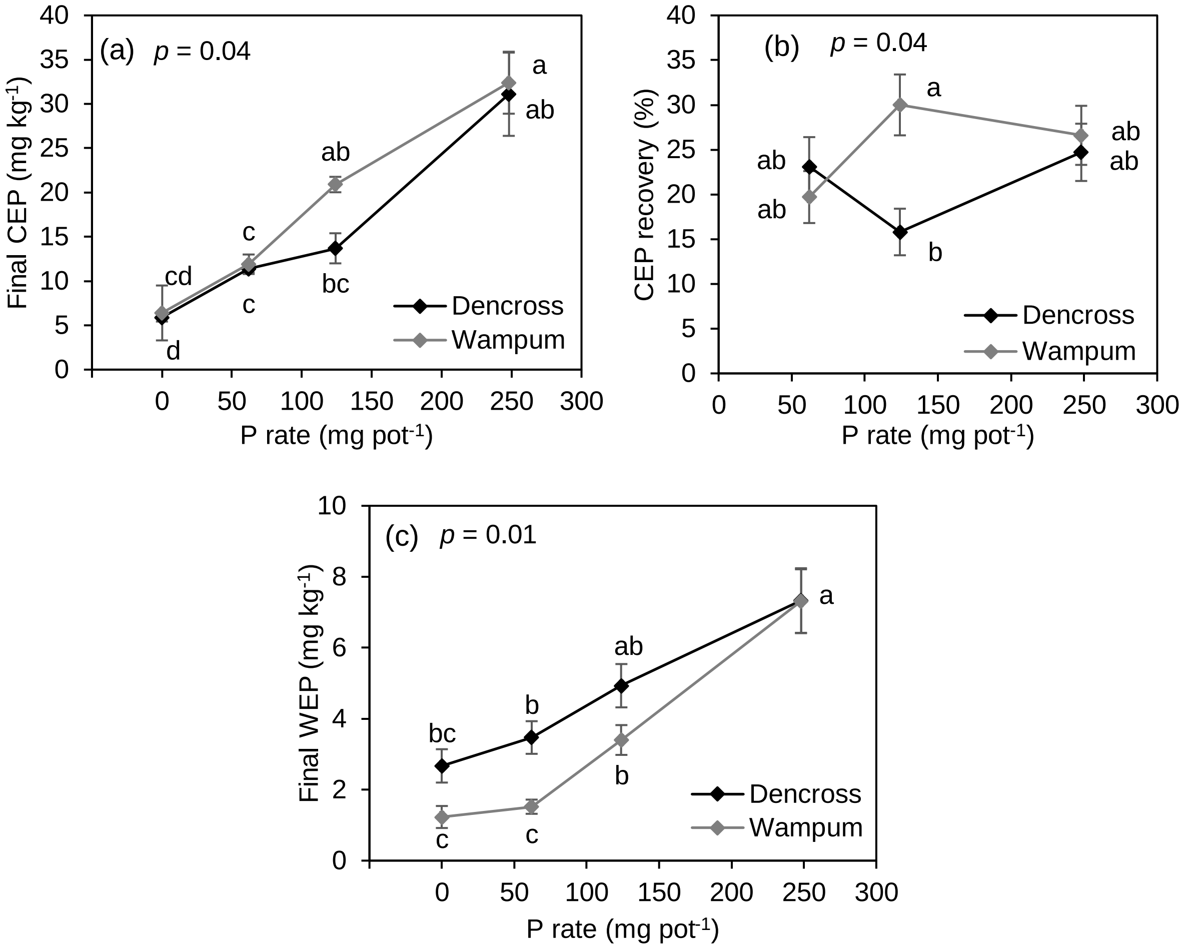

Figure 4.5 Interactive effects of soil (Dencross clay, Wampum sandy clay loam) and P application rate (0, 62, 124, 248 mg pot^{-1}) on final CEP concentration (a), CEP recovery (b), and WEP concentration (c). Data points represent least-squares means and error bars represent standard error of the mean. Lower-case letters indicate mean groupings across factors within each panel according to Tukey's test.

Based on the ratings in the Manitoba Soil Fertility Guide (Manitoba Agriculture 2007), the final Olsen-P concentrations in the P124 and P248 application rates are considered very high (≥20 mg kg^{-1}), while the Olsen-P in the P62 rate is considered high (15-19 mg kg^{-1}), indicating a relatively low likelihood of crop response to additional P fertilizers in these treatments. However, as Olsen-P increases above ~10–20 mg kg^{-1}, the P loss in runoff from rainfall or snowmelt also tends to increase in Manitoba soils (Kumaragamage et al. 2011; Liu et al. 2021). The highest mean

Olsen-P concentrations observed in fertilized treatments were 40 and 66 mg kg^{-1} for struvite and MAP, respectively (both in the Dencross soil), which could pose a substantial risk of runoff losses of dissolved P. In Manitoba, restrictions on P application to land begin to apply when soil Olsen-P reaches 60 mg kg^{-1} (Province of Manitoba 2008) due to the high risk of P losses.

The proportion of applied fertilizer-P recovered by the three soil P extractions was always <50% (Table 4.4; Figure 4.4 b, c; Figure 4.5b) but was substantially greater than the proportion of fertilizer-P recovered in plant biomass (<10%; Table 4.2, Figure 4.1). Where significant effects of treatment on P recovery in soil extractions occurred, they reflected patterns observed in soil P concentrations (Table 4.4; Figures 4.4, 4.5). Some of the P unrecovered in soil extractions and shoot biomass was undoubtedly contained in root biomass, which was not measured, but much of it was likely retained in soil in more recalcitrant forms through adsorption and/or precipitation, or possibly in undissolved struvite. The Olsen-P recovery we observed is greater than the 17% recovery reported for a low-P alkaline Manitoba soil amended with MAP at a wide range of rates and incubated without plants for 32 wk (Kashem et al. 2004). The shorter duration of the present study (18 wk) may explain this difference, as Olsen-P tends to decline with time after P application due to P retention in soil.

Averaged across soils, the Olsen-P concentration in the unfertilized control increased by about 4 mg kg^{-1} from the baseline to the final samples, despite plant offtake, while WEP and CEP in the unfertilized control remained relatively unchanged (compare Tables 4.1 and 4.4). These results demonstrate the potential to mobilize P from other P pools into the Olsen-P pool, perhaps due to rewetting the air-dried soil (Turner and Haygarth 2001) or through processes associated with the activity of plant roots or soil microorganisms.

4.4.4 Arbuscular Mycorrhizal Fungi

Root colonization was measured as the proportion of root length where arbuscular structures were present, as arbuscules are the fungal structure involved in P exchange and may be more sensitive to P dynamics than other fungal structures (Menge et al. 1978). Mean AMF root colonization with arbuscules was <10% for all treatments and was affected significantly by interactive effects of soil and fertilizer type, soil and P rate, and fertilizer type and P rate (Table 4.5). This rate of root colonization is lower than that observed in field-grown alfalfa in Ontario (19–42%) (Schneider et al. 2017) and in an alfalfa experiment at the site from which the Dencross soil was collected (19–25%; Appendix I). In most pot experiments investigating AMF colonization, plants are treated with fungal inoculum, so relying on native AMF inoculum in highly disturbed, air-dried soils may have caused the relatively low rate of root colonization in the present study.

Addition of P fertilizers generally suppressed AMF colonization to some degree, with the strength of the response depending on soil type, fertilizer type, and P rate (Figure 4.6). Overall, root colonization depended more on soil type at low P application rates and on fertilizer type at high application rates. Root colonization was greater in the Wampum soil than the Dencross soil in the P0 and P62 fertilizer rates, but both soils declined to similar colonization rates at the higher rates of P application (Figure 4.6a). The reason for the differences in root colonization between the soil types at the P0 and P62 rates is unclear. Both soils were low in Olsen-P and WEP, so factors other than P likely played a role.

Table 4.5 Effects of soil type, fertilizer type, and P application rate on the occurrence of mycorrhizal arbuscules in alfalfa roots (root colonization) after 130 d of growth under controlled conditions

Factor	**Root colonization**
	%
Soil	
Dencross	3.16 (0.31) [a]
Wampum	4.95 (0.38)
Fertilizer	
Control	6.40 (0.65)
Struvite	3.12 (0.26)
MAP	1.89 (0.22)
P Application Rate	
0 mg pot^{-1}	6.40 (0.65)
62 mg pot^{-1}	4.19 (0.42)
124 mg pot^{-1}	1.99 (0.25)
248 mg pot^{-1}	1.70 (0.25)
ANOVA	*p*-value
Soil (S)	0.005
Fertilizer (F)	0.001
Rate (R)	<0.0001
S × F	0.04
S × R	0.002
F × R	0.03
S × F × R	0.79

[a] Values presented are means with SEM in parentheses. Significant interactions are plotted in Figure 4.6.

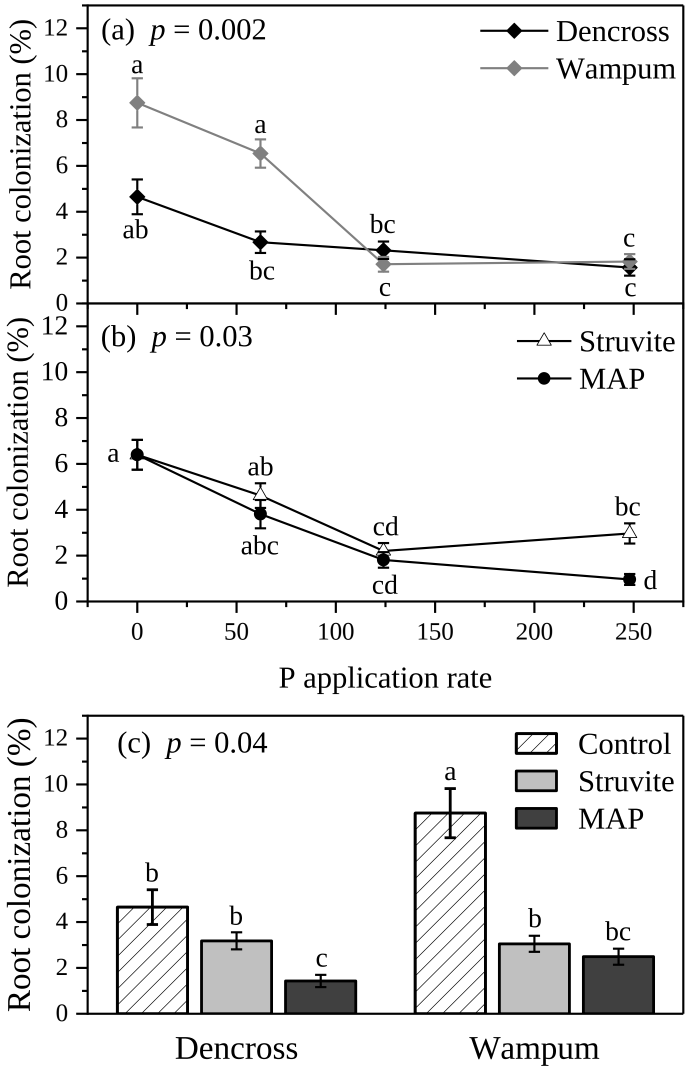

Figure 4.6 Interactive effects of soil type (Dencross clay, Wampum sandy clay loam) and P rate (0, 62, 124, 248 mg P pot^{-1}) (a), fertilizer type (struvite, MAP) and P rate (b), and soil type and fertilizer type (c) on the percent of alfalfa root length at which fungal arbuscules were present (% root colonization). Data points or bar height represent least-squares means and error bars represent standard error of the mean. Lower-case letters indicate mean groupings across factors within each panel according to Tukey's test.

Both P sources tended to reduce AMF colonization to a greater degree when applied at higher rates. However, the highest rate of struvite did not suppress root colonization as much as the highest rate of MAP (Figure 4.6b). Final extractable P concentrations were not significantly different among fertilizer types at the highest application rate, so the difference in colonization at this rate could be due to differences in P availability earlier in the experiment (e.g., slow struvite dissolution). Another possible explanation is that the extractable P pools measured in this experiment over-estimated P availability from struvite, due to undissolved struvite in finely ground soil samples, as discussed in Chapter 3.

The effect of fertilizer type on AMF root colonization was also evident in interaction with soil type. In the Dencross soil, root colonization in the struvite treatment was similar to the control and greater than the MAP treatment, whereas in the Wampum soil, root colonization with struvite was similar to MAP and both fertilizer treatments were lower than the control (Figure 4.6c). Previous research on tomato fertilized with the equivalent of 20 kg P ha^{-1} as struvite or MAP in a clay loam soil with near-neutral pH reported no difference in root colonization among fertilizer treatments (Di Tomassi et al. 2021), possibly due to the relatively low rate of P application. A possible explanation for the difference between soil types in the present study is that the alkaline conditions in the Dencross soil restricted struvite dissolution, creating soil P conditions more similar to the control than to the MAP treatment, whereas in the neutral-pH Wampum soil struvite dissolved to a greater extent and behaved more like MAP. Using large struvite granules rather than the small ones used here would likely amplify the differences between struvite and MAP in their effect on AMF in these two soil types.

Root colonization was negatively correlated with final Olsen P, final WEP, final CEP, and P application rate in both soils (Table 4.6), as expected based on commonly observed patterns (Hamel and Strullu 2006). These relationships were weaker for the Dencross soil than for the Wampum soil, again suggesting that factors other than P played an important role in root colonization in the Dencross soil. However, the relatively small range in percent root colonization in the Dencross soil may have contributed to the weak relationship. Within fertilizer types, root colonization in MAP treatments was negatively correlated with all final P pools and P application rate, but struvite treatments showed a significant negative correlation only with final Olsen-P and a weak negative relationship with P application rate (Table 4.6). These results indicate that the typical relationship between root colonization and available P in soil or fertilizers may be altered by struvite's slow-release properties, contrary to the findings of Di Tomassi et al. (2021), or that the P extractions in the present study, conducted on finely ground samples, did not accurately reflect P availability from struvite during plant growth.

Table 4.6 Spearman rank correlations (*r*) and *p*-values between root colonization by arbuscular mycorrhizal fungi (AMF) and soil water-extractable P (WEP), Olsen-P, citrate-extractable P (CEP), and P fertilizer application rate

Sample Group	***n***	**AMF & WEP**	**AMF & Olsen-P**	**AMF & CEP**	**AMF & P rate**
All samples	54				
r		–0.56	–0.62	–0.53	–0.58
p		<0.0001	<0.0001	<0.0001	<0.0001
Dencross soil	27				
r		–0.49	–0.53	–0.50	–0.46
p		0.01	0.004	0.008	0.01
Wampum soil	27				
r		–0.60	–0.69	–0.68	–0.74
p		0.001	<0.0001	<0.0001	<0.0001
Control	7				
r		–0.50	0.14	0.07	-
p		0.25	0.76	0.88	-
Struvite	24				
r		–0.32	–0.51	–0.34	–0.39
p		0.13	0.01	0.10	0.06
MAP	23				
r		–0.73	–0.64	–0.55	–0.68
p		<0.0001	0.001	0.007	0.0003

4.4.5 Patterns in Plant, Soil, and AMF Dynamics

The overall similarity between struvite and MAP for most parameters in the greenhouse study suggests that fertilizer application guidelines for MAP may also be used with struvite. However, the small granule size used and mixing of fertilizers through the soil may alter dissolution dynamics relative to those expected with banded application of large struvite granules under field conditions; thus, further testing is needed.

The P application rate required to increase plant growth and P uptake above the unfertilized control was four times the rate recommended by the Manitoba Soil Fertility Guide for alfalfa and was only effective in one of the two soils. However, the increase in labile soil P pools with increasing fertilizer rates indicates that plant growth was probably limited by factors other than P and that a substantial portion of the P applied from both struvite and MAP was indeed available for plant uptake. At the high application rate, a much larger proportion of the applied P was recovered in soil Olsen-P at the end of the experiment than in the incremental increase of plant biomass over the control, suggesting that the P supplied by fertilizers was much greater than plant requirements during the 130-d growth period, even after accounting for P retention in non-plant-available pools. This "excess" P remaining in soil could theoretically provide a supply of P for several additional seasons of crop growth as seen in a field study with alfalfa (Chapter 2), assuming similar plant P uptake in future seasons and negligible additional net transformation of soil P to pools that are unavailable to plants. However, such a multi-year approach to fertilizer application must also consider the risks of P loss in runoff due to high P application rates. Whether the increases in Olsen-P observed in this pot study would also occur in a field setting is unclear, but preliminary evidence indicates that application of large struvite granules does not increase soil Olsen-P dramatically under field conditions, even when applied at a high rate (Appendix I).

At the only P application rate that significantly increased plant P uptake (P248), applying P as struvite was beneficial for AMF root colonization, relative to MAP, although both fertilizers suppressed AMF from levels in the unfertilized control. It is noteworthy that applying a moderate

rate of P as either struvite or MAP (P124) also suppressed AMF substantially in the Wampum soil, whereas root colonization at the P62 rate did not differ significantly from the unfertilized controls (Figure 4.6a, b). Root colonization at the low levels we observed was unlikely to have substantially increased P supply to the plants. However, if the patterns showing less suppression of AMF by struvite at high application rates extend to field conditions and greater overall levels of root colonization, the use of struvite as a P source may allow for adequate P supply to plants without the same degree of AMF suppression as from MAP. In a field study at the site where the Dencross soil was collected, application of granular struvite at 30–90 kg P ha^{-1} increased alfalfa–grass forage productivity (Chapter 2) while having no effect on AMF root colonization measured in the year after struvite application (Appendix I).

The importance of AMF in supporting crop yields has sometimes been questioned (Ryan and Graham 2018), as the benefit of crop P supply from fertilizers likely outweighs the loss of direct P benefit provided by AMF (Bittman et al. 2006). However, benefits from AMF for plant nutrition are thought to occur in low-input cropping systems relying more heavily on soil P reserves or functioning at lower STP concentrations (Mäder et al. 2000; Entz et al. 2004; Schneider et al. 2017), and may also be relevant when using sparingly soluble fertilizers such as struvite. With growing interest in reducing legacy P in soils (Rowe et al. 2016) and increasing P use efficiency in agriculture (Schneider et al. 2019), it may be necessary to rely more heavily on plant P acquisition mechanisms that more fully exploit existing soil P. In addition, AMF are known to provide other benefits, including provision of water and other nutrients to plants, enhanced crop stress tolerance, and improved soil quality (Hamel and Strullu 2006), but these benefits are typically not considered when evaluating the effect of P application on AMF in terms of crop productivity. Thus, it may be worthwhile to promote AMF colonization of crop roots, even when application of P fertilizers renders the direct benefit to plant P nutrition negligible.

4.5 Conclusions

Determining the effects of struvite on a holistic set of plant and soil parameters is necessary for developing appropriate management guidelines for this novel fertilizer. In a comparison of struvite to the commonly used soluble fertilizer MAP, we found the two fertilizers to have very similar effects on plant growth and nutrient uptake, as well as on labile soil P pools. Soil type had a greater effect than fertilizer type on plant and soil parameters, reinforcing the need to develop P management guidelines based on soil properties for all fertilizer types. In particular, the frequent occurrence of interactions between soil type and P application rate in the present study demands greater attention to the role of P application rates in soil–fertilizer–plant P dynamics, not only in relation to meeting plant P requirements but in understanding the fate of P in the soil and optimizing P management. A high application rate was needed to increase plant productivity, but this rate could also increase soil P concentrations to the point of posing a risk of P loss in runoff. Although AMF root colonization was low in all treatments, our results provide evidence that struvite applied at a high rate may have less of an inhibitory effect on AMF than MAP. These results provide preliminary evidence that the slow-release properties of struvite may help to optimize P supply to crops, soil P status, and the symbiotic relationship between plants and AMF. Field studies are needed to determine whether these relationships exist under realistic growing conditions and crop management practices.

4.6 References

Anderson, R., Brye, K.R., Greenlee, L., and Gbur, E. 2020. Chemically precipitated struvite dissolution dynamics over time in various soil textures. Agric. Sci. **11**: 567–591. doi:10.4236/as.2020.116036.

Balzergue, C., Chabaud, M., Barker, D.G., Bécard, G., and Rochange, S.F. 2013. High phosphate reduces host ability to develop arbuscular mycorrhizal symbiosis without affecting root calcium spiking responses to the fungus. Front. Plant Sci. **4**: article 426. doi:10.3389/fpls.2013.00426.

Bittman, S., Kowalenko, C.G., Hunt, D.E., Forge, T.A., and Wu, X. 2006. Starter phosphorus and broadcast nutrients on corn with contrasting colonization by mycorrhizae. Agron. J. **98**: 394–401. doi:10.2134/agronj2005.0093.

Degryse, F., Baird, R., Silva, R., and McLaughlin, M. 2017. Dissolution rate and agronomic effectiveness of struvite fertilizers - effect of soil pH, granulation and base excess. Plant Soil **410**: 139–152. doi:10.1007/s11104-016-2990-2.

DeLuca, T.H., Glanville, H.C., Harris, M., Emmett, B.A., Pingree, M.R.A., de Sosa, L.L., Cerdá-Moreno, C., and Jones, D.L. 2015. A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. Soil Biol. Biochem. **88**: 110–119. doi:10.1016/j.soilbio.2015.05.016.

Di Tomassi, I., Chatterjee, N., Barrios-Masias, F.H., Zhou, Q., Gu, C., and Margenot, A.J. 2021. Arbuscular mycorrhizae increase biomass and nutrient uptake of tomato fertilized with struvite compared to monoammonium phosphate. Plant Soil **464**: 321–333. doi:10.1007/s11104-021-04957-2.

Duboc, O., Santner, J., Golestani Fard, A., Zehetner, F., Tacconi, J., and Wenzel, W.W. 2017. Predicting phosphorus availability from chemically diverse conventional and recycling fertilizers. Sci. Total Environ. **599–600**: 1160–1170. doi:10.1016/j.scitotenv.2017.05.054.

Duminda, D.M.S., Kumaragamage, D., Indraratne, S.P., and Flaten, D. 2017. Fertilizer-induced phosphorus dynamics in alkaline-calcareous soils as influenced by soil chemical properties. Can. J. Soil Sci. **97**: 159–170. doi:10.1139/cjss-2016-0055.

Entz, M.H., Penner, K.R., Vessey, J.K., Zelmer, C.D., and Thiessen Martens, J.R. 2004. Mycorrhizal colonization of flax under long-term organic and conventional management. Can. J. Plant Sci. **84**: 1097–1099. doi:10.4141/P04-055.

Everaert, M., Degryse, F., McLaughlin, M.J., De Vos, D., and Smolders, E. 2017. Agronomic effectiveness of granulated and powdered P-exchanged Mg–Al LDH relative to struvite and MAP. J. Agric. Food Chem. **65**: 6736–6744. doi:10.1021/acs.jafc.7b01031.

Gu, C., Zhou, Q., Cusick, R.D., and Margenot, A.J. 2021. Evaluating agronomic soil phosphorus tests for soils amended with struvite. Geoderma **399**: 115093. doi:10.1016/j.geoderma.2021.115093.

Hall, R.L., Boisen Staal, L., Macintosh, K.A., McGrath, J.W., Bailey, J., Black, L., Gro Nielsen, U., Reitzel, K., and Williams, P.N. 2020. Phosphorus speciation and fertiliser performance characteristics: A comparison of waste recovered struvites from global sources. Geoderma **362**: 114096. doi:10.1016/j.geoderma.2019.114096.

Hamel, C., and Strullu, D.-G. 2006. Arbuscular mycorrhizal fungi in field crop production: Potential and new direction. Can. J. Plant Sci. **86**: 941–950. doi:10.4141/P05-099.

Haney, R.L., Haney, E.B., Harmel, R.D., Smith, D.R., and White, M.J. 2016. Evaluation of H3A for determination of plant available P vs. FeAlO strips. Open J. Soil Sci. **6**: 175–187. doi:10.4236/ojss.2016.611017.

Hertzberger, A.J., Cusick, R.D., and Margenot, A.J. 2020. A review and meta-analysis of the agricultural potential of struvite as a phosphorus fertilizer. Soil Sci. Soc. Am. J. **84**: 653–671. doi:10.1002/saj2.20065.

Hertzberger, A.J., Cusick, R.D., and Margenot, A.J. 2021. Maize and soybean response to phosphorus fertilization with blends of struvite and monoammonium phosphate. Plant Soil **461**: 547–563. doi:10.1007/s11104-021-04830-2.

Hilt, K., Harrison, J., Bowers, K., Stevens, R., Bary, A., and Harrison, K. 2016. Agronomic response of crops fertilized with struvite derived from dairy manure. Water. Air. Soil Pollut. **227**: 388. doi:10.1007/s11270-016-3093-7.

Huygens, D., and Saveyn, H.G.M. 2018. Agronomic efficiency of selected phosphorus fertilisers derived from secondary raw materials for European agriculture. A meta-analysis. Agron. Sustain. Dev. **38**: 52. doi:10.1007/s13593-018-0527-1.

Ige, D.V., Akinremi, O.O., Flaten, D.N., Ajiboye, B., and Kashem, M.A. 2011. Phosphorus sorption capacity of alkaline Manitoba soils and its relationship to soil properties. Can. J. Soil Sci. doi:10.4141/S04-064.

Jalali, M., and Jalali, M. 2016. Relation between various soil phosphorus extraction methods and sorption parameters in calcareous soils with different texture. Sci. Total Environ. **566–567**: 1080–1093. doi:10.1016/j.scitotenv.2016.05.133.

Kashem, Md.A., Akinremi, O.O., and Racz, G.J. 2004. Extractable phosphorus in alkaline soils amended with high rates of organic and inorganic phosphorus. Can. J. Soil Sci. **84**: 459–467. doi:10.4141/S03-085.

Kratz, S., Vogel, C., and Adam, C. 2019. Agronomic performance of P recycling fertilizers and methods to predict it: A review. Nutr. Cycl. Agroecosystems **115**: 1–39. doi:10.1007/s10705-019-10010-7.

Kumaragamage, D., Akinremi, O.O., Flaten, D., and Heard, J. 2007. Agronomic and environmental soil test phosphorus in manured and non-manured Manitoba soils. Can. J. Soil Sci. **87**: 73–83. doi:10.4141/S06-030.

Kumaragamage, D., Flaten, D., Akinremi, O.O., Sawka, C., and Zvomuya, F. 2011. Soil test phosphorus changes and phosphorus runoff losses in incubated soils treated with livestock manures and synthetic fertilizer. Can. J. Soil Sci. **91**: 375–384. doi:10.4141/cjss10004.

Leytem, A.B., and Westermann, D.T. 2005. Phosphorus availability to barley from manures and fertilizers on a calcareous soil: Soil Sci. **170**: 401–412. doi:10.1097/01.ss.0000169914.17732.69.

Liu, J., Elliott, J.A., Wilson, H.F., Macrae, M.L., Baulch, H.M., and Lobb, D.A. 2021. Phosphorus runoff from Canadian agricultural land: A cross-region synthesis of edge-of-field results. Agricultural Water Management **255**: 107030. doi:10.1016/j.agwat.2021.107030.

Mäder, P., Edenhofer, S., Boller, T., Wiemken, A., and Niggli, U. 2000. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. Biol. Fertil. Soils **31**: 150–156. doi:10.1007/s003740050638.

Manitoba Agriculture 2007. Manitoba soil fertility guide. Province of Manitoba. [Online] Available: https://www.gov.mb.ca/agriculture/crops/soil-fertility/soil-fertility-guide/pubs/soil_fertility_guide.pdf [2021 Apr. 11].

Menge, J.A., Steirle, D., Bagyaraj, D.J., Johnson, E.L.V., and Leonard, R.T. 1978. Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. New Phytol. **80**: 575–578. doi:10.1111/j.1469-8137.1978.tb01589.x.

Meyer, G., Frossard, E., Mäder, P., Nanzer, S., Randall, D.G., Udert, K.M., and Oberson, A. 2018. Water soluble phosphate fertilizers for crops grown in calcareous soils – an outdated paradigm for recycled phosphorus fertilizers? Plant Soil **424**: 367–388. doi:10.1007/s11104-017-3545-x.

Murphy, J., and Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta **27**: 31–36. doi:10.1016/S0003-2670(00)88444-5.

do Nascimento, C.A.C., Pagliari, P.H., Faria, L. de A., and Vitti, G.C. 2018. Phosphorus mobility and behavior in soils treated with calcium, ammonium, and magnesium phosphates. Soil Sci. Soc. Am. J. **82**: 622. doi:10.2136/sssaj2017.06.0211.

Nicksy, J., and Entz, M.H. 2021. Recycled nutrients as a phosphorus source for Canadian organic agriculture: a perspective. Can. J. Soil Sci. **101**: 571–580. doi:10.1139/cjss-2021-0014.

Olsen, S.R., Cole, C.V., F. S. Watanabe, and Dean, L.A. 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. United States Department of Agriculture, Washington, DC. [Online] Available: http://hdl.handle.net/2027/uiug.30112019277042.

Pote, D.H., Daniel, T.C., Moore, P.A., Nichols, D.J., Sharpley, A.N., and Edwards, D.R. 1996. Relating extractable soil phosphorus to phosphorus losses in runoff. Soil Sci. Soc. Am. J. **60**: 855–859. doi:10.2136/sssaj1996.03615995006000030025x.

Province of Manitoba 2008. Nutrient management regulation. Province of Manitoba. [Online] Available: https://web2.gov.mb.ca/laws/regs/current/_pdf-regs.php?reg=62/2008 [2022 Apr. 5].

Reid, K., and Schneider, K. 2021. Modelling dissolved phosphorus losses from accumulated soil phosphorus and applied fertilizer and manure for a national risk indicator. Can. J. Soil Sci.: 1–14. doi:10.1139/cjss-2021-0049.

Rowe, H., Withers, P.J.A., Baas, P., Chan, N.I., Doody, D., Holiman, J., Jacobs, B., Li, H., MacDonald, G.K., McDowell, R., Sharpley, A.N., Shen, J., Taheri, W., Wallenstein, M., and Weintraub, M.N. 2016. Integrating legacy soil phosphorus into sustainable nutrient management strategies for future food, bioenergy and water security. Nutr. Cycl. Agroecosystems **104**: 393–412. doi:10.1007/s10705-015-9726-1.

Ryan, M.H., and Graham, J.H. 2018. Little evidence that farmers should consider abundance or diversity of arbuscular mycorrhizal fungi when managing crops. New Phytol. **220**: 1092–1107. doi:10.1111/nph.15308.

Schneider, K.D., Lynch, D.H., Bünemann, E.K., and Voroney, R.P. 2017. Vegetative composition, arbuscular mycorrhizal fungi root colonization, and biological nitrogen fixation distinguish organic and conventional perennial forage systems. Agron. J. **109**: 1697–1706. doi:10.2134/agronj2016.12.0700.

Schneider, K.D., Thiessen Martens, J.R., Zvomuya, F., Reid, D.K., Fraser, T.D., Lynch, D.H., O’Halloran, I.P., and Wilson, H.F. 2019. Options for improved phosphorus cycling and use in agriculture at the field and regional scales. J. Environ. Qual. **48**: 1247–1264. doi:10.2134/jeq2019.02.0070.

Schoenau, J.J., and O’Halloran, I.P. 2008. Sodium bicarbonate-extractable phosphorus. Pages 89–94 *in* M.R. Carter and E.G. Gregorich, eds. Soil Sampling and Methods of Analysis, 2nd edition. CRC Press, Boca Raton, FL, USA.

Schwalb, S.A., Hemkemeyer, M., Watson, C., and Wichern, F. 2021. Mycorrhiza reduces phosphorus uptake from struvite in rye (*Secale cereale* L.) plants. J. Soil Sci. Plant Nutr. **21**: 3451–3460. doi:10.1007/s42729-021-00619-5.

Self-Davis, M.L., Moore, Jr., P.A., and Joern, B.C. 2009. Water- or dilute salt-extractable phosphorus in soil. Pages 22–24 *in* J.L. Kovar and G.M. Pierzynski, eds. Methods of Phosphorus Analysis for Soils, Sediments, Residuals, and Waters, 2nd edition. Southern Extension/Research Activity - Information Exchange Group 17, Blacksburg, VA.

Ström, L., Owen, A.G., Godbold, D.L., and Jones, D.L. 2005. Organic acid behaviour in a calcareous soil: implications for rhizosphere nutrient cycling. Soil Biol. Biochem. **37**: 2046–2054. doi:10.1016/j.soilbio.2005.03.009.

Syers, J.K., Johnston, A.E., and Curtin, D. 2008. Efficiency of soil and fertilizer phosphorus use: Reconciling changing concepts of soil phosphorus behaviour with agronomic information. Food and Agriculture Organization of the United Nations, Rome.

Szymańska, M., Sosulski, T., Bożętka, A., Dawidowicz, U., Wąs, A., Szara, E., Malak-Rawlikowska, A., Sulewski, P., van Pruissen, G.W.P., and Cornelissen, R.L. 2020. Evaluating the struvite recovered from anaerobic digestate in a farm bio-refinery as a slow-release fertiliser. Energies **13**: 5342. doi:10.3390/en13205342.

Szymańska, M., Szara, E., Wąs, A., Sosulski, T., Pruissen, G.W. van, and Cornelissen, R.L. 2019. Struvite—an innovative fertilizer from anaerobic digestate produced in a bio-refinery. Energ. Basel **12**. doi:10.3390/en12020296.

Talboys, P.J., Heppell, J., Roose, T., Healey, J.R., Jones, D.L., and Withers, P.J.A. 2016. Struvite: a slow-release fertiliser for sustainable phosphorus management? Plant Soil **401**: 109–123. doi:10.1007/s11104-015-2747-3.

Turner, B.L., and Haygarth, P.M. 2001. Phosphorus solubilization in rewetted soils. Nature 411: 258–258. doi:10.1038/35077146.

Vogel, T., Nelles, M., and Eichler-Löbermann, B. 2015. Phosphorus application with recycled products from municipal waste water to different crop species. Ecol. Eng. **83**: 466–475. doi:10.1016/j.ecoleng.2015.06.044.

Vogel, T., Nelles, M., and Eichler-Löbermann, B. 2017. Phosphorus effects of recycled products from municipal wastewater on crops in a field experiment. Plant Soil Environ. **63**: 475–482. doi:10.17221/513/2017-PSE.

Wang, Y.T., Zhang, T.Q., Hu, Q.C., Tan, C.S., Halloran, I.P.O., Drury, C.F., Reid, D.K., Ma, B.L., Ball-Coelho, B., Lauzon, J.D., Reynolds, W.D., and Welacky, T. 2010. Estimating dissolved reactive phosphorus concentration in surface runoff water from major Ontario soils. J. Environ. Qual. **39**: 1771–1781. doi:10.2134/jeq2009.0504.

Weissengruber, L., Möller, K., Puschenreiter, M., and Friedel, J.K. 2018. Long-term soil accumulation of potentially toxic elements and selected organic pollutants through application of recycled phosphorus fertilizers for organic farming conditions. Nutr. Cycl. Agroecosystems **110**: 427–449. doi:10.1007/s10705-018-9907-9.

Withers, P.J.A. 2019. Closing the phosphorus cycle. Nat. Sustain. **2**: 1001–1002. doi:10.1038/s41893-019-0428-6.

Wollmann, I., Gauro, A., Müller, T., and Möller, K. 2018. Phosphorus bioavailability of sewage sludge-based recycled fertilizers. J. Plant Nutr. Soil Sci. **181**: 158–166. doi:10.1002/jpln.201700111.

5. OVERALL SYNTHESIS

5.1 Summary of Findings and Contributions to Knowledge

Recycling P from waste streams to agricultural land as a fertilizer can help close regional and global P cycles, reducing P losses to the environment as well as reliance on fertilizers sourced from mined P deposits. Although recycling human waste to farmland as fertilizer will not eliminate the current need for mined fertilizers globally or within Canada, it is an important aspect of efficient and responsible use of non-renewable P resources (Chen and Graedel 2016; Withers et al. 2018; Nicksy and Entz 2021). Advances in P recovery technology have led to the development of commercial struvite fertilizers derived from municipal wastewater (e.g., Crystal Green®, Ostara Nutrient Recovery Technologies, Inc.), making participation in the circular economy for nutrients more accessible for farmers. Organic cropping systems may be particularly well-positioned to use recycled P sources due to the current lack of suitable P sources to address soil P deficits, along with organic farmers' access to premium prices to help offset the currently higher cost of recycled fertilizers relative to conventional fertilizers (Möller et al. 2018; Nicksy and Entz 2021). However, regulatory barriers need to be removed for fertilizers derived from human waste to be permitted in organic production.

Optimal management of any P amendment, including struvite, in organic systems requires thorough understanding of effects not only on crop productivity but also on environmental and soil health. For novel fertilizers such as struvite, which behave differently from soluble fertilizers and recalcitrant P sources such as phosphate rock, an in-depth understanding of soil–fertilizer–crop dynamics is needed, including not only how struvite compares to other common fertilizers but also its unique properties and behaviour and the associated challenges and opportunities. While research to date on struvite fertilizer generally indicates it is a promising P fertilizer (Huygens and Saveyn 2018; Hertzberger et al. 2020), interactions among soil types, crop types, and struvite properties (e.g., Talboys et al. 2016; Degryse et al. 2017; do Nascimento et al. 2018) demonstrate the need to clarify the P dynamics associated with use of struvite. It is particularly important to devote attention to optimization of struvite use, evaluating a suite of outcomes beyond crop growth and P uptake. Accordingly, the purpose of this research was to investigate the potential of struvite as a P source for organically managed field crops in Manitoba, considering effects on crop productivity, labile soil P pools, and effects on crop root colonization with arbuscular mycorrhizal fungi (AMF), using a combination of field experiments (Chapter 2), a soil incubation (Chapter 3), and a plant growth study under controlled conditions (Chapter 4).

The field study presented in Chapter 2 is among only a handful of field studies conducted using struvite in North America, with even fewer conducted under organic management conditions. Examining the effect of commercial granular struvite application rates on three crop types, this study demonstrated the efficacy of struvite as a P source for spring wheat and alfalfa–grass forage (but not flax), even under alkaline soil conditions where granular struvite solubility was expected to be low. In contrast, alfalfa did not respond to either struvite or MAP in the alkaline Dencross soil from the field study site in the pot experiment (Chapter 4). However, the relatively high Olsen-P concentration remaining at the end of the pot study demonstrates the potential of struvite to support additional seasons of plant growth, though perhaps no differently than MAP.

The promising findings from the field study were apparently supported in an unexpected way by the results from the incubation of alkaline and neutral-pH soils (Chapter 3) demonstrating labile P concentrations that were often as high or higher in soils amended with struvite compared to those amended with MAP, especially in the first 28 d after application. Struvite and MAP also increased Olsen-P to similar concentrations at the end of the pot experiment (Chapter 4). However, the presence of relatively intact struvite granules in soil in the early sampling dates of the incubation suggests that sample preparation and extraction procedures increased the extractability of the P in undissolved struvite. Whether the extractable P concentrations accurately reflect P bioavailability and risk of loss in runoff is the present study is unknown, but evidence from other studies (Everaert et al. 2017, 2018) suggests that extractions of labile P from struvite-amended soils overestimate the risk of P loss in runoff shortly after fertilizer application. Thus, the extractable P concentrations in the present incubation experiment may reflect the artificially elevated solubility of struvite in extractions conducted on finely ground samples, misrepresenting its solubility in soil.

Nonetheless, dissolution of struvite appeared to be slower than that of MAP, as P concentrations at the fourth day of incubation suggested that MAP had already fully dissolved and undergone considerable transformation to non-labile forms whereas struvite granules were still largely intact. The lower plant P uptake of alfalfa at the first cut of the pot experiment also suggests that P supply was slightly restricted in the initial plant growth period. In the incubation, transformation of fertilizer P to less labile soil pools over time appeared to follow similar general patterns for the two fertilizers but proceeded at a slower pace and stabilized at a lower Olsen-P concentration for struvite than for MAP. However, differences in Olsen-P between soils amended with struvite and MAP were not significant at the lower application rate in the incubation (equal to the highest rate in the pot experiment), which may explain the similar response of alfalfa and soil P pools to the two fertilizers when assessed over the entire plant growth period of the pot experiment. Overall, fewer significant effects of fertilizer type were found in the pot experiment than the incubation, probably because sampling occurred only at the end of the pot experiment; in the incubation, most differences between fertilizers occurred early in the incubation period.

Soil properties influenced the labile P pools in the incubation, as well as the P pools, alfalfa growth and P uptake, and root colonization by AMF in the pot experiment, often in interaction with other factors. The effects of soil type on labile P pools were very similar in the incubation and the pot experiment. Olsen-P tended to be greater in the Dencross soil than the Wampum soil in both experiments. The small but significant differences in WEP between the two soils were a function of fertilizer application rate in both studies, with the coarse-textured Wampum soil showing greater changes in WEP in response to P application rate than the fine-textured Dencross soil, possibly reflecting differences in P retention capacity. Soil properties also modulated the effect of struvite or MAP application on the relationship between WEP and Olsen-P pools in the incubation. These results reinforce the need to investigate the P dynamics associated with struvite in a wide range of soils.

In all experiments, the P application rate produced significant effects, often in interaction with other factors. The positive linear relationships observed between P application rate and crop productivity for spring wheat and alfalfa–grass forage in the field study demonstrated the need for application rates greater than the annual P recommendations to attain high crop yields. Nevertheless, the 3-yr alfalfa-grass experiment showed that the residual effects of struvite lasted at least 2 yr past the year of application, indicating that a single high-rate application of struvite can be an effective multi-year P source. The incubation experiment revealed potential negative effects of very high rates of struvite application, however, with elevated concentrations of WEP at early incubation dates. In the pot experiment, the rate of P application required to elicit a plant response in the pot study also elevated soil Olsen-P at the end of the plant growth period to concentrations that could pose a risk of P losses through runoff. However, applying this high rate of P to alfalfa as struvite had a smaller suppressive effect on AMF root colonization than when it was added as MAP. These results demonstrate the importance of investigating the effect of differing application rates on soil–fertilizer–crop P dynamics, especially when evaluating a suite of outcomes with the goal of determining optimal management practices.

Contrary to expectations, the weak citrate extraction did not appear to provide significant additional insights into struvite behaviour in soil compared to the water and Olsen extractions in the soil incubation or the pot study, as WEP and Olsen-P also followed the pattern predicted for CEP in these finely ground soil samples. However, the citrate extraction may provide valuable information in extractions on samples that are prepared differently and is thus worthy of further investigation.

5.2 Practical Implications of the Research

The results of this research demonstrate the substantial P-supplying potential of struvite fertilizer, even in alkaline soil conditions, but highlight the importance of soil properties and fertilizer application rate in determining the outcome of struvite application. The information provided by this set of studies is useful to farmers and agronomists in their decision-making processes regarding struvite fertilizer management practices, such as the appropriate struvite application rate, to optimize P management considering a range of outcomes. However, caution is required in interpreting results of soil extractions conducted on struvite-amended soils due to uncertainties regarding the relationship between P extractability in tests and actual struvite dissolution in soil and availability to plants under field conditions.

The significant increases in crop yield and P uptake in the year of application for both spring wheat and alfalfa–grass forage under field conditions using large struvite granules indicate that crop P supply is enhanced significantly within the first growing season, but that struvite application rates 2–3 times the recommended P application rate may be needed to maximize yield. The increasing strength of crop response observed in the alfalfa–grass experiment in the second and third years of application suggests that P supply to crops is somewhat delayed, but that P applied as struvite continues to contribute to crop P supply over several growing seasons, perhaps justifying the use of high rates in the initial struvite application. The delayed transformation of labile P (WEP, Olsen-P) to non-extractable forms for struvite relative to MAP, as shown in the incubation, may in fact enhance crop P supply from struvite over time. However, whether the temporal patterns observed with small struvite granules under controlled conditions apply to large granules under realistic field conditions is not yet known.

If dissolution of struvite is indeed delayed relative to soluble fertilizers, this may hardly be a concern in organic cropping systems, where struvite could very well be the more soluble option available if wastewater-derived struvite is added to the list of permitted P sources. In fact, slow initial dissolution of struvite (i.e., within the first few days after application) is a property that may make it particularly well suited to organic cropping systems, based on the overarching principles aligning organic crop production practices with naturally occurring nutrient cycling processes (IFOAM n.d.). However, if high concentrations of labile P due to struvite application increase the risk of P loss, struvite may be less compatible with organic principles than expected. The smaller suppressive effect of struvite on AMF colonization in the pot study, relative to MAP, demonstrates the potential of struvite to provide adequate crop P with reduced impact on soil organisms, though further testing is required. Using large struvite granules will likely reduce the impact of added P on AMF even further.

For organic cropping systems, the yield gains over the unfertilized control observed with the highest struvite application rates in wheat (~35%) and alfalfa-grass forage (~130%, averaged across a 3-yr sequence) in the field study represent economically important benefits for farmers. However, the lack of flax yield response to struvite, with relatively good yields across all treatments in the very low-STP soil of the Libau study site, emphasizes the need to clarify P dynamics for different crop types so that available P amendments can be used strategically in crop sequences to provide the greatest overall benefit. A disproportionate increase in the biomass production of alfalfa relative to grass in the forage mixture where struvite was applied reinforces the importance of P supplementation for the productivity of legumes in organic cropping systems, with ensuing N benefits to other crops in rotation.

The increase in labile soil P pools after struvite application, even in the absence of plants, adds important information in relation to crop P supply as well as the risk of P loss to aquatic ecosystems. Although further clarification is needed regarding the behaviour of undissolved struvite particles in agronomic and environmental soil tests, agronomists and farmers (organic and conventional alike) need to be aware that struvite applied to soil may be more reactive than expected based on its purported low solubility in water. The relatively slow transformation of P from struvite to non-labile forms, as demonstrated in the soil incubation, may provide a benefit to crops but also has the potential to increase the risk of P loss if labile P concentrations continue to be elevated during periods of low crop P demand such as fall and early spring.

The significant interactions between soil type and fertilizer type in the soil incubation and in the AMF root colonization in the pot study have implications for struvite management recommendations in differing soil types. The differences between struvite and MAP were often larger in the alkaline Dencross clay than in the neutral Wampum sandy clay loam, supporting findings from past research (Degryse et al. 2017; Everaert et al. 2017; do Nascimento et al. 2018). Differences between soil types could be expected to be even larger when using the large struvite granules typically used for crop production rather than the small granules used in the incubation and pot study. Thus, the need for high rates of struvite application, as observed in the field study on an alkaline soil, may not apply to the same degree in neutral-pH soils.

5.3 Recommendations for Further Study

The present research demonstrates the potential for struvite as a P source in the alkaline-calcareous soils of Manitoba, providing a foundation and identifying priorities for future studies. In general, areas of research that now deserve greater attention include clarifying the underlying factors and mechanisms that contribute to the soil–fertilizer–crop dynamics associated with struvite and developing management approaches to optimize struvite fertilizer use in cropping systems. While comparisons between struvite and soluble fertilizers continue to be useful, clarifying the behaviour of struvite as a fertilizer in its own right is necessary.

One of the key themes emerging from this research is the need to understand the fate of the P from struvite in soil, not only in terms of the P pools characterized using soil extractions but also the identity, properties, and behaviour of the P compounds that remain or are formed over time after struvite application. The presence of granule remnants in soil after an extended period (>1 yr in the field studies) indicates that at least a portion of the applied P remains in the chemical form of struvite, rather than dissolving rapidly and being retained in soil through adsorption and precipitation as occurs with soluble fertilizers. Further research is required to confirm the identity of P compounds in granule remnants and the surrounding soil and to characterize the solubility and reactivity of these compounds, especially over time spans longer than a single growing season. The resulting knowledge will contribute to more nuanced agronomic recommendations for struvite fertilizer management that account for the multi-year P supply, as well as the potential risk of P loss from slowly dissolving struvite during periods of low or no crop uptake.

A closely related knowledge gap pertains to the extractability of struvite P in standard agronomic and environmental soil tests and implications for the relevance of these tests for struvite-amended soils. Recent research has shown that a portion of the P in struvite is extracted in standard agronomic tests, including the Olsen test, as well as in water (Gu et al. 2021). Preliminary evidence in the present study (very high WEP and Olsen-P concentrations at early soil incubation dates when granules were still largely intact) and in previous research (conflicting results between soil extractions and P losses in simulated rainfall (Everaert et al. 2017, 2018)) suggests that readily dissolved P extracted from struvite-amended soils does not accurately represent the true P dynamics occurring in soil. Additional research is needed to investigate the roles of sample collection, preparation, and extraction techniques in determining how much struvite-P is detected in various tests; this knowledge will guide the choice of procedures in future studies. The next step will be to establish whether struvite-derived P detected in these tests behaves similarly to P extracted from soils amended with other P sources, in terms of crop P supply and risk of loss. If not, alternative sampling or analysis procedures or alternative tests will be needed to provide meaningful guidance on struvite fertilizer management in cropping systems.

The frequent interactions observed in the present study and past research among fertilizer types (struvite compared with soluble fertilizers), P application rates, soil types, and crop types highlight the need for more systematic investigation of the factors and mechanisms underlying these patterns. For example, soil–struvite dynamics in the present research and elsewhere have typically been studied in a single soil or in soils with multiple contrasting properties, making it difficult to determine the roles of specific soil properties in struvite dissolution and transformation processes. Soil pH and P retention capacity appear to be among the most important properties affecting the P dynamics associated with struvite and need to be investigated in greater depth. Similarly, while differences among crop types in their responsiveness to struvite have been noted (Katanda et al. 2016; Talboys et al. 2016; Rech et al. 2019), information is lacking on the specific P acquisition mechanisms involved in plant P uptake from struvite fertilizers. How struvite fertilizers impact P supply dynamics in the interactive relationship between plants and AMF is another important question worthy of further study. Given the demonstrated importance of granule size in struvite dissolution, especially in alkaline soils, such research needs to be conducted using the granule size relevant to the target crop production system. For most grain and forage production systems, this will be relatively large granules (>2 mm diameter).

Integrating knowledge of the P dynamics of struvite with the practical considerations of commercial cropping systems can lead to the development of struvite management guidelines that align with a holistic set of agronomic and environmental goals. Optimizing the use of struvite fertilizer in cropping systems will require additional knowledge of fertilizer management practices such as application rates, placement, and timing of application under field conditions, which have received very little attention to date. Understanding the roles of environmental conditions such as temperature and moisture in interaction with crop- and soil-related processes in struvite dissolution, transformation, and P uptake processes is also necessary. The evidence for multi-year crop P supply in the present field study (Chapter 2) highlights the need to investigate the effects of struvite management practices over periods longer than a single growing season and with a range of crop types and sequences. Such research should consider variables representing a diverse set of outcomes including crop performance, P use efficiency, soil biological health, and the risk of P loss. These considerations are important in all cropping systems but may be particularly relevant in research on organic systems in which such outcomes are explicitly stated in production principles. Regardless of what production system recycled struvite is used in, research to better understand and optimize its use will help to advance the circular economy for nutrients and contribute to sustainable P management.

5.4 References

Chen, M., and Graedel, T.E. 2016. A half-century of global phosphorus flows, stocks, production, consumption, recycling, and environmental impacts. Glob. Environ. Change **36**: 139–152. doi:10.1016/j.gloenvcha.2015.12.005.

Degryse, F., Baird, R., Silva, R., and McLaughlin, M. 2017. Dissolution rate and agronomic effectiveness of struvite fertilizers - effect of soil pH, granulation and base excess. Plant Soil **410**: 139–152. doi:10.1007/s11104-016-2990-2.

Everaert, M., Degryse, F., McLaughlin, M.J., De Vos, D., and Smolders, E. 2017. Agronomic effectiveness of granulated and powdered P-exchanged Mg–Al LDH relative to struvite and MAP. J. Agric. Food Chem. **65**: 6736–6744. doi:10.1021/acs.jafc.7b01031.

Everaert, M., da Silva, R.C., Degryse, F., McLaughlin, M.J., and Smolders, E. 2018. Limited dissolved phosphorus runoff losses from layered double hydroxide and struvite fertilizers in a rainfall simulation study. J. Environ. Qual. **47**: 371–377. doi:10.2134/jeq2017.07.0282.

Gu, C., Zhou, Q., Cusick, R.D., and Margenot, A.J. 2021. Evaluating agronomic soil phosphorus tests for soils amended with struvite. Geoderma **399**: 115093. doi:10.1016/j.geoderma.2021.115093.

Hertzberger, A.J., Cusick, R.D., and Margenot, A.J. 2020. A review and meta-analysis of the agricultural potential of struvite as a phosphorus fertilizer. Soil Sci. Soc. Am. J. **84**: 653–671. doi:10.1002/saj2.20065.

Huygens, D., and Saveyn, H.G.M. 2018. Agronomic efficiency of selected phosphorus fertilisers derived from secondary raw materials for European agriculture. A meta-analysis. Agron. Sustain. Dev. **38**: 52. doi:10.1007/s13593-018-0527-1.

IFOAM n.d. The four principles of organic agriculture. [Online] Available: https://www.ifoam.bio/en/organic-landmarks/principles-organic-agriculture [2022 Mar. 28].

Katanda, Y., Zvomuya, F., Flaten, D., and Cicek, N. 2016. Hog-manure-recovered struvite: Effects on canola and wheat biomass yield and phosphorus use efficiencies. Soil Sci. Soc. Am. J. **80**: 135–146. doi:10.2136/sssaj2015.07.0280.

Möller, K., Oberson, A., Bünemann, E.K., Cooper, J., Friedel, J.K., Glæsner, N., Hörtenhuber, S., Løes, A.-K., Mäder, P., Meyer, G., Müller, T., Symanczik, S., Weissengruber, L., Wollmann, I., and Magid, J. 2018. Improved phosphorus recycling in organic farming: Navigating between constraints. Adv. Agron. **147**: 159–237. doi:10.1016/bs.agron.2017.10.004.

do Nascimento, C.A.C., Pagliari, P.H., Faria, L. de A., and Vitti, G.C. 2018. Phosphorus mobility and behavior in soils treated with calcium, ammonium, and magnesium phosphates. Soil Sci. Soc. Am. J. **82**: 622. doi:10.2136/sssaj2017.06.0211.

Nicksy, J., and Entz, M.H. 2021. Recycled nutrients as a phosphorus source for Canadian organic agriculture: a perspective. Can. J. Soil Sci. **101**: 571–580. doi:10.1139/cjss-2021-0014.

Rech, I., Withers, P.J.A., Jones, D.L., and Pavinato, P.S. 2019. Solubility, diffusion and crop uptake of phosphorus in three different struvites. Sustainability **11**: 134. doi:10.3390/su11010134.

Talboys, P.J., Heppell, J., Roose, T., Healey, J.R., Jones, D.L., and Withers, P.J.A. 2016. Struvite: a slow-release fertiliser for sustainable phosphorus management? Plant Soil **401**: 109–123. doi:10.1007/s11104-015-2747-3.

Withers, P., Doody, D., and Sylvester-Bradley, R. 2018. Achieving sustainable phosphorus use in food systems through circularisation. Sustainability **10**: 1804. doi:10.3390/su10061804.

APPENDICES

I. Labile Soil Phosphorus Pools and Mycorrhizal Colonization in Field Experiments

Soil P concentrations and plant root colonization with arbuscular mycorrhizal fungi (AMF) were assessed in selected years of the field study reported in Chapter 2. This study evaluated the effects of P application rate as struvite on plant and soil variables in spring wheat, flax, and alfalfa-grass forage. Spring wheat and flax experiments were repeated over three years (2017–2019). In the alfalfa-grass experiment, struvite was applied to an existing forage stand in May 2017 and monitored over three years (2017–2019). Soil and root sampling in the wheat and flax experiments occurred about 8 wk after seeding and struvite application. In contrast, sampling in the alfalfa-grass forage in 2018 and 2019 occurred approximately 14 and 26 months after struvite was applied in 2017.

Methods

Soil samples were collected from all experiments in 2018 and 2019 for analysis of labile P pools. Samples were collected from all crops at the flowering stage, on July 16, 2018 and July 16, 2019 for wheat and flax, and June 28, 2018 and July 2, 2019 for alfalfa-grass forage. In each plot, 9 cores were collected to a depth of 15 cm and mixed thoroughly to create a composite sample. Soils were air-dried and sieved to 2 mm and stored prior to analysis. The concentration of P in water-extractable (WEP), Olsen-P, and citrate-extractable (CEP) pools was determined using the same methods as in the soil incubation study (Chapter 3) and the alfalfa pot study (Chapter 4), except that soil samples from the field study were not finely ground prior to extraction.

On the same dates in 2018 only, plant roots were collected for determination of colonization by AMF. In wheat and flax, groups of three plants were excavated to a depth of 15 cm from three places in each plot, creating three subsamples per plot. In alfalfa, three plants from each plot were excavated to 15 cm and retained as three subsamples per plot. Soil was washed from roots by hand, and fine roots (<~3 mm diameter) were stored in 70% (v/v) ethanol at ~4 °C. Roots were cleared and stained using the ink and vinegar method (Vierheilig et al. 1998), as in Chapter 4. Root samples were mounted in glycerol on microscope slides and the proportion of root length at which fungal arbuscules were present was assessed using the magnified intersections method (McGonigle et al. 1990). Subsamples from each plot were prepared and assessed separately, but the resulting data was summed to obtain a single value for each plot.

An analysis of variance was conducted using PROC GLIMMIX of SAS® software for each soil P pool and for AMF colonization to determine the effect of P application rate. For soil P concentrations, P application rate was a fixed effect and experiment year was treated as a random effect for the wheat and flax experiments and thus the effects of year are not reported for these crops. P application rate, year of sampling, and their interaction were treated as fixed effects for the alfalfa-grass forage experiment. Root colonization by AMF was analyzed as a binomial distribution, with resulting means and SEM back-transformed to the original scale using the ILINK function of the LSMEANS statement. Post-hoc means comparison was conducted using Tukey's test. All effects were considered significant at $p < 0.05$.

Results

Soil P concentrations in the wheat and flax experiments were very low and were unaffected by the rate of P application in the two years of each experiment (Tables I-1, I-2). In the alfalfa-grass forage, a significant effect of P application rate was found for Olsen-P and CEP, but not for WEP (Table I-3), with the highest P concentrations observed at the highest application rate. Year did not have a significant effect on soil P concentrations in the alfalfa-grass forage experiment.

Root colonization by AMF was affected by P application rate in wheat (Table I-1), with evidence of AMF suppression at the highest application rate. Root colonization of flax and alfalfa was unaffected by P application rate (Tables I-2, I-3).

Table I-1 Effect of P application rate as struvite on the concentrations of water-extractable P (WEP), Olsen-P, and citrate-extractable P (CEP), averaged across two experimental years (2018 and 2019), and root colonization by arbuscular mycorrhizal fungi (AMF) in spring wheat in 2018 only

P application rate	**WEP**	**Olsen-P** [a]	**CEP**	**AMF**
	mg kg^{-1}			%
0 kg P ha^{-1}	0.90 (0.29) [b]	3.72 (0.21)	1.39 (0.50)	9.74 (1.40) ab [c]
20 kg P ha^{-1}	1.16 (0.29)	3.87 (0.24)	1.89 (0.50)	14.0 (1.90) a
30 kg P ha^{-1}	0.96 (0.29)	4.78 (0.76)	2.86 (0.50)	12.2 (1.64) ab
40 kg P ha^{-1}	1.18 (0.29)	5.28 (0.91)	2.64 (0.50)	8.90 (1.32) b
p-value	0.48	0.21	0.17	0.02

[a] Olsen-P did not meet the assumptions of normal distribution of residuals, even with log-transformation (W=0.85)

[b] Values are means with SEM in parentheses.

[c] Means within columns followed by the same letter are not significantly different according to Tukey's test ($p < 0.05$).

Table I-2. Effect of P application rate as struvite on the concentrations of water-extractable P (WEP), Olsen-P, and citrate-extractable P (CEP), averaged across two experimental years (2018 and 2019), and root colonization by arbuscular mycorrhizal fungi (AMF) in flax in 2018 only

P application rate	**WEP**	**Olsen-P**	**CEP**	**AMF**
	mg kg^{-1}			%
0 kg P ha^{-1}	1.13 (0.17)	3.71 (0.35)	1.74 (0.87)	50.4 (2.2)
20 kg P ha^{-1}	1.21 (0.17)	3.77 (0.36)	1.83 (0.87)	50.0 (2.2)
30 kg P ha^{-1}	1.09 (0.17)	3.67 (0.35)	1.14 (0.87)	51.1 (2.2)
40 kg P ha^{-1}	1.33 (0.17)	4.40 (0.42)	2.14 (0.87)	47.0 (2.2)
p-value	0.76	0.42	0.46	0.17

[a] Values are means with SEM in parentheses.

Table I-3. Effects of P application rate and year of sampling on the concentrations of water-extractable P (WEP), Olsen-P, and citrate-extractable P (CEP) measured in 2018 and 2019, and root colonization by arbuscular mycorrhizal fungi (AMF) measured in 2018 only, in alfalfa-grass forage fertilized with struvite in 2017

Factor	**WEP**	**Olsen-P**	**CEP**	**AMF**
	mg kg^{-1}			%
P application rate				
0 kg P ha^{-1}	1.28 (0.30) [a]	3.67 (0.57) b [b]	3.62 (0.87) b	22.5 (2.5)
30 kg P ha^{-1}	1.50 (0.30)	5.88 (0.91) b	6.06 (1.18) ab	21.0 (2.5)
60 kg P ha^{-1}	1.75 (0.30)	6.12 (0.94) b	5.69 (0.92) b	24.6 (2.7)
90 kg P ha^{-1}	2.26 (0.30)	10.8 (1.66) a	12.99 (2.54) a	19.0 (2.3)
Year				
2018	1.43 (0.24)	5.66 (0.66)	6.26 (0.89)	-
2019	1.96 (0.24)	6.59 (0.59	6.30 (0.89)	-
ANOVA	*p*-values			
Rate	0.08	0.0003	0.006	0.11
Year	0.052	0.30	0.98	-
Rate × Year	0.80	0.93	0.73	-

[a] Values are means with SEM in parentheses.

[b] Means within columns and factors followed by the same letter are not significantly different according to Tukey's test ($p < 0.05$).

References

McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., and Swan, J.A. 1990. A new method which gives an objective measure of colonization of roots by vesicular—arbuscular mycorrhizal fungi. New Phytol. **115**: 495–501. doi:10.1111/j.1469-8137.1990.tb00476.x.

Vierheilig, H., Coughlan, A.P., Wyss, U., and Piché, Y. 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. Appl. Environ. Microbiol. **64**: 5004–5007.

II. Final P Concentrations and Recovery in Incubated Soil

Extractable phosphorus (P) concentrations and recovery data from the final date of the soil incubation study reported in Chapter 3 were analyzed separately to allow for more meaningful comparisons to the companion pot experiment using the same soils and fertilizers (Chapter 4). The objective of this separate analysis was to assess the effect of soil type (Dencross clay with pH 8.1; Wampum sandy clay loam with pH 7.0), fertilizer type (granular struvite; granular monoammonium phosphate (MAP)), and fertilizer application rate (100 or 200 mg P kg^{-1}), in a factorial-plus-control design with a nil-P control, on the final extractable P concentrations and recovery after 112 d of incubation.

Methods

Soils were amended with fertilizers and incubated for 112 d, and the concentrations and percent fertilizer-P recovered were determined for water-extractable (WEP), bicarbonate-extractable (Olsen-P), and citrate-extractable P (CEP) as described in Chapter 3. An analysis of variance (ANOVA) was conducted for each variable using PROC GLIMMIX of SAS software, with soil type, fertilizer type, and fertilizer application rate as fixed effects. Extractable P concentration data were analyzed as Gaussian distributions and P recovery data as beta distributions. Effects were considered significant at $p < 0.05$.

Results

The final P concentrations and proportion of fertilizer-P extracted were influenced by interactions among soil type, fertilizer type, and application rate, in various combinations (Table II-1), with patterns sometimes differing from the data from all sampling dates due to the strong influence of high P concentrations in the early sampling dates on overall means.

Table II-1 Effect of soil type, fertilizer type, and fertilizer application rate on the concentrations and percent fertilizer-P recovery of water-extractable P (WEP), Olsen-P, and citrate-extractable P (CEP) in soil samples after 112 d of incubation

	WEP		Olsen-P		CEP	
Factor	**Conc.**	**Recov.**	**Conc.**	**Recov.**	**Conc.**	**Recov.**
	mg kg^{-1}	%	mg kg^{-1}	%	mg kg^{-1}	%
Soil						
Dencross	4.9 (0.2) [a]	3.8 (0.3)	29.3 (0.7)	28.3 (1.0)	20.1 (1.5)	18.8 (1.0) b [b]
Wampum	4.6 (0.2)	4.3 (0.3)	25.4 (0.7)	20.5 (0.9)	23.5 (1.5)	24.4 (1.1) a
Fertilizer						
Control	1.3 (0.1)	-	8.5 (0.2)	-	4.7 (1.9)	-
Struvite	7.2 (0.5)	3.4 (0.3)	38.6 (0.8)	19.8 (0.9)	34.3 (1.4)	18.9 (1.0) b
MAP	9.2 (0.5)	4.8 (0.3)	53.8 (1.6)	29.2 (1.0)	43.5 (1.3)	24.2 (1.1) a
Rate						
0	1.3 (0.1)	-	8.5 (0.2)	-	4.7 (1.9)	-
100	4.9 (0.3)	3.4 (0.3)	32.2 (1.4)	22.9 (0.9)	23.9 (1.4)	18.8 (1.0) b
200	11.5 (0.6)	4.9 (0.5)	60.3 (1.1)	25.6 (0.9)	53.9 (1.3)	24.4 (1.1) a
ANOVA	*p*-values					
Soil (S)	0.94	0.29	0.0002	<0.0001	0.005	0.001
Fertilizer (F)	0.01	0.002	<0.0001	<0.0001	<0.0001	0.002
Rate (R)	<0.0001	0.001	<0.0001	0.053	<0.0001	0.001
S x F	0.002	0.0002	0.002	0.004	0.18	0.10
S x R	0.002	0.002	0.87	0.06	0.005	0.35
F x R	0.91	0.63	0.01	0.97	0.006	0.31
S x F x R	0.21	0.59	0.97	0.31	0.50	0.43

[a] Values are means with standard error of the mean in parentheses.

[b] Means within columns and factors followed by the same letter are not significantly different, where main effects are not superseded by a significant interaction. Significant interactions are plotted in Figures II-1 to II-5.

Both the concentration and recovery of WEP and Olsen-P were affected by interactions between soil types and fertilizer types (Figures II-1 and II-2). Concentrations of WEP and Olsen-P were greater for MAP than for struvite in the Dencross soil but the fertilizers did not differ in the Wampum soil. Though the fertilized treatments followed expected patterns of WEP and Olsen-P increases in the two soil types, based on soil properties contributing to P retention capacity, the unfertilized controls exhibited the opposite pattern, with the unfertilized Dencross soil having greater WEP and lower Olsen-P than the unfertilized Wampum soil at the end of the experiment (Figures II-1a and II-2a).

All other significant interactive effects on final P concentrations involved fertilizer application rate. Final WEP concentration and recovery followed the same pattern as in the means across all sampling dates with a disproportionate increase in the Wampum soil at the high application rate (Figure II-3). For final Olsen-P and CEP concentrations, interactive effects involving application rate were a result of approximately proportional increases at the higher rate relative to the low rate, as expected (Figures II-4 and II-5).

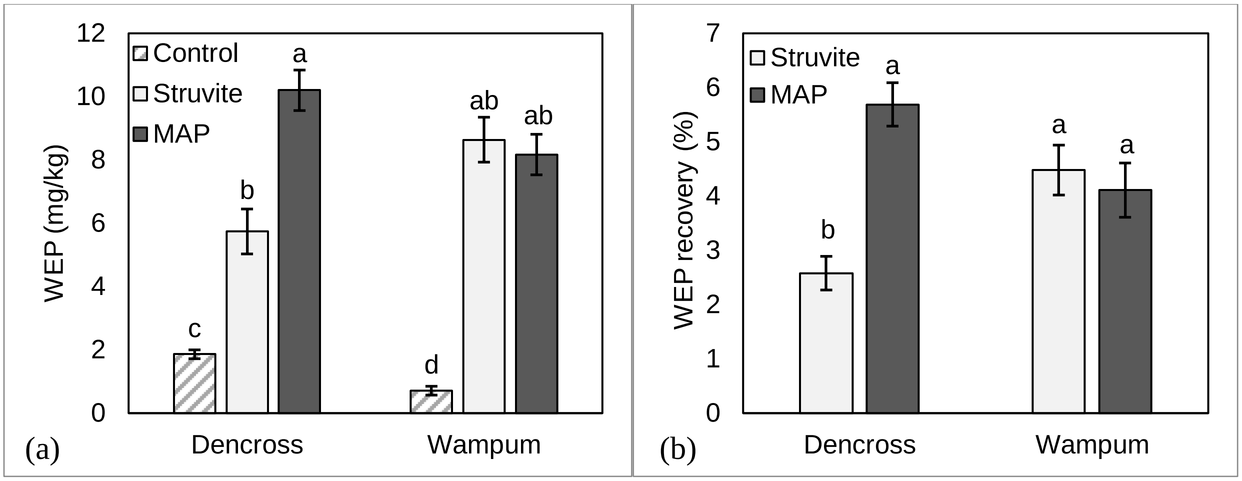

Figure II-1. Interactive effect of soil type and fertilizer type on final WEP concentration (a) and percent fertilizer-P recovery (b) in soils incubated 112 d without plants. Bar height and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across soil and fertilizer treatments within each panel according to Tukey's test.

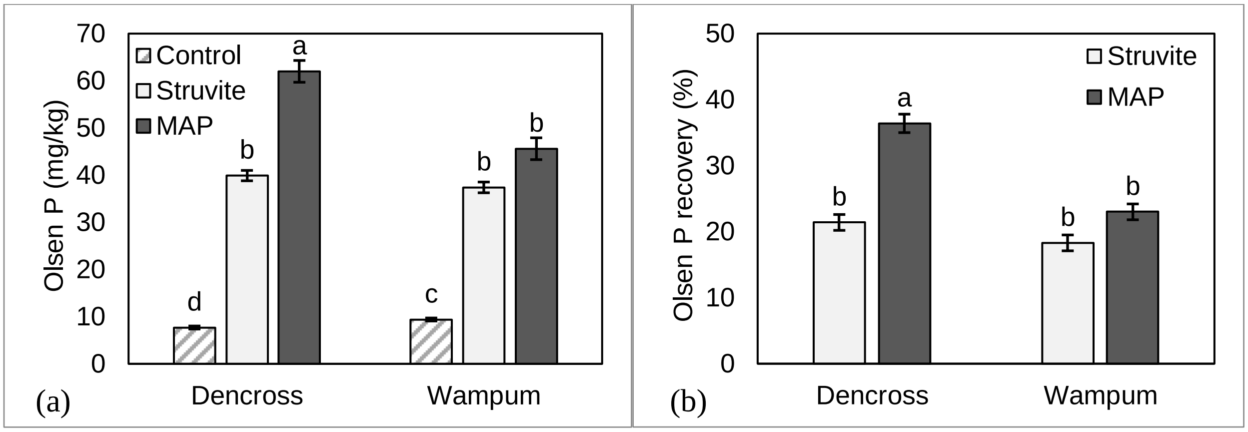

Figure II-2. Interactive effect of soil type and fertilizer type on final Olsen-P concentration (a) and percent fertilizer-P recovery (b) in soils incubated 112 d without plants. Bar height and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across soil and fertilizer treatments within each panel according to Tukey's test.

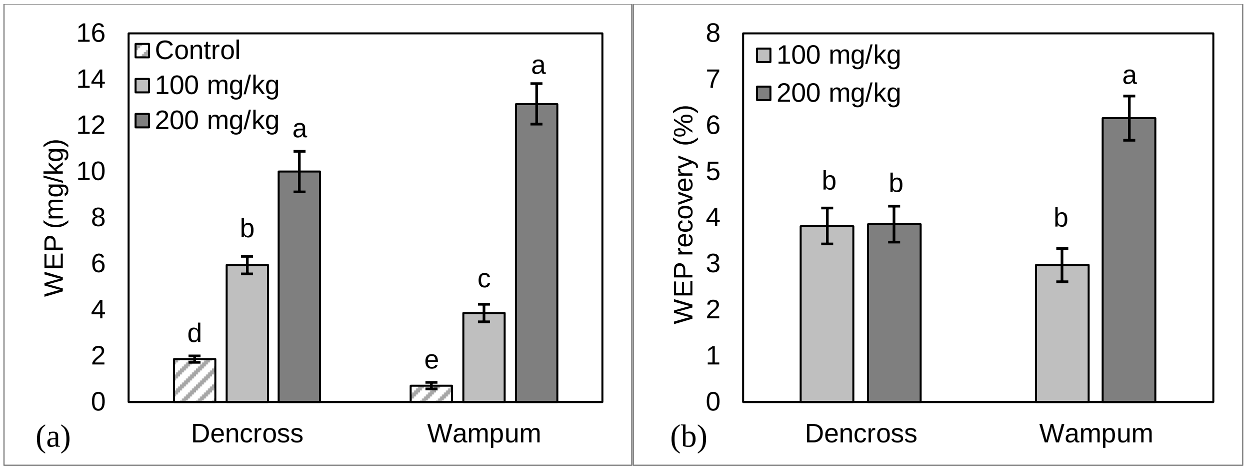

Figure II-3. Interactive effect of soil type and fertilizer application rate on final WEP concentration (a) and percent fertilizer-P recovery (b) in soils incubated 112 d without plants. Bar height and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across soil and fertilizer treatments within each panel according to Tukey's test.

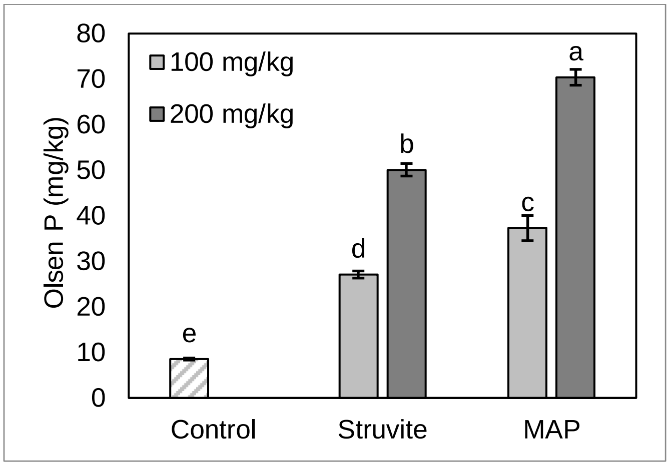

Figure II-4. Interactive effect of fertilizer type and application rate on final Olsen-P concentration in soils incubated 112 d without plants. Bar height and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across soil and fertilizer treatments within each panel according to Tukey's test.

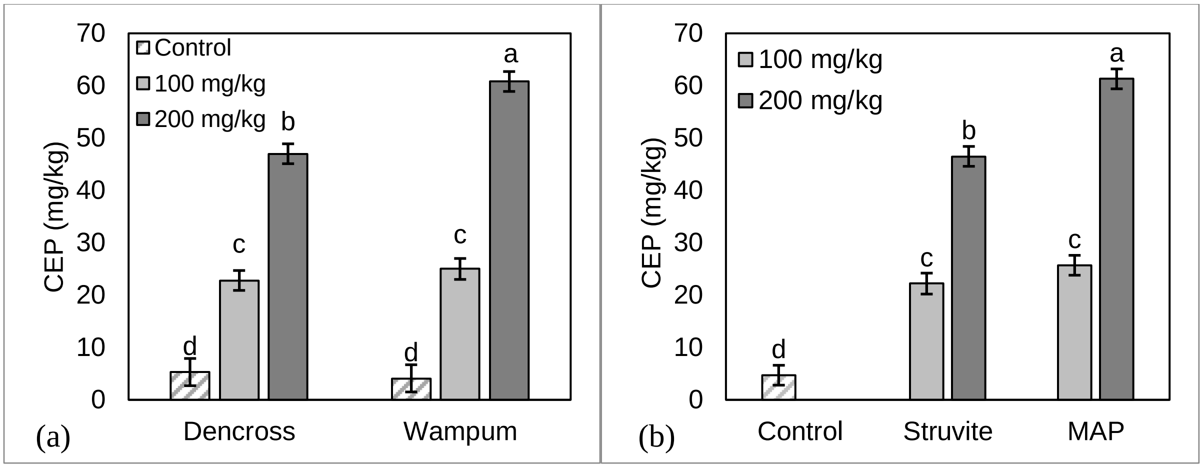

Figure II-5. Interactive effects of soil type and fertilizer application rate (a) and of fertilizer type and application rate (b) and percent fertilizer-P recovery (b) on final CEP concentration in soils incubated 112 d without plants. Bar height and error bars represent the mean and standard error of the mean, respectively. Lower-case letters indicate mean groupings across soil and fertilizer treatments within each panel according to Tukey's test.

III. Effect of Supplemental Nitrogen on Alfalfa under Controlled Conditions

In the alfalfa pot study reported in Chapter 3, no supplemental nitrogen (N) was added to soils as alfalfa was expected to be N-sufficient due to biological N fixation. An additional treatment with supplemental N added to the lowest struvite application rate (62 mg P pot^{-1}) in both soil types was included alongside the main experiment to help determine whether plants were limited by N.

Supplemental N was added as commercial urea–ammonium nitrate (UAN) dissolved in irrigation water. The total rate of supplemental N was 156 mg N pot^{-1}, equivalent to 84 kg ha^{-1}, bringing the total N applied including N in struvite to the equivalent of 100 kg ha^{-1}. Nitrogen was applied in three equal portions over the duration of the 130-d pot experiment—during pot study set-up, after Cut 1, and after Cut 2.

Addition of supplemental N did not affect alfalfa shoot biomass, tissue P or N concentration, shoot P or N uptake, or P recovery efficiency in biomass compared to the treatment with the same struvite application rate and no N added (Table III-1).

Table III-1 Effect of N addition and soil type on alfalfa shoot biomass, P and N concentration, P and N uptake, and P recovery efficiency after 130 d of growth in the supplemental experiment

Factor	Shoot biomass	Tissue P conc.	Shoot P uptake	P recov. efficiency	Tissue N conc.	Shoot N uptake
	g pot^{-1}	mg g^{-1}	mg pot^{-1}	%	mg g^{-1}	mg pot^{-1}
Fertilizer						
Struvite	9.36 (0.54) [a]	2.64 (0.04)	24.8 (1.2)	8.7 (1.9)	39.6 (0.32)	370 (19)
Struvite+N	10.7 (0.58)	2.65 (0.04)	28.5 (1.8)	14.7 (2.9)	39.6 (0.35)	423 (21)
Soil						
Dencross	9.74 (0.58)	2.66 (0.04)	26.0 (1.7)	11.1 (2.7)	40.1 (0.35)	390 (21)
Wampum	10.4 (0.54)	2.63 (0.04)	27.3 (1.3)	12.2 (2.2)	39.1 (0.32)	403 (19)
ANOVA			*p*-value			
Fertilizer (F)	0.11	0.84	0.12	0.12	0.99	0.08
Soil (S)	0.45	0.59	0.56	0.77	0.06	0.65
F × S	0.12	0.26	0.09	0.09	0.25	0.13

[a] Values are least-squares means with SEM in parentheses.