

Potential use of reclaimed struvite as a phosphorus fertilizer source and effects of low molecular weight organic acids (LMWOAs) in the rhizosphere on its performance in three contrasting soils

by

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Abstract

Phosphorus (P) is a crucial macronutrient for crop growth, often sourced from rock phosphate-based fertilizers. Closing the P cycle through recycling and reusing is vital. Struvite, a reclaimed P product from wastewater treatment plants, offers promise as an alternative P fertilizer, promoting sustainability and reducing reliance on nonrenewable resources. The role of low molecular weight organic acids (LMWOAs) exuded in the rhizosphere is recognized as potentially aiding struvite dissolution in crop fields. This thesis research was conducted to address the knowledge gap regarding struvite's effectiveness as a phosphorus fertilizer across diverse soil pH, especially when compared to conventional phosphorus fertilizers, and to examine how adding low molecular weight organic acids influences struvite. The objectives of the first study were to investigate dissolution, P mobility, and potential plant availability of reclaimed struvite in comparison to conventional P fertilizers: monoammonium phosphate (MAP), diammonium phosphate (DAP), and ammonium polyphosphate (APP) in three different soils (mildly calcareous, acidic, and neutral) using short-term laboratory incubation studies combined with wet chemical analysis and synchrotron-based X-ray techniques. Soils were incubated for 1, 2, 5, 9, and 12 weeks in Petri dishes with four fertilizer treatments, reclaimed struvite (Str), MAP, DAP, APP, and a control. The objectives of the second study were to assess the impact of LMWOAs on struvite P dissolution, mobility, and potential plant bioavailability and the fate of LMWOAs in the same three soils using short-term laboratory incubation studies combined with wet chemical analysis and synchrotron-based X-ray techniques. Soils were incubated with three different soils for 1, 5, 9, and 12 weeks in Petri dishes with struvite (Str), with and without LMWOAs addition with a control. The citric acid (CA), oxalic acid (OA), malic acid (MA), and acetic acid (AA) were used as LMWOAs and were added as single organic

acids at two rates (41mM-Low and 82mM-High) or as an acid mixture (CA + OA or CA + OA + MA + AA) at one rate (82mM- High). At the end of the incubation, dishes were sectioned into three concentric rings at different distances from the point of application (POA). The pH, resin extractable P to assess potential plant available P, and total P to determine P diffusion from the POA were analyzed. Percent P added (PPA) and Percent resin P (PRP) values were calculated based on total P and resin extractable P. To determine the reaction products of P X-ray absorption near edge structure (XANES) analysis was performed. Based on the results of the first study, reclaimed struvite showed comparative performance to conventional fertilizers after 9 weeks of incubation in neutral and acidic soils, but not in mildly calcareous soils. The formation of insoluble secondary P minerals via precipitation and sorption into CaCO_3 were the culprits of lower P mobility and availability in P fertilizers in calcareous soils. Modification or blending of struvite with conventional P fertilizers may enhance struvite performance under calcareous soil conditions. The results of the second study showed that in general, the efficacy of struvite in terms of mobility, and/or solubility was enhanced in the presence of LMWOAs in neutral and acid soils, while most of the LMWOAs added into mildly calcareous soil were degraded or sorbed. Compared to struvite alone (Str) treatment (20 and 40%), Str + CA + OA + MA + AA High treatment (40% and 50%) showed superiority in P diffusion into further sections, respectively, in neutral and acid soils after 12 weeks. Phosphorus availability (PRP per Petri dish) in neutral (39% and 44% of PRP) and acid soils (66 and 78% of PRP) after 12 weeks of incubation in struvite alone (Str) and Str + CA + OA + MA + AA High treatments, respectively. This is likely because the mixture of ligands is more successful in chelation and ligand exchange reactions. Moreover, relative to struvite alone (Str) treatment, P availability of Str + CA High treatment was higher in acid soil (PRP per dish 66% vs 71%, respectively), and Str + OA High

treatment showed higher P availability in neutral soil (PRP per dish 40% vs 57%, respectively) after 12 weeks of incubation. This is likely because CA promotes P release through the chelation of Al and Fe in acid soils while OA promotes P release in less acidic/ neutral soils due to the high acidic strength of oxalic acid. Future studies with plants will give better insights into how these treatments impact crops.

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Dedication

To my loving husband, parents, and sister!

Your unwavering love and support have been my guiding light throughout this journey!



Chapter 1 - Introduction

Phosphorus (P) is vital for all living organisms (Shim et al., 2020). In terms of crop production, P is one of the growth-limiting nutrients and it is second only to nitrogen (N) as mineral nutrients (Petriman et al., 2010). Even though most soil consists of significant reserves of total P content, the bioavailable fraction is limited in proportion to total P (Stevenson & Cole M.A., 1999). This low plant availability is due to insoluble forms of P. Phosphorus needs to be in soluble forms to be bioavailable. However, the monobasic (H_2PO_4^-) and dibasic (HPO_4^{2-}) ions are dominant orthophosphate species under usual soil pH conditions (Konesky et al., 1989). So, the producers are applying the required P primarily as soluble P fertilizers. Globally, 79% of rock phosphate is used to make fertilizers, 11% for feed additives, and 7% for detergents. Rock phosphate is a nonrenewable source, and natural P reserves are located mainly in Morocco, China, the United States, and South Africa, which fulfill 80% of world P demand. The demand for P is increasing by 1.5% each year, and the natural P reserves are estimated to be depleted in the next 90 years (Liu et al., 2013). Since the mineral fertilizers are highly soluble and provide excess phosphate ions into the soil solution, there is a risk of P losses into the waterbodies either by erosion or surface runoff. The excessive nutrients (primarily N and P) in the water can cause eutrophication (Kataki et al., 2016).

To overcome these issues, the requirement of sustainable and renewable P sources is more promising as a better, long-lasting solution. Struvite has been identified as an environmentally friendly, recycled/ reclaimed P fertilizer (Liu et al., 2013). The struvite precipitation and crystallization from wastewater are popular in the fertilizer industry. Struvite is a crystal of magnesium ammonium phosphate, which can be used as a good source of P. The theoretical fertilizer value of struvite varies based on the source and recovery process. Usually, it is 12.5%

P, 5.7% N, and 9.9% Mg (Ahmed et al., 2018). Less heavy metal contamination and slow P release into the soil solution are potential benefits of struvite as a P fertilizer. The slow-releasing behavior of struvite is especially advantageous in grasslands, forests, and coastal agricultural lands where highly soluble fertilizers are undesirable (Liu et al., 2013). Several plants showed the ability to grow under the struvite application such as broad bean, lettuce, ryegrass, tomato, soybean, and maize (Diwani et al., 2007; Korchef et al., 2011; Laboski & Boerboom, 2006; Uysal et al., 2014).

However, in high pH soils, the struvite dissolution is challenging and has less ability to provide an adequate amount of available P for plant uptakes. This is because struvite precipitation occurs under alkaline conditions. Compared to conventional P fertilizers, water solubility of struvite is low and more constrained. For example, when monoammonium phosphates are soluble (23.5%) in water struvite's water solubility is 2-3% (Hertzberger et al., 2020). Due to its low water solubility, struvite is unable to fulfill the P demand for the early development stages of some plants. Some studies have identified that plant growth is limited in some plants such as canola, wheat, and potato with struvite application compared to conventional P fertilizers (Ackerman et al., 2013; Talboys et al., 2016). However, under acidic soil conditions, struvite dissolution is greatest (Hertzberger et al., 2020). When struvite solubility is higher soil solution P concentrations are also higher. The soil solution P concentration is controlled by P mineral solubility. Under different soil pH conditions forms of secondary P minerals vary. The most common P minerals in calcareous soils are Ca-P minerals while Al-P and Fe-P minerals are dominant in acidic soil conditions. The solubility of these minerals represents ion concentration in soil solution, and it depends on the solubility product of the mineral (K_{sp}). Overall, struvite solubility is affected by different soil properties such as soil pH, cation exchange capacity, soil

native P concentration, number and type of cations present in soils. Struvite dissolution is favorable under higher in acidic soil pHs, low soil solution P concentrations, soils with high cation exchange capacity, soils with low amounts of divalent (Ca^{2+} , Mg^{2+}) and trivalent (Al^{3+} , Fe^{3+}) cations (Havlin J. & Tisdale S. L., 2014).

Finding ways to make struvite a suitable P fertilizer under different soil conditions is important.

Research has shown that the roots exude organic acids has a great impact on struvite dissolution in soils (Talboys et al., 2016). Rhizosphere acidification due to the presence of organic acid

impacts struvite dissolution. Hence struvite solubility is higher a high P mobility and P

extractability would be observed. Organic acids in the soils are considered organic molecules

that contain one or more carboxylic groups which are a major constituent of root exudates in the rhizosphere and can alter the rhizosphere environment via a series of reactions (Adeleke et al.,

2017). Generally, organic acids can be classified into two groups, those are high molecular

weight organic acids (HMWOAs) and low molecular weight organic acids (LMWOAs). The

molecular weight of HMWOAs ranges from a few hundred to millions of Daltons (DA) while

LMWOAs have low molecular weights ranging from 46 to a few 100 DA (Perminova et al.,

2003). These LMWOAs are more water soluble than HMWOAs and contain one to three

carboxylic acid groups in LMWOAs, while HMWOAs contain more than three carboxylic

groups. Humic and fulvic acids are examples of HMWOAs and citric, oxalic, malic, succinic,

acetic, malonic, and maleic are examples of LMWOAs (Adeleke et al., 2017). The concentration

of organic acids in soil varies based on the soil type and other factors. The estimated organic acid

concentrations in soil solution around the rhizosphere can range from 1 mM to 10 mM while

concentrations in bulk soils are less than 50 μM (Ryan et al., 2014). However, this concentration

can change based on the soil and plant type (Jones et al., 2003). Organic acids play major roles in

mineral mobilization. Organic acids can induce the release of P from stable P pools and mobilize P in sandy loam soils. The reduced pH conditions lead to the release of unavailable minerals in the soil such as P, and Fe (Arcand & Schneider, 2006; Calvaruso et al., 2006). Moreover, among the organic acids, dicarboxylic acid, and tricarboxylic organic acids (malate, citrate, oxalate) are more efficient than monocarboxylic acid in terms of P mobilization (Gang et al., 2012).

Organic acids are effective on P availability until they stay in the soil solution without breaking down for several reasons. In terms of the half-life of organic acids, adsorption and desorption reactions play a key role since organic acid anions are negatively charged, and sorption reactions can occur. The general trend of sorption is decreased in this order: phosphate > oxalate > citrate > malate > sulfate > acetate (Earl et al., 1979; Jones et al., 1996; Jones & Kochian, 1996).

Organic acids are nutrient-rich sources and microbes can utilize those by breaking them down (Adeleke et al., 2017).

However, there is a research gap in the mechanistic understanding of how dissolution, transformation, and availability of struvite differ under different soil conditions compared to commonly used conventional P fertilizers and the efficacy of the co-addition of LMWOAs on struvite dissolution, diffusion, and potential availability under different soil conditions compared to non-LMWOAs added struvite. In this thesis, two studies were conducted to explore the potential use of reclaimed struvite as a P fertilizer source compared to conventional P fertilizers and with the addition of low molecular weight organic acids (LMWOAs).

Research Objectives

1. The objectives of the first study were to investigate reclaimed struvite P dissolution, mobility, and potential plant availability in comparison to conventional P fertilizers in three different soils (mildly calcareous, acid, and neutral) using short-term laboratory

incubation studies combined with wet chemical analysis and synchrotron-based X-ray absorption near-edge structure spectroscopy technique.

2. The objectives of the second study were to investigate the impact of added LMWOAs on struvite P dissolution, mobility, and potential plant availability and the fate of LMWOAs in soils (mildly calcareous, acid, and/or neutral) with contrasting pH using short term laboratory incubation studies combined with wet chemical analysis and synchrotron-based X-ray absorption near-edge structure spectroscopy technique.

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Chapter 2 - Review of Literature

2.1. Soil phosphorus cycle

Phosphorus (P) is one of the major essential macronutrients for all life forms and a key structural and functional component of essential biochemical reactions (Westheimer, 1987). Phosphorus is a significant structural component in cell membranes (phospholipids) and bones. Further, P is a component of genetic materials (DNA, RNA) and energy transfer molecules (ATP). Mostly, P is not directly accessible for organisms since it is locked up in sediment, bedrock, and soil - unavailable P. However, converting these forms into orthophosphates by geochemical and biochemical reactions of the global P cycle makes it available to living forms. Weathering of bedrock dissolves P-bearing minerals -apatites [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH}, \text{F}, \text{Cl})_2$]- increasing the P availability acting as a natural P source for vegetation (Cosgrove, 1977; Frossard E., 2000). Apatites are known as the primary mineral source of P (Thomas & Pierzynki, 2005). Plants uptake solubilized phosphates from soil. Phosphorus is stored in plants as organic P forms and returned to the soil by decomposition (Likens et al., 1981).

Soil P can be divided into three major pools namely labile (available), slowly cycling, and occluded (Richter et al., 2006). The labile P supplies the short-term demand for plants since it is readily available. Slowly cycling P moieties are known as moderately labile P and can easily be transformed into labile P by mineralizing organic matter, desorption by clay and other mineral surfaces and dissolution of metal phosphates. Slowly cycling P is pH dependent and makes Al and Fe complexes at pH 5.0 and pH 3.5 forming respectively and form Al and Fe phosphates. Moreover, phosphates make calcium phosphates at higher pHs. The formation of secondary minerals limits the P availability for plants (Vu et al., 2010). The occluded (stable) P remains in

the soil without being available to the organism for the long term (Johnson et al., 2003; Niederberger et al., 2019).

Phosphorus losses from arable soils by surface runoff and soil erosion can cause eutrophication in freshwater bodies. P concentrations associated with eutrophication are 0.01 - 0.03 mg dissolved P L⁻¹ and 0.035 - 0.10 mg total P L⁻¹ (Correll, 1998). Eutrophication reduces the surface water quality by forming algal blooms (Djodjic et al., 2004).

2.1.1. Phosphorus availability in native soil

Soil solution phosphates or soil native Ps are considerably limited due to phosphorus sorption reactions. Sorption reactions limit P bioavailability to plants. Iron (Fe) and aluminum (Al) hydroxides or oxyhydroxides-rich soils are increasingly prone to P sorption (Niinemets & Kull, 2005). Usually, soil P concentration ranges from 100 and 3000 mg/ kg of soil, mostly stored as orthophosphate compounds (Frossard E., 2000). Soil P concentration in topsoil (0-15 cm) varies from 50-3000 mg kg⁻¹. However, these P concentrations depend upon the parent material, type of soil, and soil management practices such as fertilization, manuring, and cropping system (Foth & Ellis, 1997; Gary et al., 1995). Soil solution P concentration ranges from less than 0.01 mg L⁻¹ in very infertile soil to 1 mg L⁻¹ in well-fertilized soils. In some cases, soils with recent fertilizer or organic by-product addition show a high soil solution P concentration of 7 to 8 mg L⁻¹. For optimal plant growth, 0.2 mg L⁻¹ of soil solution P concentration is required. However, some plants give optimum yields when the concentration is around 0.03 mg L⁻¹ (Sims & Pierzynski, 2018).

2.2. Phosphorus reactions in soils

The sorption reactions, dissolution-precipitation, and mineralization-immobilization are the three major reactions of P in soils (figure 1.1). These reactions are affected by the concentration of soil

solution P, soil solid phases, and the available P amount for plant uptake and microbial utilization (Sims & Pierzynski, 2018).

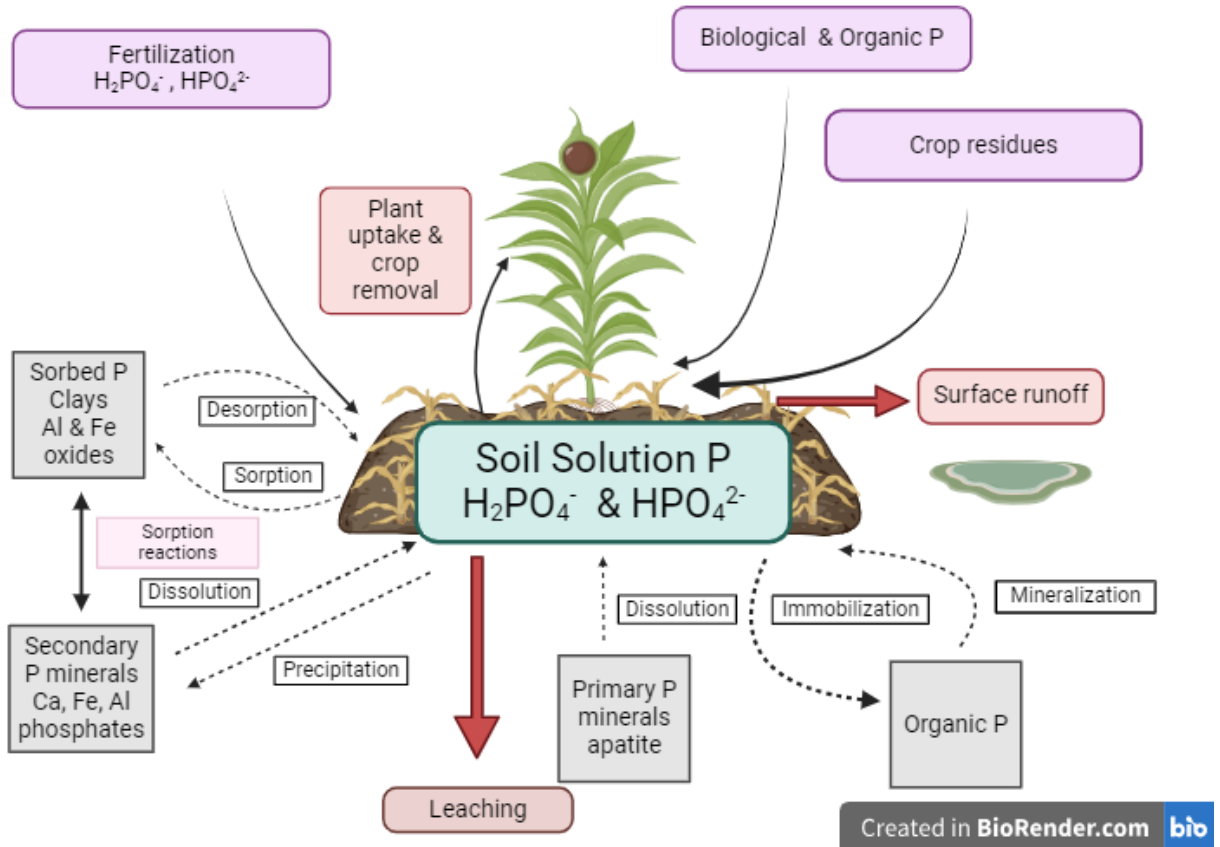


Figure 2.1. The soil P cycle

2.2.1. Sorption reactions

In general, sorption is used as a general term to refer to several chemical processes that result in the retention of some sorbate (eg: orthophosphate) at the sorbent's surface (eg: soil colloids).

Sorption includes several processes such as adsorption, surface precipitation, and polymerization (Sparks, 1995). Moreover, when the exact mechanism of retention of sorbate by sorbent is

unknown the sorption term is frequently used in the case studies of chemical reactions of P with soils. Adsorption is defined as the net accumulation of matter at an interface between a solid phase and an aqueous phase. Adsorption is due to physical and chemical processes. Van der

Waals forces and electrostatic outer sphere complexes (anion exchange) are the main physical processes associated with the nonspecific adsorption of P. Chemical processes are considered specific adsorption/chemisorption due to covalent and H bonding in the inner-sphere ligand exchange reactions. Adsorption is a two-dimensional process. Inner sphere complexes are not reversible in mechanisms since they consist of powerful adsorptive forces compared to ligand exchange reactions (monodentate). Ligand exchange reactions can result in monodentate, bidentate, or binuclear forms of adsorbed P species (Sims & Pierzynski, 2018).

Desorption is the release of adsorbed/sorbed forms from a sorbent into the solution phase.

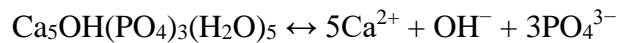
Usually, the P desorption process is less reversible since sorbed materials are tightly bound into the adsorbent or sorbent. This condition is called hysteresis. However, the P sorption process is crucial due to its importance of the availability of native or added P into the plants and P loss into the waterbodies by runoff (Sims & Pierzynski, 2018).

Desorption or P release is usually induced in the presence of organic acids. According to Sims & Pierzynski. (2018) P sorption by soils is inhibited in the presence of organic acids (humic, fulvic, citrate, malate, oxalate), and P concentration in organic amendments will affect P availability since some of P will be released into the soil by mineralization and dissolution reactions.

However, desorption is highly dependent on higher organic acids presence in soil. At least $> 100 \mu\text{M}$ for citrate, and $>1 \text{ mM}$ for oxalate, malate, and tartrate are required to mobilize a considerable amount of P into the soil solution (Earl et al., 1979; Jones & Darrah, 1994a). The P mobilization does not occur in some soils which are known to have a very low P concentration with the addition of organic acids (Jones & Darrah, 1994a).

2.2.2. Dissolution /precipitation and forming secondary minerals

In moderately weathered soils, sparingly soluble apatites $\text{Ca}_{10}\text{X}(\text{PO}_4)_6$ ($\text{X} = \text{OH}^-$, F^- , Cl^- , or carbonates (CO_3^{2-}) and other more soluble calcium phosphates (octacalcium phosphates, monetite, brushite) are dominated in P mineralogy. As soils go through the weathering process, apatites are dissolved and phosphates are released into the soil solution which are then absorbed by plants or lost via leaching.



The Al phosphates (berlinite, variscite, sterretite, tarakanite) and Fe phosphates (strengite, tenticite, vivianite) dominate highly weathered acidic soils. When P is added as organic or inorganic fertilizer into the soil, secondary solid phases are formed by coprecipitation of P with soluble Al, Fe, and Ca in the soil. Those minerals are usually amorphous and have a mixed composition.

Precipitation is a three-dimensional process. The solubility of P minerals highly depends upon pH. Under high pH conditions, P limits solubility by forming calcium phosphate minerals. Comparatively, the low pH conditions limit P solubility by forming aluminum and iron phosphates (Sims & Pierzynski, 2018).

However, organic acid-mediated dissolution of P minerals is accelerated by low soil solution pH. According to a study of Dinkelaker et al. (1989), the decreased pH phenomenon in soil solution is associated with roots experiencing P deficiency.

2.2.3. Mineralization and immobilization

Organic P in plants and microorganisms is hydrolyzed by enzymes into inorganic P (plant-available P) is called P mineralization. Immobilization is the reverse of mineralization, which is a conversion of soil inorganic P to organic P in plant or microbial biomass. Both processes are

biological and the balance between both processes controls the concentration of P in soil solution. However, mineralization and immobilization are influenced by factors such as moisture, temperature, pH, redox, and nutrient availability which affect biological activity in an ecosystem (Sims & Pierzynski, 2018).

2.3. Speciation of phosphorus

Different soil conditions affect the different P mineral formations in soils. The soil mineral solubility determines the solution P concentration. Highly weathered acidic soils are rich in Fe and Al hydroxides and oxyhydroxides. The Al-P and Fe-P minerals are common in acid soil mainly due to adsorption reactions. Under neutral and acidic conditions, Ca-P minerals are more common as a result of precipitation reactions in addition to adsorption reactions with CaCO_3 . The forms/ speciation of P determines the mobility, availability, transformations, fate and reaction products of P in soils (Beauchemin et al., 2003). There are different P speciation techniques for determining P in soils such as X-ray absorption spectroscopy (XAS), X-ray diffraction (XRD), Nuclear magnetic resonance (NMR) spectroscopy, Fourier transform infrared resonance (FTIR) spectroscopy and X-ray absorption near edge spectroscopy (XANES). Synchrotron-based X-ray absorption spectroscopy (XAS) techniques provide a clear and detailed insight into structural and chemical information of an absorbing element in situ without modifying, pretreatment, or processing (Hunter et al., 1998). Among those techniques, only XANES analysis is focused on this literature review. XANES analysis is one of the available tools for understanding P speciation in soils (Eriksson et al., 2016).

2.3.1. X-ray absorption near edge spectroscopy (XANES) analysis

The XANES technique is a non-destructive and element-specific chemical speciation technique (Beauchemin et al., 2003). The P XANES spectra have unique features in the pre and post-edges

depending on the P-associated mineral species (Khare et al., 2004). Based on the different electron configurations and electronic transitions at the X-ray absorption edge of P spectra, different metal-associated P can be identified. As an example, distinct features on the low energy side of an intensive white line peak (near 2150 eV) were observed in P K-edge XANES spectra of P minerals associated with Fe³⁺ and other transition metals (Hesterberg et al., 1999). However, this feature is absent on Al-P spectra (Khare et al., 2004). The P K-edge XANES spectra from bulk soils can usually be fit with only three or four model standards of P. Those standards assume that the main P species are present in the bulk soil (Hettiarachchi et al., 2008; Lombi et al., 2006).

The XANES technique is used by different scientists to investigate P speciation in fertilized soils (Lombi et al., 2006; Pierzynski & Hettiarachchi, 2018; Weeks & Hettiarachchi, 2020). The XANES technique was used to determine the reaction products of granular and fluid P fertilizers in calcareous soils (Lombi et al., 2006; Weeks & Hettiarachchi, 2020). Moreover, Pierzynski & Hettiarachchi. (2018) used XANES to identify reaction products of commonly used conventional P fertilizers (monoammonium phosphate, diammonium phosphate, and ammonium polyphosphate) with fertilizer enhancement products in acid soils. To identify the adsorbed phosphate species between ferrihydrite and boehmite in aqueous mixtures of these minerals, P K-edge XANES analysis was used (Khare et al., 2004). To identify and characterize different calcium phosphate minerals and iron phosphate minerals in soils, Hesterberg et al. (1999) used this technique.

However, if the XANES speciation data is complemented with the data from other techniques (wet chemical data) a better explanation can be provided regarding reaction mechanisms and fate of P minerals in soils.

2.4. P fertilizers

Fertilization is the major anthropogenic factor that can be influenced by soil P concentrations in the solution and the solid phase of soils. P fertilization is the major P input strategy in crop production since many soils lack bioavailable phosphates (Nyborg et al., 1999).

Fertilization is an essential component of soil management in arable conditions since plants absorb P from soil solution and soluble P fraction is more prone to runoff losses. In addition, labile P dissolves or desorbs from the solid phase to the solution phase. Hence, a new addition of phosphorus is needed to provide sufficient labile P to meet the plant P demand (Sims & Pierzynski, 2018).

If soils are unfertilized over time, labile P concentration is decreased through crop removal or P losses due to erosion and runoff. Those are the reasons for the soil's inability to meet the crops' P demands. Furthermore, excessively fertilized soils have a greater risk for P losses to the surface and ground waters significantly via runoff and erosion (Gburek et al., 2015; Van der Molen et al., 1998).

Chemical and organic fertilizers are the main P sources. Chemical fertilizers are typically composed of N, P, and K, which are highly soluble and readily available to plants. Plants can uptake phosphates and complete their life cycles. Most chemical P fertilizers are made from rock phosphate. Chemical fertilizers are available in different forms such as granular, water-soluble, and liquid forms. Phosphorus is often applied in the form of phosphate salt granules in agriculture that dissolve in soil pore water and enhance plant P uptake (Talboys et al., 2016). Monoammonium phosphate (MAP), Diammonium phosphate (DAP), Superphosphate, and Triple superphosphate are

granular forms of P fertilizer while Ammonium polyphosphates (APP) are in liquid form.

Organic fertilizers are typically agricultural by-products such as cow manure, poultry litter, and plant residues which are needed to decompose to be plant bioavailable (Green, 2015).

2.4.1. Recovered nutrient products

Conventional mineral P fertilizers are manufactured using phosphate rock (PR). However, PR is a non-renewable natural finite reserve, and continuous usage of PR can cause the depletion of PR reserves in the world. Based on the estimations, current global reserves of PR will decline during the next century (Cordell et al., 2009, 2011). Hence, good quality PR is diminished and becoming more expensive (Reijnders, 2014). There is a concern about the affordability of P fertilizer and its impact on food security (Cordell & Neset, 2014; Haygarth et al., 2014). To overcome these issues closing the P cycle by recycling and recovering P is more convenient (Elser and Bennett, 2011; Sharpley et al., 2015a).

Globally, 1.3 Mt P /year is removed either by chemical P removal (CPR) or enhanced biological P removal (EBPR) from wastewater streams (Lin et al., 2017). So, increasing P demand for P by the fertilizer industry shows higher interest in developing and integrating P recovery techniques for removing P from wastewater (Solon et al., 2019).

Recovered nutrient products used as P fertilizers are more sustainable in agriculture (Lederer et al., 2014; Sharpley et al., 2015b).

2.5. Struvite as a reclaimed nutrient product

Struvite is one of the recovered nutrient products from wastewater treatment plants that can be used as an alternative P fertilizer. Struvite is typically reclaimed from wastewater treatment plants as a byproduct (figure 1.2). Swine, dairy manure, landfill leachate, urine, anaerobic

effluent, and sludge can be used as sources for struvite reclamation. Hence the composition of nutrients varies among the type of wastewater used for the recovering process of struvite (Ahmed et al., 2018).

When the Mg^{2+} , NH_4^+ , and PO_4^{3-} ion activities are in excess in solution than the solubility product, struvite precipitation occurs spontaneously. However, the degree of precipitation depends on solution pH, supersaturation, temperature, and the presence of foreign ions (Koutsoukos et al., 2007).

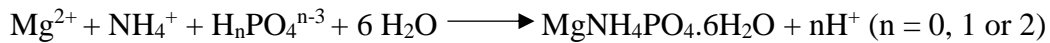
In the treatment plant, pipes are clogged with struvite precipitates with rapid pressure changes (Jaffer et al., 2002). However, controlling the struvite precipitation with the use of specialized reactors allows the alleviation of the problem with sludge digestion (Ashley et al., 2009). During this process, struvite granules are formed as a P fertilizer with the formula of magnesium ammonium phosphate ($NH_4MgPO_4 \cdot 6H_2O$) which can be used in agriculture. In addition, struvite has other environmental benefits including but not limited to reducing the sewage P concentration in watercourses (Schauer et al., 2011).

Struvite crystallization occurs through nucleation and crystal growth which is a complex process. It is affected by physical and chemical factors such as temperature, mixing strength, pH, supersaturation, and the presence of foreign ions (Wu L. et al., 2021).

2.6. Struvite as an alternative P fertilizer in the sustainable agriculture system

Struvite is a crystalline salt and can be used as a raw material for P production (Koutsoukos et al., 2007). Currently, struvite precipitation in enhanced biological P removal (EBPR) is the most common method to recover P as fertilizer. Full-scale EBPR commercialization can be seen in wastewater resource recovery facilities around the world (Etter et al., 2011; Ye et al., 2017).

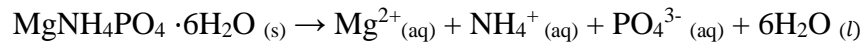
Struvite is a white crystalline substance made up of ammonium, magnesium, and phosphorus. However, the ammonium ions in the struvite can be replaced by potassium with the presence of high K^+ concentrations in the solution, and potassium struvite ($MgKPO_4 \cdot 6H_2O$) can be formed (Rech et al., 2019). Fertilizer grade struvite has a molecular formula of $(NH_4MgPO_4 \cdot 6H_2O)$ and has an orthorhombic crystal structure. The formation of the struvite can be written as the following chemical reaction:



Struvite can easily be spread, has a high P content, and is produced with less heavy metal contamination compared to other mineral fertilizers (Antonini et al., 2012).

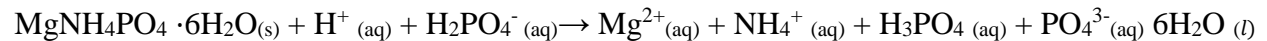
2.7. Struvite solubility/ dissolution

Struvite dissolution reaction can be written as below:

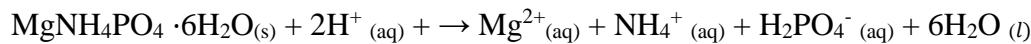


The struvite dissolution equation can be written as below at different pHs:

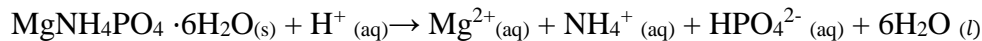
pH below 7.2



pH at 7.2



pH above 7.2



Struvite is less soluble and slowly releases P into the soil solution with compared to commonly used P fertilizers with a solubility constant of $10^{-13.26}$ (Ohlinger et al., 1998). It has been

investigated that struvite is readily soluble in acidic environments ($0.033 \text{ g } 100^{-1} \text{ mL}^{-1}$ in 0.01 M HCl and $0.178 \text{ g } 100^{-1} \text{ mL}^{-1}$ in 0.01 M HCl at 25°C (Ye et al., 2014).

The struvite solubility is determined by different soil factors such as soil pH, buffering capacity of soil, cation exchange capacity, amount of divalent and trivalent cations in soil solutions, and initial soil solution P concentration in soils. When soil pH is low high proton (H^+) concentration in soil solution is there and forward reaction of struvite dissolution is facilitated, and solubility is increased. Under alkaline conditions, a low amount of H^+ inhibits the forward reaction, and struvite solubility will be low. The soil buffering capacity is also an important property of soils in terms of nutrient availability. Buffering capacity is the ability or capacity of the soil to resupply an ion to the soil solution. Soil buffering capacity is increased in high CEC soil (Havlin J. & Tisdale S. L., 2014). High clay soil has high CEC and high buffering capacity, and a high amount of fertilizer (P) needs to be added into the soil with high buffering capacity since soil can maintain a relatively stable pH despite the presence of acidifying or alkaline factors. So, the struvite dissolution may be lower in the soils with higher buffering capacity since soils with high buffering capacity can maintain a relatively stable pH despite external influences. High CEC can promote the forward reaction of struvite dissolution since more cations can be exchangeable in high CEC soil. However, the valency of cations determines the P adsorption into clays. Divalent cations on the CEC enhance P adsorption relative to monovalent cations. When the initial (native) soil solution P concentration is high in soil struvite dissolution reaction is slower. Other than these soil properties, the granular size of struvite, fertilizer placement method, and soil moisture can determine the struvite solubility.

The slow release of P is advantageous due to its lower environmental impact on runoff. Alternatively, it is disadvantageous especially in high pH soils due to low P-releasing ability, with low solubility particularly in the early growing season of crops (Massey et al., 2009).

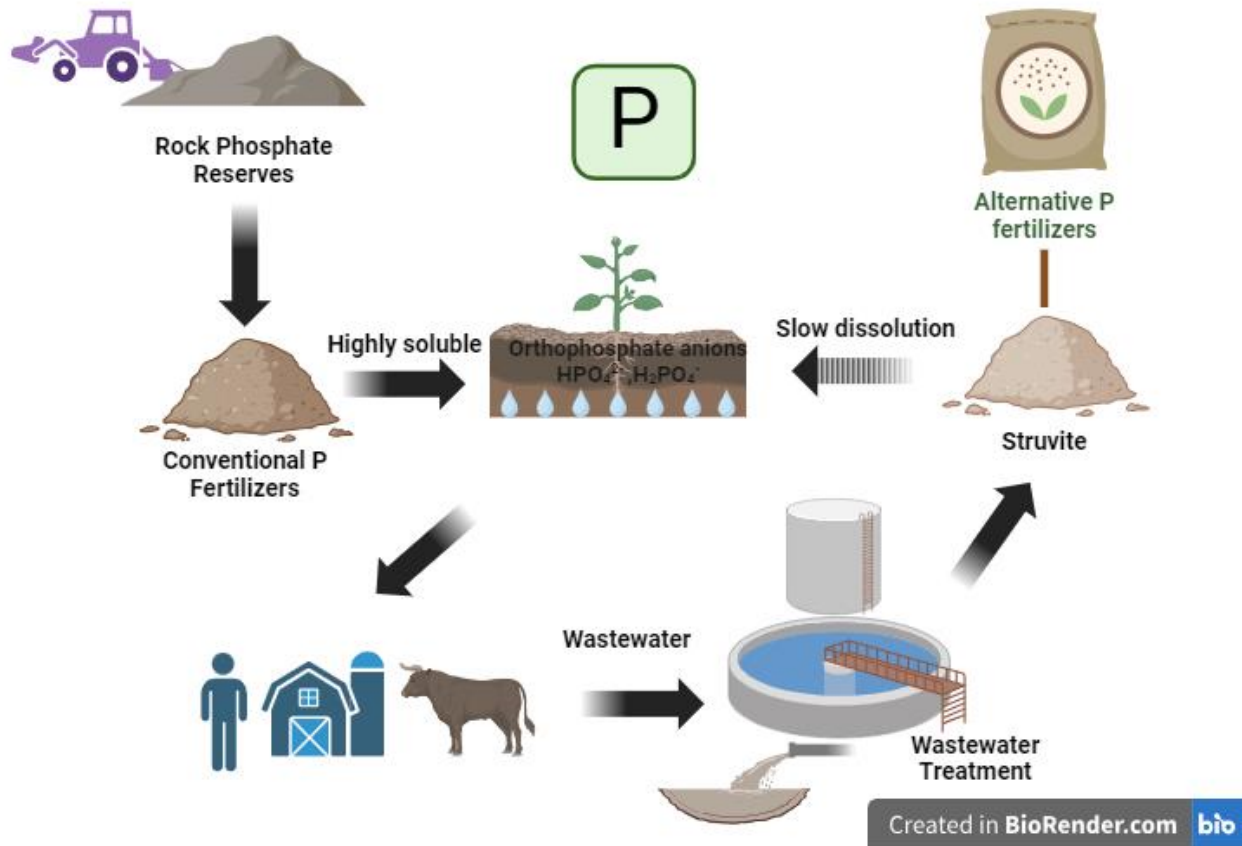


Figure 2.2. Struvite as an alternative P fertilizer

2.8. Low molecular weight organic acids (LMWOAs)

Plants release root exudates into the rhizosphere and change its conditions. LMWOAs are a class of root exudates that have significant importance (Oburger et al., 2009a).

Generally, organic acids are C, H, and O, containing low-molecular weight compounds found in all organisms and composed of one or more carboxyl groups. The release of a vast number of organic acids into the rhizosphere induces the growth of pre-existing rhizosphere bacteria and

acts as chemotaxis of motile microbes towards the roots in fungal hyphae and flagellate bacteria (Barbour et al., 1991).

Organic acids have various potential functions in the rhizosphere (Jones, 1998). Citrate, malate, and oxalates are the most common and well-studied organic acid anions, while malonate and shikimate are less studied even though they have a functional significance and are highly concentrated in root exudates (Oburger et al., 2009b).

Di and tri carboxylic acids have the potential to solubilize recalcitrant P species bonded to Al or Fe oxides and increase the P concentration in rhizosphere soil solution (Gardner et al., 1983).

Some plant species can secrete specific organic acids as an adaptive mechanism for P-limited soils (Susan J. Graystons S.J. & Campbell C.D., 1996; Zhang et al., 1997).

2.8.1. Importance of LMWOAs

Some organic acids such as citrate and malate are involved in energy production as intermediates in the tricarboxylic acid (TCA) cycle. In addition, malate, malonate, and oxalate are present in cells and responsible for cation exchange balancing and maintaining the osmotic potential. The composition of organic acid content in the plants is determined by their C fixation pathway (either CAM, C3, or C4), age, and nutritional stage. Furthermore, organic acids are released under P, Al, and Fe deficiencies in plants (Jones, 1998).

Plants growing under calcareous and silicate soil conditions have major challenges for growth due to Fe and P deficiency; hence plants should have mechanisms to solubilize P and Fe in the rhizosphere. Previous studies have investigated that Calcicole plants have a greater ability to release organic acids in calcareous soils. Calcifuge plants can thrive better in silicate soils (Ström et al., 1994).

When Fe is deficient in plants, citrate, and malate have the potential to complex with Fe in soil and facilitate the dissolution of ferric oxyhydroxides to become more available in calcareous soil conditions ($\text{pH} > 7$) (Jones et al., 1996). In addition, researchers have investigated a direct connection between Fe deficiency and high organic acid accumulation in root tissues (about a 5-fold increment). Similar trends were identified for H^+ induction (5-10-fold) and organic acids excretion (Cuerinot & Yi, 1994; De Vos et al., 1986). The combination effect of acidification power and the complexing ability of citrate facilitate Fe mobilization in the rhizosphere in calcareous soils (Jones, 1998).

Some dicotyledonous plant roots and non-mycorrhizal plants (*Lupinus albus*, *Brassica napus*) can release a vast number of organic acids in the presence of P deficiency (Hoffland et al., 1992; Imas et al., 1997; Lipton et al., 1987; Schwab et al., 1983). Under P deficiency primary components released by the plant roots are malate and citrate (Jones, 1998). Organic acids can inhibit P sorption reactions in soils (Sims & Pierzynski, 2018).

Aluminium (Al) phytotoxicity is a huge challenge for production agriculture since that condition can inhibit root development and cell division (Pellet et al., 1995). Some plants have adapted resistance mechanisms outside and inside the root to detoxify Al concentrations higher than $5\mu\text{M}$ through complexation reaction with organic acids with aluminum (Henn & Zang, 1997; Pellet et al., 1995).

Organic acids are released under various other stress conditions such as K^+ deficiency, general nutrient deficiency, N nutrition, Ca^{2+} , and Zn^{2+} deficiency (Imas et al., 1997; Jones et al., 1996). Moreover, plants have adapted to rapid changes in C metabolism and organic acid transport under hypoxic conditions (Marschner, 2011).

Additionally, Mn mobilization is also enhanced by organic acids. Organic acids can release Mn to the rhizosphere (Godo & Reisenauer, 1980). Citrate and malate have the potential to release Mn from synthetic MnO₂ via a combination of oxidation and complexation reactions (Jauregui & Reisenauer, 1982). However, Mn dissolution is highly dependent upon the pH. In conditions above pH 5 Mn release amount is negligible compared to other elements (Fe and Al) by organic acids in soil (Jones et al., 1996; Mench et al., 1991).

According to some studies, organic acids can induce mineral dissolution rate by 2-4 folds compared to rainwater alone. However, the mineral dissolution is highly dependent on the pH, mineral type, organic acid type, and Al content of the mineral type (Jones & Kochian, 1996; Mccoll & Pohlman, 1986) .

2.8.2. Composition of the rhizosphere in different plant species

Plant roots contain different organic acids which have varying chain lengths. Lactate, acetate, oxalate, succinate, fumarate, malate, citrate, isocitrate, and aconitate are respective anions of primary organic acids. Total organic acid concentration in roots is known to be 10-20 mM (1-4% total dry weight). The primary determining factor of organic acid concentrations in roots is the degree of cation-anion imbalance (Jones, 1998). For example, plant roots may need more anions to balance when taking up cations (K⁺) by roots. The anions needed are typically provided by organic acids (citrate, malate, malonate, aconitate) (Chang & Roberts, 1991). However, the composition of root exudates is highly dependent upon many factors like species of the plant, age of the plant, and physicochemical environment. Researchers have investigated citrate exudation from rice roots into the soil at 2 to 3% w/w (dry weight basis) over the 14-day growing period (Kirk et al., 1999). The following table is adapted by (Adeleke et al., 2017).

Table 2.1. Rhizosphere organic acid composition in different plants

Plant	Organic acid composition	Concentration (μM)
Soybean	Citric acid	4.0
Chickpea	Citric, oxalic, and malic acids	0.01 – 84
Maize	Acetic and lactic acids	1.5 – 3.5
Wheat	Malic acid	0.25
Cotton	Citric, oxalic and tartaric	5.0
Tomato	Citric acid	0.13
Pinus	Citric, acetic and tartaric acids	1.0
Pigeon pea	Citric acid	17

2.8.3. Mechanism of LMWOAS on struvite solubility

2.8.3.1. Complexation

Organic acids can form complex metal ions in solution. Different organic acids carry varying negative charges allowing metal cation complexation and displacement of anions from the soil matrix depending on the number of carboxylic groups and dissociation constants. Usually, organic acids containing one carboxyl group such as lactate, formate, and acetate have little capacity for metal complexation. In addition, the pH of the soil solution, concentration, and type of metal ions also determine the degree of complexation. Stability constant is another determining factor. For instance, malate, oxalate, and citrate have a higher affinity for Al^{3+} and Fe^{3+} - like trivalent metal cations that are mobilized in most soils by organic acids (Jones & Kochian, 1996; Mccoll & Pohlman, 1986). However, soil pH influences the complexation of Fe by malate and oxalate. At higher soil pH, no or less complexation occurs. However, above

mentioned organic acids have a higher potential to precipitate in the presence of Ca^{2+} ions such as calcium oxalate, calcium citrate, and calcium malate hence reducing the potential of complex formation and plant P availability. The reduction of complex formation in the presence of calcium-containing minerals such as apatite is an example of this phenomenon (Jones, 1998).

The presence of citrate, oxalate, and malate in soil can accelerate the phosphate dissolution rate increasing soil solution P concentrations 10-1000-fold depending on the type of soil, concentration, and speciation of organic acids (Earl et al., 1979; Jones et al., 1996).

Generally, inorganic P extraction efficiency by the organic acids is citrate > oxalate > malate > acetate in decreasing order. The P release depends on the anion's ability to make a complex with Al (i.e. a $\log K_{a1}$ greater than 3.5) (Lan et al., 1995; Stumm & Furrer, 1987). However, pH and soil mineralogy are the factors affecting the organic acid-induced P release. Ligand exchange and complexations are the main mechanisms of P release. Citrate anions directly replace P (PO_4^{3-}) on ligand exchange surfaces when crystalline aluminum hydroxide ($\text{Al}(\text{OH})_3$) or iron hydroxide ($\text{Fe}(\text{OH})_3$) are present. In higher organic acid concentrations, citric acid can complex with and solubilize solid phase Al as Al-OM complex and then decrease the P sorption capacity (Sims & Pierzynski, 2018). Complexation of Ca^{2+} in the rock phosphate and Fe^{3+} in $\text{Fe}(\text{OH})_3$ occurs with the P-holding exchange matrix. Usually, the complexation is dominant to ligand exchange (Earl et al., 1979; Jones et al., 1996).

Some studies agree that the P mobilized by citrate may stay in soil solution for a considerable time, usually more than ten weeks. However, the plant availability of that P is unknown (Fox & Comerford, 1990; Lan et al., 1995).

2.8.3.2. Ligand exchange

Ligand exchange reactions occur when organic ligand exchanges for inorganic P on the mineral surfaces, which can facilitate the release of PO_4^{3-} anions into the soil solution (He et al., 1994; Hue, 1991; Lopez-Hernandez et al., 1986). In the ligand-enhanced dissolution process of Fe oxide P, citrate, oxalate, and malate are adsorbed at a surface structural Fe site. Then enhancing slow dissolution of the Fe-oxide surface occurs and releases adsorbed P into the soil solution (Earl et al., 1979; Johnson et al., 2006; Jones et al., 1996; Kirk et al., 1999). The following figure (Figure 2.3) is adapted from (Johnson et al., 2006).

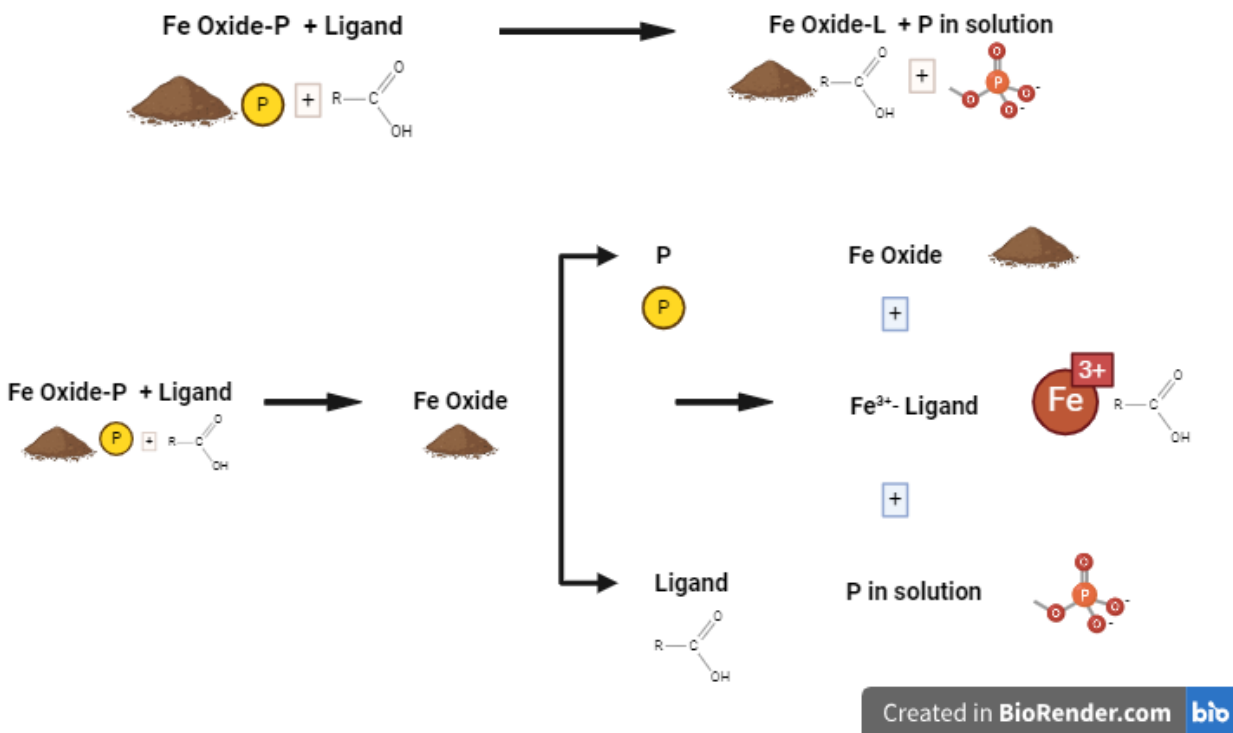


Figure 2.3. Formation of Fe-ligand complexes

Surface Fe-ligand complexes are formed in both mechanisms mentioned above. The structural stability of Fe-oxide surface sites, the surface Fe^{3+} ligand and dissolved Fe^{3+} ligand complexes

are the determining factors of the effectiveness of P release by organic acids (Stumm & Furrer, 1987).

2.8.3.3. Reductive dissolution

Reductive dissolution of oxides (Fe oxides) can occur in the presence of reducing agents such as ascorbate, dithionite, oxidizable metal or by photoreduction when oxalate is present (Ryan & Gschwend, 1991; Suter et al., 1991).

During the P recovery process in wastewater treatment plants, Fe-P sludges are formed and those are highly stable and low mobile which makes P recovery more challenging (Wilfert et al., 2015). Several studies have discovered methods to recover P from Fe-P sludge under alkaline and acidic conditions by sulfide reduction and complexing agents such as citric, tartaric, EDTA (ethylenediaminetetraacetic acid) and tannic (Chen et al., 2022; Ping et al., 2020; Wilfert et al., 2020; Zou et al., 2017). In addition to those methods, scientists are interested in recent redox manipulation to release P from Fe-P sludges (Prot et al., 2019; Wilfert et al., 2015; Wu L. et al., 2021; Xu et al., 2023).

Biological or chemical reductive dissolution of ferric phosphate can take place under low oxidation and reduction potential in CPR plants. Under reductive dissolution, Fe-bound P tends to be released (Wilfert et al., 2015; Wu et al., 2020). Ascorbic acid (AA) has been investigated as a potential reducing agent due to its high reactivity toward reactive oxygen species and active transition metals (Co^{3+} , Cu^{2+} , and Fe^{3+}) (Njus et al., 2020). Ascorbic acid donates two protons, and two electrons have the potential to reduce species and can form metal chelates in pH 2.0-12.5 (Njus et al., 2020). The AA has been identified as an effective reducing agent to reduce Fe^{3+} in iron oxyhydroxides to a more soluble Fe^{2+} form (Conrad & Schade, 1968; Hsieh & Hsieh, 2000; Martinez & Uribe, 1982; Shen et al., 2023).

Based on research by Alnimer et al. (2023), AA is considered an effective and environmentally friendly reducing agent that can facilitate the reduction of Fe^{3+} to Fe^{2+} . Moreover, Ascorbic acid can be active as both chelating and reductant to facilitate Fe-P dissolution. Studies have identified that AA has the potential to reduce 98% of solid Fe^{3+} in sludge into Fe^{2+} and release Fe^{2+} and PO_4^{3-} into the solution. This reduction occurs at a minimum Fe/AA molar ratio of 1:2 at pH 3 and pH 4 (Alnimer et al., 2023).

Oxalic acid has been investigated as a good chelating agent that facilitates solubilizing Fe-P sludge at high oxygen concentrations and is stronger in chelating Fe than ascorbic acid. When low molecular weight organic acids are used, acidic pH provides more beneficial effects on practical P-recovering applications such as a strong reducing effect compared to other common reducing agents (hydroxylamine), organic acids can form a metal complex with Fe^{2+} and enhance solubilization and stabilization of Fe^{2+} in solution. Organic acids are more environmentally sustainable compared to other reagents (Alnimer et al., 2023).

2.8.4. Organic acid sorption in soils

Organic acids consist of carboxyl groups which carry a negative charge. Organic acids can sorb rapidly and readily into the soil's solid phase. However, sorption is highly dependent upon soil type. The general sorption trend is phosphate > oxalate > citrate > malate > sulfate > acetate (Earl et al., 1979; Jones et al., 1996; Jones & Kochian, 1996). All those anions can share similar sorption sites while organic acid anions have the potential to induce P desorption or prevent newly added P sorption (Bolan et al., 1970; Earl et al., 1979; Jones et al., 1996). Organic acid anions sorption into CaCO_3 is based on the surface chemistry of it. The partial dissolution of calcium carbonate impacts its surface charges, interaction with other substances and colloidal

properties. Some studies suggested that the small organic acid anions have a great potential to adsorb onto the CaCO_3 surface (Plank & Bassioni, 2007).

Synthetic ferric and aluminum hydroxide studies show the sorption potential of organic acids is highly dependent on the pH of the solution. An increase in sorption is observed when the pH is decreased. Even though the sorption of organic acids (salicylate, maleic, succinic, phthalic) is an instantaneous process, the desorption reactions remain unknown. The degree of sorption is determined by the presence of cations in the soil solution and anions in the soil's exchange surface (Jones and Brassington, 1988).

Organic acid biodegradation is enhanced in the rhizosphere of P-deficient soil (Èm et al., 2002; Jones et al., 2003).

2.8.5. Dissolved organic carbon (DOC)

DOC content is an important component in the carbon cycle of the terrestrial environment. DOC production, decomposition, and fluxes are important major steps in the carbon cycle (Giesler et al., 2007).

2.8.5.1. DOC concentration in soils including root exudates

The amount of the major plant-originating organic acids investigated in the soil solution is 0.1-100 mM in concentration and composed of about 2% of the total dissolved organic C in podzolic soils (Krzyszowska et al., 1996; Pohlman & McColl, 1988; Shen et al., 1996)

Generally higher organic acid concentrations are found in rhizosphere soil compared to those present in the bulk soil (Jones, 1998). However, LMWOAs contribute to DOC content in less than 5%. Due to their rapid decomposition in the soil when present in low concentrations (Giesler et al., 2007).

2.8.5.2. Impact microbial activity on DOC concentration in different soils

Organic acids are the most labile C source in soils. Their half-life is characterized by the rapid breakdown and consumption by soil microbes and the sorption into the soil solid phase once they have been released into the rhizosphere. Studies have found that mineralization of root exudates can cause rapid degradation of citrate and malate with an average half-life of 2-3h in the bulk soil. However, it depends on the soil type (Jones, 1998; Jones & Darrah, 1994a). Generally, decomposition is way faster in organic surface horizons compared to low organic content subsoils, while in rhizosphere soil decomposition rates are 2-3-fold faster than in bulk soil (Jones & Darrah, 1994b). Based on the Michaelis-Menten kinetics regarding microbial decomposition of organic acids in soil and solution, 60% of organic acids are mineralized into CO₂, and 40% are incorporated into new cell biomass (Jones & Darrah, 1994b; Jones & Willett, 2006). However, other root exudates such as simple sugars and amino acids are not affected by mineralization in soils (Jones & Kochian, 1996).

Since organic acids are charged exudates, the biodegradation of organic acids seems to be highly dependent upon the amount and type of sorption to soil particles, with Al and Fe hydroxides providing a protective effect (Boudot, 1992; Boudot et al., 1986; Jones & Edwards, 1988).

Negligible biodegradation occurs when citrate is held on the surface of Fe and Al hydroxides (Boudot, 1992; Jones & Edwards, 1988).

Microbes can consume root exudates as well as produce a wide range of organic acids in situations where nutrient-limited conditions (Rózycki & Strzelczyk, 1986).

The decomposition rate of individual organic acids increases with the reduction of amounts of anionic binding sites in soils. This suggests that sorption is the major regulator of organic acid bioavailability in soils. Among oxalate, citrate and malate half-life is changing in the order of oxalate > citrate > malate. This order correlates with the adsorption strength order of oxalate >

citrate \geq malate (Oburger et al., 2009). This suggests that oxalate has the longest half-life (slowest decaying rate) and the sorption is faster, however, it is reversible, and microbes can have access to adsorbed or precipitated oxalate. The sorption rate of oxalate is higher than citrate or malate in all soils. However, when there is a high pH condition precipitation of calcium oxalate could occur and the amount of exchangeable Ca^{2+} ions could be increased. This scenario could increase the difference in citrate and oxalate sorption (Oburger et al., 2009).

However, the degree of sorption of organic acids depends on the number of carboxylate groups present, dissociation constants, their reactions with other solutes, and the nature of the soil (soil pH, soil solution ionic strength the composition, sorption characteristics of the soil) (Èm et al., 2001; Jones & Brassington, 1998; Van et al., 2002).

Alternatively, organic acid decomposition is not affected by the presence of other organic acids in the mixture. Instead, it may be due to the different uptake mechanisms in different components of the microbial community. Soils with high sorption capacity are sorbed into the solid phase and it greatly inhibits organic acid biodegradation (Oburger et al., 2009).

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Chapter 3 - Reclaimed struvite P mobility and availability in comparison to conventional P fertilizers in three different soils

Abstract

Phosphorus (P) is one of the essential macronutrients for crop growth and yield. Recycling and reusing recovered nutrient products are a remedy for preserving global rock phosphate reserves. Struvite, a phosphate mineral with a molecular formula of $\text{NH}_4\text{MgPO}_4 \cdot 6\text{H}_2\text{O}$, is reclaimed from wastewater in treatment plants and may be used as an alternative P source. This study was designed to investigate the effectiveness of reclaimed struvite as a potential P fertilizer under three contrasting soil conditions compared to conventional P fertilizers. The study goals were to compare dissolution, transformation, and potential plant P availability of reclaimed struvite with monoammonium phosphate (MAP), diammonium phosphate (DAP), and ammonium polyphosphate (APP) under mildly calcareous, neutral, and acidic soil conditions using short term laboratory incubation studies. Three different soils were incubated for 1, 2, 5, 9, and 12 weeks in Petri dishes with four fertilizer treatments: reclaimed struvite, MAP, DAP, and APP, and a control. At the end of the incubation, dishes were sectioned into three concentric rings at different distances from the point of application (POA). The pH, resin extractable P to assess potential plant available P, and total P to determine P diffusion from the POA were analyzed. Based on the results, reclaimed struvite is best suited for neutral and acidic soils since struvite performed well as conventional P fertilizers. In terms of P diffusion of struvite under calcareous, neutral, and acid soils 5%, 20% and 40%, of added P diffused into the second and third sections, respectively while MAP 13%, 86% and 75%, DAP 7%, 82% and 74% and APP showed 25%, 87% and 65% greater amounts of P diffusion into further sections at the 12 weeks of incubation period. Overall resin P extractability (percent resin P per Petri dish) of struvite showed the

highest in acid soil (67%) followed by neutral (40%) and calcareous (9.2%) soils while MAP, DAP, and APP showed, 72%, 79%, and 55% in acid, 33%, 31%, 26% in neutral and 7%, 5%, 8% in calcareous soils, respectively, at the end of 12 weeks incubation. The XANES data provided a clear insight into the fate of P fertilizers in calcareous soils. Struvite performance was significantly less than conventional P fertilizers under mildly calcareous soil conditions due to the formation of more insoluble secondary P minerals by adsorption and precipitation reactions in the presence of CaCO_3 . Modification or blending of struvite with conventional P fertilizers may enhance struvite P availability under calcareous soil conditions.

Introduction

Phosphorus (P) is one of the major essential macronutrients for all living organisms on the planet. P is considered the primary macronutrient since it can affect on limitation of growth and productivity of plant-based ecosystems (Niederberger et al., 2019). Phosphorus is only second to nitrogen in terms of growth limitation of plants (Petriman et al., 2010). Phosphorus is vital for plants and animals as a functional and structural component (Westheimer, 1987). Even though P is a widely distributed element on Earth (the 12th most abundant element on Earth's crust) the available fraction of P is very small. Since soil cannot supply an adequate amount of P for crop growth and productivity, producers are applying P as fertilizers during agricultural production to obtain better growth and yield. Mineral P fertilizers and organic-based fertilizers such as cow manure, poultry litter, and plant residues are used in agriculture depending on the soil conditions (Green, 2015). P fertilizers have the potential to provide the available P for crop production. Commercialized chemical fertilizers are in the market in different forms such as granular and liquid even though the application of granular fertilizer is most common (Talboys et al., 2016). Monoammonium phosphate (MAP), Diammonium phosphate (DAP), Superphosphate (SSP), and Triple superphosphate (TSP) are granular forms of P fertilizer while Ammonium polyphosphates (APP) are in liquid form. To overcome the limitation of P during the production of agriculture globally, MAP, DAP, TSP, and SSP are the most dominant used fertilizers (Hedley & McLaughlin, 2015). From fertilizer to fertilizer, the P content is different (Green, 2015). The production of chemical P fertilizer is based on natural rock phosphate reserves. However, rock phosphate is a non-renewable source that threatens to deplete over time with continuous usage (Ahmed et al., 2018).

Struvite is a by-product of wastewater treatment plants that can be precipitated by increasing the pH (pH 9.0) of wastewater. At the end of the precipitation process, struvite mineral ($\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$) is formed which can be used as a P fertilizer (Cerrillo et al., 2015). In sustainable agricultural practices, struvite is a good P fertilizer source and can be used as a remedy for global rock phosphate scarcity. As a P fertilizer, struvite has potential benefits during agriculture, such as the slow release of nutrients can reduce the leaching and runoff losses during intense precipitation conditions and root exudated assisted struvite dissolution can be beneficial for enhancing the timing of nutrient release (Hertzberger et al., 2020). The struvite source used in this study was reclaimed from Ostara Nutrient Recovery Technologies under the trade name Crystal Green. This granule contains struvite equivalent to 12% P (28% P_2O_5).

Based on the different chemical and physical properties of soils, P availability is diverse and affects many factors in the available P pool. The availability of P is especially limited in highly weathered acidic soil conditions and the presence of high calcium carbonate (CaCO_3) content. Calcareous soils consist of CaCO_3 from 3 to 95% and limit crop productivity because of P deficiency due to high pH (pH 7.0 - 8.5) and less microbial activity (Brownrigg et al., 2022). With reactive CaCO_3 and high pH conditions in calcareous soil, P availability is reduced by two mechanisms. In the first mechanism, P is precipitated out with calcium and/or sorption to CaCO_3 and then becomes low soluble minerals like octa calcium phosphate or in the second mechanism, forming inner-sphere complexes with iron (Fe) and aluminum (Al) oxyhydroxides and fixation of P (Weeks & Hettiarachchi, 2020). In highly weathered acidic soil conditions, Fe and Al oxyhydroxides, hydrous oxides, or both are more abundant. When P fertilizers are added into these soils, the binding capacity or affinity of P and the oxides are increased around the point of application and form surface precipitates or inner sphere complexes which results in P

being less soluble (Beauchemin et al., 2003; Hedley & McLaughlin, 2015; Khatiwada et al., 2012).

However, there is a knowledge gap regarding the mechanistic understanding of dissolution, transformation, and efficacy of struvite as a fertilizer in different soils with varying pH conditions compared to commonly used conventional P fertilizers such as MAP, DAP, and APP. This study was designed to fill that gap using Crystal Green by Ostara Nutrient Recovery Technologies as the reclaimed struvite source while MAP, DAP, and APP were used as conventional P fertilizers.

The overall aims of this study were to investigate and compare the dissolution, transformations, and potential plant availability of P in the struvite (reclaimed nutrient product from wastewater treatment facilities) with conventional P fertilizers in mildly calcareous, neutral, and acidic soils using short-term laboratory incubation studies with wet chemical analysis and X-ray absorption near edge structure (XANES) technique.

Materials and methods

Soil Collection

Mildly calcareous soil (Ulysses silt loam) from Oakley City, KS, Neutral soil (sandy loam) from Ashland Bottom, KS, and Acid soil (loamy sand) from Piracicaba, São Paulo, Brazil. The surface soils (0- 20cm) were collected, carefully broken down the large aggregates, air dried, and sieved by 2mm by stainless steel mesh/sieve.

Soil characterization

Sieved soils were used for the short-term incubation studies. The pH was measured in a 1:10 soil: MiliQ water extract (Watson and Brown, 1998). The maximum water holding capacity (MWHC) was determined using the (Jenkinson & Powlson, 1976). Resin P was extracted using

anion exchange resin (AER) membranes (Myers et al., 2005). The resin extractable P (potential plant available P) was determined using the ammonium molybdate blue colorimetric method (Murphy & Riley, 1962). Total P concentration in soil was determined using aqua regia Zarcinas et al. (1996) and this method was modified to use a digestion block instead of a microwave. Available P was measured by extraction with sodium bicarbonate according to Olsen et al. (1954) for the calcareous soil, and Mehlich-3 P was determined as described in Frank et al. (1998) for neutral and acidic soils. Cation exchange capacity was determined by the displacement method (Soil Survey Staff, 2011). Soil carbonates were determined by (Allison & Moodie, 1965). Total organic carbon and total N content of the soil was measured by the dry combustion method (Nelson & Sommers, 1996; Wright & Bailey, 2001). Soil texture was determined using the Hydrometer method (Gee & Bauder, 1979).

Design of the study

The same setup of incubation study was performed with three different types of soils (mildly calcareous, neutral, and acid) and for five different incubation periods (1 week, 2 weeks, 5 weeks, 9 weeks, and 12 weeks) as separate incubation studies.

A short-term petri dish incubation study was performed based on a completely randomized design (CRD) with four fertilizers and no P fertilizer (control) treatments with 4 replicates.

This study was composed of 20 Petri dishes per period hence 100 dishes per soil and all together 300 dishes (54mm diameter and 13.9 mm height) for the whole study.

Experimental setup and method

The soil was packed to achieve a uniform bulk density of 1.05 kg m^{-3} , 1.05 kg m^{-3} , and 1.05 kg m^{-3} for mildly calcareous, neutral, and acid soils. The soil was prewetted and packed

homogenously. After packing, soils were brought to 55% of MWHC, the Petri dishes were closed with the top covers, edges were wrapped using parafilm to mitigate moisture loss, and the set of dishes was covered with aluminum foil to prevent light exposure and inverted to equilibrate overnight (24 hours) at room temperature (~ 25°C).

The following day, dishes were unwrapped, and treatments (n=4) were carefully introduced into the center of the dish as follows:

- (i) Unfertilized control soil sample
- (ii) Granular reclaimed struvite (Crystal Green): a single granule 37.64 ± 0.05 mg (~4.6 mg P per dish)
- (iii) Granular MAP: a single granule 20.27 ± 0.05 mg (~4.6 mg P per dish)
- (iv) Granular DAP: a single granule 22.91 ± 0.05 mg (~4.6 mg P per dish)
- (v) Liquid APP: 21.93 μ L (~4.6 mg P per dish)

After treatment was added the dishes were closed, covered individually with parafilm, and stacked. Stacked dishes were wrapped in an aluminum foil and incubated over specified periods and incubated as separation incubation studies for (1 week, 2 weeks, 5 weeks, 9 weeks, and 12 weeks) inside the incubator (Precision Low Temp Incubator, Waltham, MA) in the dark at 25 °C. At the end of each incubation period, petri dishes were opened. The soil was sectioned into three sections based on the distances from the point of application 0-7.5mm, 7.5-15.5mm, and 15.3-27 mm as concentric rings. Each sample (ring) was placed in a pre-weighed plastic specimen container (Fisher Scientific, Waltham, MA). Then, the samples were oven-dried at 40 °C (Fisher Scientific drying oven, Waltham, MA). The final weight of the samples was recorded after drying and soils were ground gently with a mortar and pestle. The ground soil was used for future analysis.

Determination of total P content

The total P content of all the samples was determined by aqua regia digestion (without H₂O₂ pretreatment) and analyzed using ICP-OES (Agilent 5800). Aqua regia digestion was performed using trace metal grade hydrochloric acid and nitric acid in a 3:1 ratio. Total P content data was normalized by calculating the percentage of P added (PPA) for each dish section for all treatments according to Hettiarachchi et al. (2010) as below:

$$PPA = \frac{(P_f)S_i \times M_i}{\sum_{i=1-3} [(P_f) S_i \times M_i]} \times 100$$

Where i is the dish section (1–3), $(P_f)S_i$ is the concentration of P fertilizer in each dish section, and M_i is the soil mass in each dish section. The $(P_f)S_i$ is calculated by subtracting the total P concentration of the unfertilized soil sample from the total P concentration in the fertilized dish section.

Determination of resin extractable P

Resin P was determined using anion exchange resin (AER) membranes according to Myers et al. (2005). This method is a proxy for potential plant P availability. After extracting orthophosphate ions, those were quantified by colorimetric method using a Beckman-Coulter DU-800 spectrophotometer (Brea, CA) (Murphy & Riley, 1962). Then the percentage of resin extractable P (PRP) for each section of the dish for all the treatments was calculated based on the equation described in Pierzynski and Hettiarachchi (2018) below:

$$PRP = \frac{REP_i}{Total P_i} \times 100$$

Where i is the dish section (1-3), REP_i is the resin P concentration and $Total P_i$ is the total P concentration in the i^{th} dish section.

Determination of percent resin P per Petri dish

The percent resin P was calculated per Petri dish after each incubation period for each treatment in calcareous, neutral and acid soils. Sum of resin P in each dish section (Section 1,2, and 3) was divided by sum of total P in each section and calculated the percentage.

The XANES analysis

The X-ray absorption near-edge structure spectroscopy analysis was performed for the composite samples of 0 – 7.5 mm section for five weeks incubated mildly calcareous soil (Oakley City, KS) samples. The composite samples were prepared using equal masses of soil from four replicates. The P K-edge XANES spectra of soil samples were collected at the beamline NSLS – II- 7- ID- 2, Brookhaven National Laboratory, Upton, NY. All K-edge P spectra were collected in partial electron yield (PEY) mode. A soil pellet for each composite sample was prepared and carefully mounted on the sample holder with double-sided carbon tape (SPI Supplies, West Chester, PA) and placed into a helium-filled chamber for analysis. Two to three scans were collected for each P-treated sample and control sample at a range of 2.1 to 2.3 keV.

Background correction, normalization, and linear combination fitting of the spectra were performed using Athena (v.0.9.25) software (Ravel & Newville, 2005) according to the concept set forth by (Werner & Prietzel, 2015). Previously collected standards were also used with P standard spectra collected during each run for special alignment. Two to three scans were merged to limit the noise. Pre-edge and normalization ranges were not fixed, allowing for individual samples to maximize fit. All spectra were adjusted such that E_0 (2149 eV) corresponded to one-half the height of the white line peak. The reference spectra were eliminated if they comprised < 5% of the calculated composition and fitting was repeated.

Statistical analysis

The statistical analysis was performed using SAS version 9.4 software (SAS Institute Inc., Cary, NC, 2020). The experiment design was completely randomized. Data were analyzed by ANOVA using PROC MIXED as a split-plot arrangement, the main plot as the fertilizer treatment, and the subplot as the dish section. Tukey's HSD test was performed to compare all the treatments at a $p = 0.05$ significance level.

Results and Discussion

Soil Properties

Selected physical and chemical properties of the soils used for the incubation studies showed the characteristics of mildly calcareous (with 7.6% CaCO_3), neutral, and acidic soils (Table 3.1).

Diffusion of P

The P diffusion of struvite-treated samples showed that most of the added P remained within the first/center section in calcareous soil for all the periods (Figure 3.1). Phosphorus diffusion to the second section was superior in APP treatments followed by MAP and DAP after 5 weeks of incubation, while APP moved towards the third section at the end of 12 weeks but not in MAP or DAP. This scenario can be explained using the phosphate species' acidic nature in APP and MAP treatments Weeks & Hettiarachchi (2020). Based on the pH results of calcareous soil (Table 3.2), APP showed a dramatic acidifying effect at the center section. Similar results were observed by other researchers previously Weeks & Hettiarachchi (2020). Diammonium phosphate (DAP) has the least ability to acidify compared to APP and MAP. The acidulation effect facilitates the P diffusion towards the further sections from the point of application in calcareous soil. In addition, APP has the potential ability to move towards further sections since it is a liquid P fertilizer with less chance of forming secondary minerals by precipitation with

Table 3.1. Selected physical and chemical properties of the soils used for incubation studies.
Selected physical and chemical properties of the soils used for incubation studies.

Parameter	Calcareous soil	Neutral soil	Acidic soil
pH	8.54 ± 0.04	6.28 ± 0.06	5.17 ± 0.07
CEC, cmol _c /kg	21.3	6.8	3.74
CaCO ₃ , %	7.6	N/A	N/A
Total P, mg/kg	582 ± 4.4	329 ± 0.95	101 ± 1.2
Available P, mg/kg	31 (Olsen P)	53.8 (Mehlich)	8 (Mehlich)
Resin P, mg/kg	12.38 ± 0.78	35 ± 1.5	7.2 ± 0.3
Total Organic C, %	0.64	0.6	1.041
Total -N %	0.09	0.08	0.102
MWHC	58.6 ± 0.04	49 ± 0.2	35.43 ± 0.1
Texture	Silt loam	Sandy loam	Loamy Sand
(Sand, Silt, and Clay%)	12.7, 65.5, 21.8	50.9, 43.6, 5.5	82, 6, 12

CEC, Cation Exchange Capacity; MWHC, Maximum Water Holding Capacity

Ca²⁺ ions and due to adsorption reactions than granular P fertilizers. This scenario was discussed in McBeath et al. (2005). Moreover, due to the hygroscopic nature of the granular fertilizers, P diffusion was comparatively less than liquid fertilizers (Hettiarachchi et al., 2006; Lombi et al., 2004). In APP treatment showed P movement (25% of added P) into the second and third sections after 12 weeks of incubation. Even after 12 weeks, APP could maintain the acidulation effect and favor the P diffusion since polyphosphate presence in the APP may be hydrolyzed over a long term and inhibit the calcium phosphate mineral formation through the disruption of crystal lattice formation by the large and condensed phosphate molecules in polyphosphate (Amer & Mostafa, 1981). Even though the conventional P fertilizers have moved, struvite did

not show a significant P diffusion in high alkaline soils (pH 8.54) (Table 3.1). This can be explained by the initial struvite dissolution process uptake of protons from surrounding soil solution and further decreasing pH with slow dissolution of struvite in alkaline soil conditions (Ahmed et al., 2018). According to Le Corre et al. (2009), struvite solubility is minimum within the pH range of 8.2-8.8. Overall, the struvite's fertilizing efficiency depends on the soil type due to the varying solubility and sorption properties of soils (Kataki et al., 2016). However, overall P diffusion reduction in all the nutrients might be due to the formation of Ca-P minerals by precipitation reactions and sorption reactions into soil colloids, solid CaCO_3 with the silt loam texture in the calcareous soil right after application. Studies have investigated that the calcareous properties of soils determine the fate of soil P due to the fixation of P in soils with CaCO_3 via mineral precipitation and surface adsorption (Weeks & Hettiarachchi, 2020). This P fixation resulted in removing and limiting P from the plant-available P pools (Leytem & Mikkelsen, 2005). This Ca-P mineral formation is the reason for the overapplication of fertilizers to overcome the low P content and increase the available P pools in calcareous soils (Westermann, 1992). The soil used for the study contained 7.6% CaCO_3 (Table 3.1). According to the meta-analysis of Hertzberger et al. (2020), when struvite was applied to alkaline soils, calcium P fixation was greatest. In calcareous soil conditions, several factors such as different Ca/P ratios, formulation of P, pH at the moment of the precipitation, and carbonate contents determine the formation of Ca-P and their amorphous or crystalline nature (Weeks & Hettiarachchi, 2020). Phosphorus diffusion after 1-week incubation results showed that all the conventional fertilizers moved up to the third section while struvite movement was restricted at the point of application in neutral soil (Figure 3.2). In MAP treatment (80% of added P moved) showed the superior mobility of P followed by APP (64%) and DAP (61%) in the second and third sections.

Table 3.2. The pH after 1, 2, 5, 9, and 12 weeks of incubation in calcareous soil. Means within a soil section for each treatment in each time period containing the same letter are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

Incubation Period	Treatment	pH		
		0.0-7.5 mm	7.5-15.5 mm	15.5-27.0 mm
1 week	Control	8.54 ± 0.03 a	8.56 ± 0.05 a	8.58 ± 0.04 ab
	Str	8.38 ± 0.04 b	8.51 ± 0.05 ab	8.62 ± 0.08 a
	MAP	8.02 ± 0.01 c	8.37 ± 0.03 b	8.41 ± 0.04 b
	DAP	8.12 ± 0.04 cd	8.45 ± 0.03 ab	8.42 ± 0.02 ab
	APP	7.9 ± 0.04 d	8.39 ± 0.04 ab	8.45 ± 0.02 ab
2 weeks	Control	8.54 ± 0.03 a	8.58 ± 0.01 a	8.55 ± 0.01 a
	Str	8.45 ± 0.02 b	8.51 ± 0.01 b	8.49 ± 0.01 a
	MAP	8.04 ± 0.01 d	8.36 ± 0.02 c	8.40 ± 0.01 a
	DAP	8.24 ± 0.01 c	8.36 ± 0.01 c	8.44 ± 0.1 a
	APP	8.01 ± 0.01 d	8.39 ± 0.01 c	8.35 ± 0.01 a
5 weeks	Control	8.67 ± 0.01 ab	8.65 ± 0.01 a	8.60 ± 0.01 a
	Str	8.63 ± 0.09 a	8.45 ± 0.01 b	8.52 ± 0.04 a
	MAP	8.07 ± 0.02 c	8.35 ± 0.03 b	8.40 ± 0.03 a
	DAP	8.23 ± 0.05 bc	8.34 ± 0.01 b	8.37 ± 0.01 a
	APP	8.09 ± 0.02 c	8.38 ± 0.04 b	8.45 ± 0.14 a
9 weeks	Control	8.55 ± 0.02 a	8.47 ± 0.07 a	8.50 ± 0.04 a
	Str	8.46 ± 0.02 a	8.43 ± 0.03 a	8.38 ± 0.01 a
	MAP	8.24 ± 0.02 b	8.35 ± 0.01 a	8.38 ± 0.02 a
	DAP	8.24 ± 0.01 b	8.33 ± 0.01 a	8.37 ± 0.01 a
	APP	8.18 ± 0.02 b	8.37 ± 0.04 a	8.38 ± 0.02 a
12 weeks	Control	8.49 ± 0.05 a	8.51 ± 0.02 a	8.33 ± 0.03 a
	Str	8.40 ± 0.03 ab	8.44 ± 0.02 a	8.33 ± 0.01 a
	MAP	8.29 ± 0.02 bc	8.32 ± 0.02 b	8.30 ± 0.01 ab
	DAP	8.28 ± 0.01 bc	8.3 ± 0.07 b	8.19 ± 0.04 ab
	APP	8.24 ± 0.04 c	8.31 ± 0.02 b	8.29 ± 0.03 ab

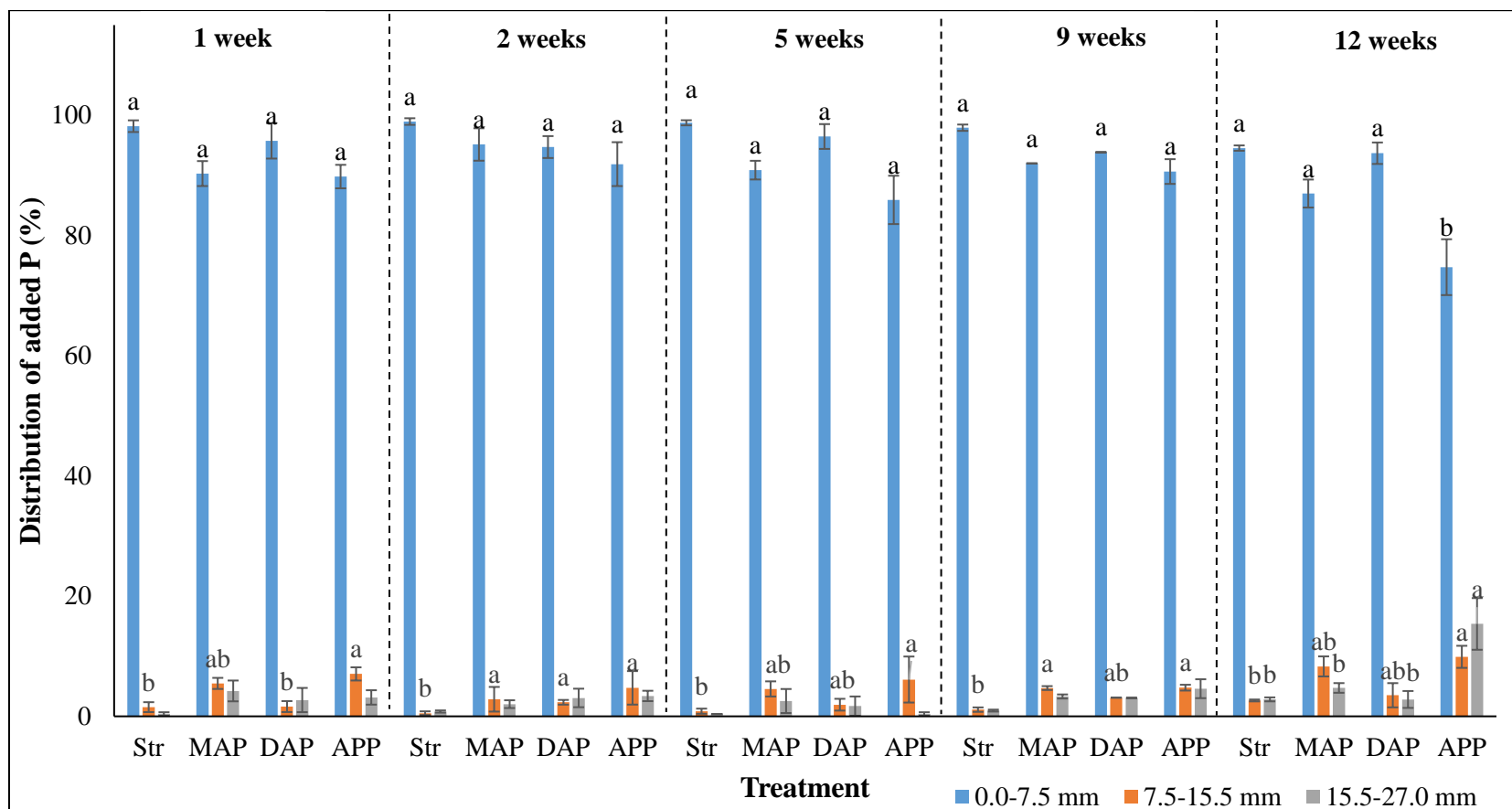


Figure 3.1. Distribution of added P (%) after 1 week, 2 weeks, 5 weeks, 9 weeks and 12 weeks of incubation in calcareous soil. Means within a soil section for each treatment in each time period containing the same letter are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

This can be explained by the initial acidulation effect of commercial P fertilizer (Figure 3.2). Struvite has an alkaline effect at the point of application (Table 3.3). The first section of struvite-treated samples showed a significant pH enhancement compared to other treatments. The alkalinity effect of struvite might limit the P diffusion at the beginning of incubation and almost all the P remaining in the first section. This is because the consuming protons during the dissolution process of struvite from the surrounding soil solution make the initial pH increase as discussed by Ahmed et al. (2018). However, after 12 weeks incubation showed 15% of added P had moved towards the second section in struvite while more than 65% of the added P in APP, MAP (62%), and DAP (61%) had moved towards the third section. Compared to calcareous soil overall P diffusion in nutrients was diffused towards the further sections in neutral soil. In neutral to calcareous soils, P retention is controlled by precipitation reactions with CaCO_3 , and this CaCO_3 in calcareous soil is the major limiting factor of P availability (Brownrigg et al., 2022). Soil texture wise neutral soil was a sandy loam while calcareous soil was a silt loam, so there was less potential of occurring adsorption reactions in neutral soil compared to calcareous soil and allowing the P distribution further away from the point of application without restrictions.

Phosphorus diffusion after 1 week of incubation showed that all the conventional fertilizers started to diffuse into the third section while almost all the added P in the struvite treatment remained at the point of application in acid soil (Figure 3.3). In MAP (73% of added P moved), DAP (71% of added P moved), and APP (63% of added P moved) treatments showed higher P mobility into the second and third sections compared to struvite (15% of added P moved) treatment up to a 5-week incubation period.

Table 3.3. The pH after 1, 2, 5, 9, and 12 weeks of incubation in neutral soil. Means within a soil section for each treatment in each time period containing the same letter are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

Incubation Period	Treatment	pH		
		0.0-7.5 mm	7.5-15.5 mm	15.5-27.0 mm
1 week	Control	6.3 ± 0.01 b	6.34 ± 0.01 a	6.28 ± 0.01 a
	Str	8.24 ± 0.1 a	6.92 ± 0.36 a	6.64 ± 0.31 a
	MAP	6.91 ± 0.17 b	6.72 ± 0.19 a	6.76 ± 0.19 a
	DAP	6.87 ± 0.17 b	6.72 ± 0.13 a	6.77 ± 0.17 a
	APP	6.82 ± 0.14 b	6.81 ± 0.17 a	7.07 ± 0.4 a
2 weeks	Control	6.68 ± 0.16 b	6.53 ± 0.06 a	6.67 ± 0.14 a
	Str	8.09 ± 0.05 a	5.98 ± 0.08 b	5.94 ± 0.11 ab
	MAP	6.24 ± 0.05 c	5.72 ± 0.05 c	5.53 ± 0.03 bc
	DAP	6.30 ± 0.06 bc	5.48 ± 0.02 d	5.07 ± 0.37 c
	APP	6.08 ± 0.05 c	5.52 ± 0.03 cd	5.42 ± 0.05 bc
5 weeks	Control	6.42 ± 0.01 b	6.18 ± 0.01 a	6.12 ± 0.01 a
	Str	7.69 ± 0.25 a	5.32 ± 0.50 a	5.49 ± 0.60 a
	MAP	5.54 ± 0.04 c	5.31 ± 0.01 a	5.25 ± 0.01 a
	DAP	5.25 ± 0.007 c	5.02 ± 0.02 a	5.02 ± 0.02 a
	APP	5.51 ± 0.02 c	5.35 ± 0.04 a	5.33 ± 0.02 a
9 weeks	Control	6.1 ± 0.01 b	6.11 ± 0.01 a	6.01 ± 0.01 a
	Str	6.77 ± 0.01 a	5.52 ± 0.006 b	5.86 ± 0.006 a
	MAP	5.41 ± 0.03 d	5.26 ± 0.02 c	5.14 ± 0.08 b
	DAP	4.94 ± 0.02 e	4.91 ± 0.04 d	4.89 ± 0.03 c
	APP	5.72 ± 0.02 c	5.68 ± 0.05 b	5.66 ± 0.06 a
12 weeks	Control	6.35 ± 0.01 b	6.13 ± 0.01 a	6.03 ± 0.01 a
	Str	7.02 ± 0.06 a	5.52 ± 0.003 ab	5.79 ± 0.02 a
	MAP	5.23 ± 0.17 c	5.06 ± 0.08 bc	5.05 ± 0.03 b
	DAP	4.91 ± 0.04 d	4.67 ± 0.16 c	4.79 ± 0.05 b
	APP	5.09 ± 0.06 cd	5.08 ± 0.04 bc	4.85 ± 0.16 b

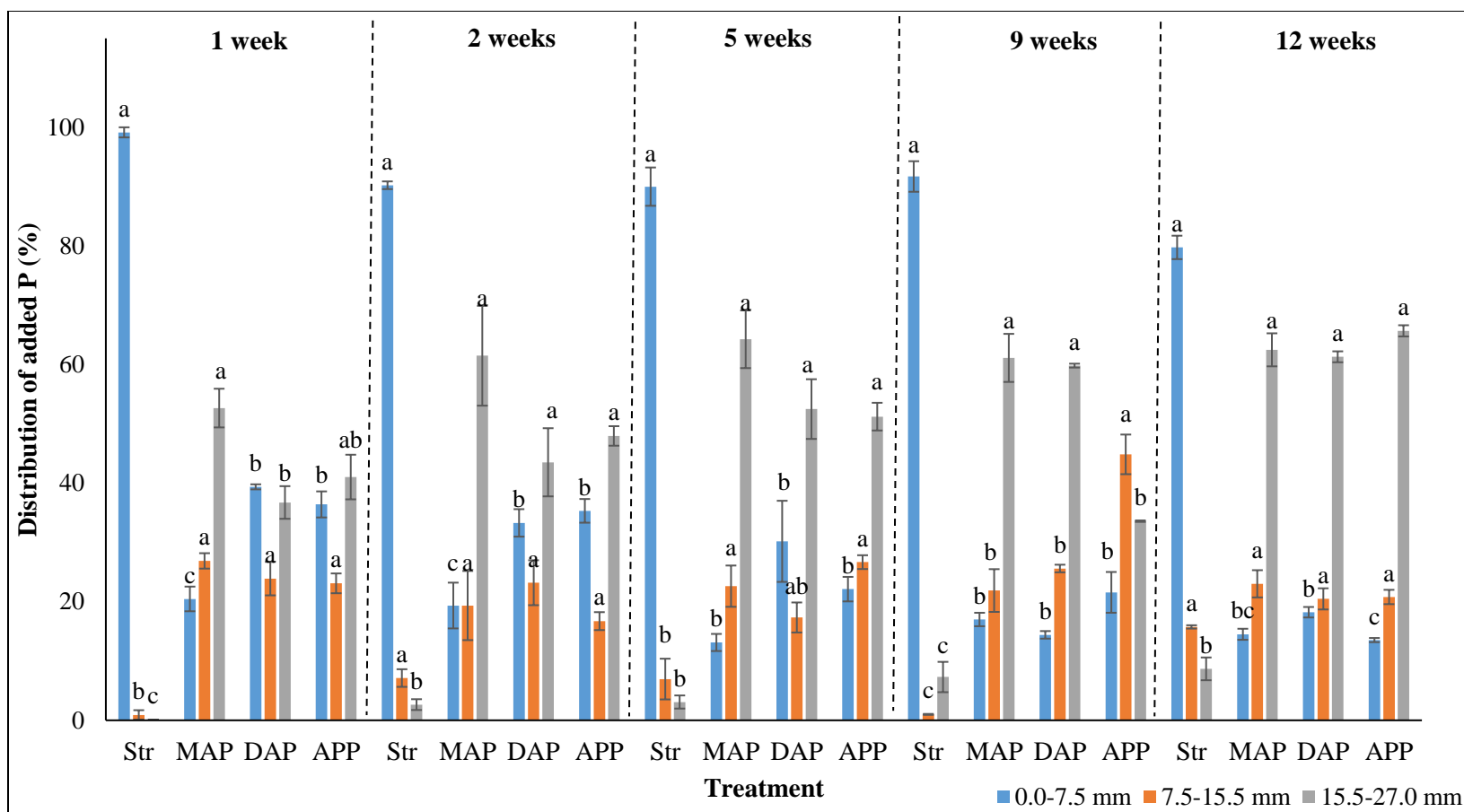


Figure 3.2. Distribution of added P (%) after 1 week, 2 weeks, 5 weeks, 9 weeks, and 12 weeks of incubation in neutral soil. Means within a soil section for each treatment in each time period containing the same letters are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

However, after 5 weeks (9 and 12 weeks) of incubation, struvite started to move (<40 % of added P) towards the further sections. At the end of 12 weeks of incubation, MAP (<75%), DAP (<73%), and APP (<65%) treatment's added P had moved further while more than 40% of added P in struvite moved into the second and third sections (Figure 3.3). The alkalinity effect of struvite might be favorable in terms of P diffusion in acid soil (Table 3.4). Since struvite precipitation occurs under alkaline conditions, dissolution rates are higher under acidic soil conditions when applied as a fertilizer source (Hertzberger et al., 2020). According to Talboys et al. (2016) when the struvite is dissolving, a potential pH increment can be observed by two pH units in acidic soil due to the consumption of two protons for its dissolution process. After 9 and 12 weeks of incubation duration, DAP showed a significantly higher percent P added (40-50%) in the third section compared to other fertilizers. When monoprotonated anion was added to the acid soil, protons were consumed and the surrounding proton concentration was reduced raising the pH (Pierzynski & Hettiarachchi, 2018). In higher pH conditions, there is less chance to solubilize Fe and Al which contributes to form precipitation with fertilizer P. So, more P diffusion could be seen in DAP and struvite treatments after 9 weeks of incubation. In addition to the pH effect, the textural condition of soils is also a major factor for P diffusion. The loamy sand texture of acid soil might be favorable for P diffusion compared to the other two soils. The study by Katakai et al. (2016) discussed that the solubility of struvite is improved under acidic conditions and increasing fertilizer efficiency of struvite. Struvite solubility can increase up to 1 - 10 mM at a pH less than pH 5.0 (Le Corre et al., 2009).

Table 3.4. The pH after 1, 2, 5, 9, and 12 weeks of incubation in acid soil. Means within a soil section for each treatment in each time period containing the same letter are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

Incubation Period	Treatment	pH		
		0.0-7.5 mm	7.5-15.5 mm	15.5-27.0 mm
1 week	Control	5.56 ± 0.05 d	5.53 ± 0.04 b	5.58 ± 0.01 a
	Str	7.81 ± 0.04 a	5.79 ± 0.2 ab	5.67 ± 0.14 a
	MAP	6.13 ± 0.09 c	5.93 ± 0.03 ab	5.93 ± 0.07 a
	DAP	6.46 ± 0.02 b	6.23 ± 0.01 a	5.8 ± 0.27 a
	APP	6.13 ± 0.07 c	5.86 ± 0.11 ab	5.86 ± 0.06 a
2 weeks	Control	5.39 ± 0.10 d	5.28 ± 0.05 d	5.37 ± 0.06 c
	Str	7.99 ± 0.06 a	5.6 ± 0.06 c	5.41 ± 0.06 bc
	MAP	6.06 ± 0.003 c	5.91 ± 0.02 b	5.69 ± 0.1 ab
	DAP	6.5 ± 0.04 b	6.31 ± 0.03 a	5.95 ± 0.06
	APP	6.23 ± 0.13 bc	5.94 ± 0.1 b	5.74 ± 0.05 a
5 weeks	Control	5.05 ± 0.07 b	5.02 ± 0.04 a	5.05 ± 0.04 a
	Str	7.42 ± 0.03 a	4.95 ± 0.04 a	4.86 ± 0.1 a
	MAP	4.84 ± 0.10 bc	4.54 ± 0.03 b	4.45 ± 0.04 b
	DAP	4.78 ± 0.04 bc	4.55 ± 0.04 b	4.5 ± 0.05 b
	APP	4.60 ± 0.06 c	4.53 ± 0.03 b	4.54 ± 0.04 b
9 weeks	Control	4.79 ± 0.05 b	4.91 ± 0.04 a	4.85 ± 0.14 a
	Str	6.12 ± 0.01 a	4.84 ± 0.04 a	4.75 ± 0.03 ab
	MAP	4.77 ± 0.04 b	4.55 ± 0.04 ab	4.51 ± 0.005 ab
	DAP	4.14 ± 0.24 c	4.37 ± 0.15 b	4.06 ± 0.3 b
	APP	4.46 ± 0.02 bc	4.42 ± 0.06 b	4.32 ± 0.13 ab
12 weeks	Control	5.16 ± 0.09 b	4.99 ± 0.06 a	5.07 ± 0.06 a
	Str	6.63 ± 0.06 a	4.86 ± 0.06 b	4.87 ± 0.006 b
	MAP	4.83 ± 0.009 d	4.64 ± 0.006 d	4.55 ± 0.006 d
	DAP	4.56 ± 0.006 e	4.49 ± 0.02 e	4.51 ± 0.003 e
	APP	4.96 ± 0.01 c	4.81 ± 0.003 c	4.65 ± 0.01 c

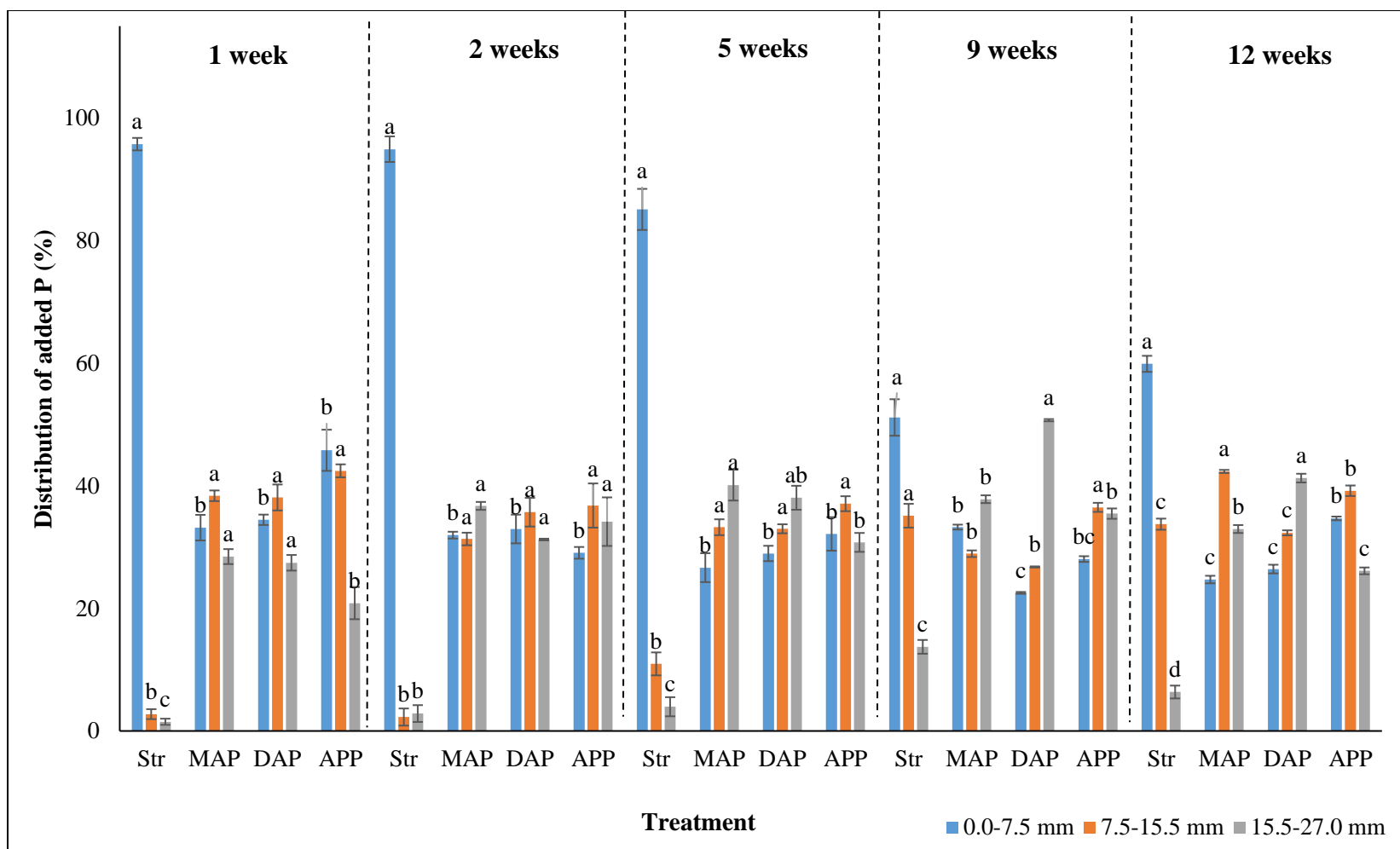


Figure 3.3. Distribution of added P (%) after 1 week, 2 weeks, 5 weeks, 9 weeks, and 12 weeks of incubation in acid soil. Means within a soil section for each treatment in each time period containing the same letters are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

Potential plant available P and pH

Plant-available P species are orthophosphate anions and potential plant-available P may be estimated indirectly by extraction procedures. Labile P in soils is anion exchange resin extractable and therefore, anion resin extractable P method mimics the soil solution/plant root model (Myers et al., 2005). The soil pH has a significant impact on P concentration, aboveground biomass, and P uptake response ratios (Hertzberger et al., 2020).

In calcareous soil, after 1 week of incubation, the highest percent resin P was in the first section of the MAP followed by struvite (Figure 3.4). Soil available P concentration (Olsen P) in struvite treatment was much lower than the MAP treatment in the first and second concentric samples under alkaline soil conditions due to slow dissolution and diffusion of struvite through the soil compared to MAP in the study of (Martens et al., 2023). A larger PRP (percent resin P) indicated the greater plant P availability (P extractability). When the soil pH is higher than pH 7.2, the diprotonated anion form (H_2PO_4^-) is converted into the monoprotonated form (HPO_4^{2-}) in the MAP treatments by releasing H^+ and the medium becomes slightly acidic, and this lower pH may facilitate the orthophosphate extractability. This effect was uncommon in the DAP treatment since it is already in monoprotonated anion form and there are no protons to donate (Weeks & Hettiarachchi, 2020). As observed in P diffusion results, P moved into the second section in MAP and APP, and labile P was higher in the second section of the same treatments. According to Lombi et al. (2004), liquid P fertilizer forms are isotopically more exchangeable and have more potential to become plant-available compared to granular P forms in highly calcareous Australian soils.

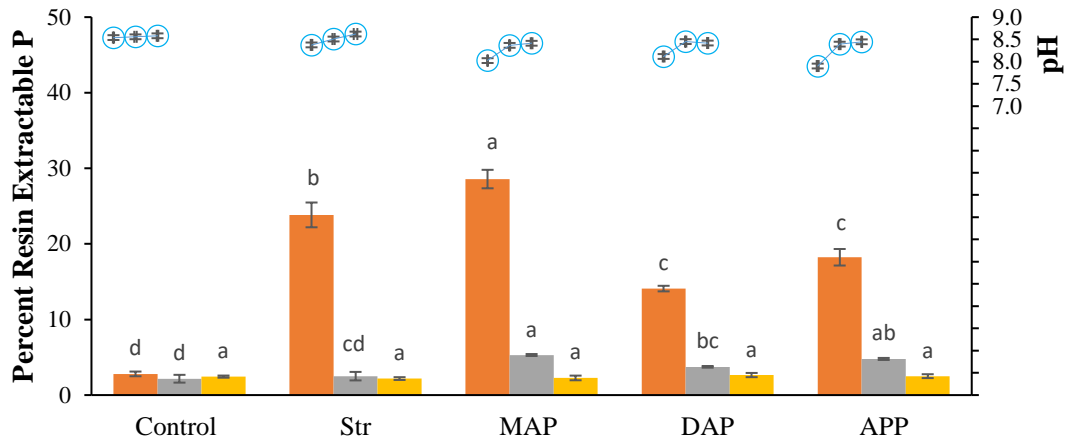
However, after 12 weeks, almost all the added P remained in the first section of struvite treatments, and higher resin extractable P was only seen in that section. Around 10% of PRP was

shown in the MAP and APP treatments in the 2nd section and around 5% in the DAP treatment. Degryse et al. (2017) discussed that higher soil solution P was observed in the first section (POA) of struvite treatment than MAP as observed after 9 weeks of this study. In conventional P fertilizers, NH_4^+ in the fertilizers goes through a nitrification process and provides protons into the medium by microbial reactions and this scenario is beneficial to maintain the lower pH in the soil after application of conventional fertilizers. According to Degryse et al. (2017), struvite addition increases the soil pH. High pH impact on low struvite solubility.

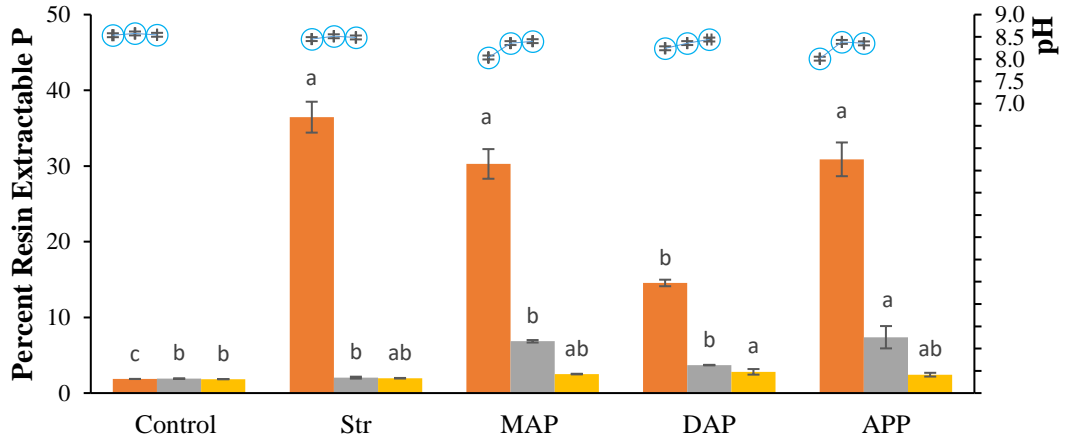
According to overall resin P extractability results (Figure 3.7a), all fertilizer treatments in calcareous soil showed (6-12 %) while control soil showed (around 2%) PRP per Petri dish. Results suggested that under alkaline and calcareous soil conditions, the available P amount was low since low struvite solubility impacted low P extractability.

In addition, the low solubility of struvite was responsible for low P concentration in soil solution. This low P concentration could be the reason for little P diffusion from the POA and restricted almost all the P there. Hence less potential available P was shown in the second and third sections of struvite's sample. A similar discussion was in Martens et al. (2023). However, under field conditions plant P uptake may be enhanced with struvite treatments over a long period since plant uptake provides a P sink and contributes to dissolving struvite continuously whereas conventional P has completed the dissolution after a certain period and plant uptake (Martens et al., 2023). A pot experiment indicated that the P recovery efficiency of wheat biomass was 8.9% for struvite while MAP was (12.4%) for MAP under Manitoba alkaline soils (Katanda et al., 2016).

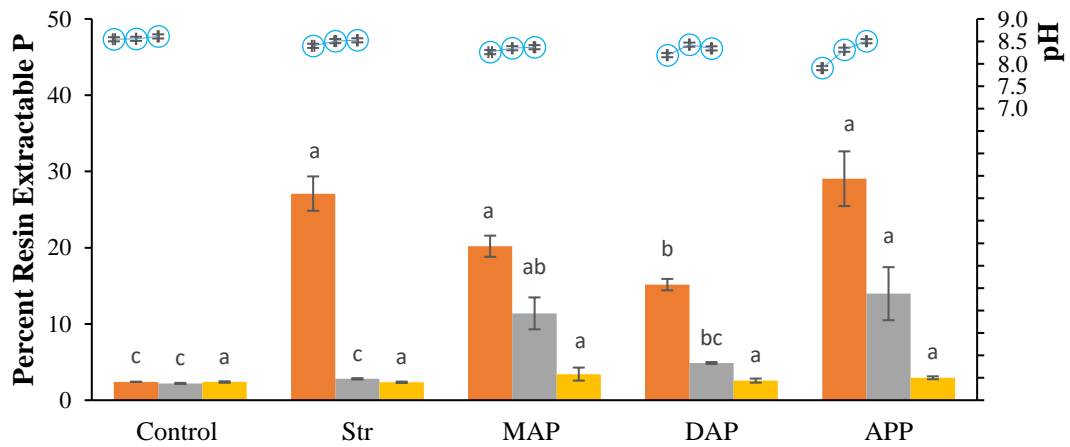
(a) 1 week



(b) 2 weeks



(c) 5 weeks



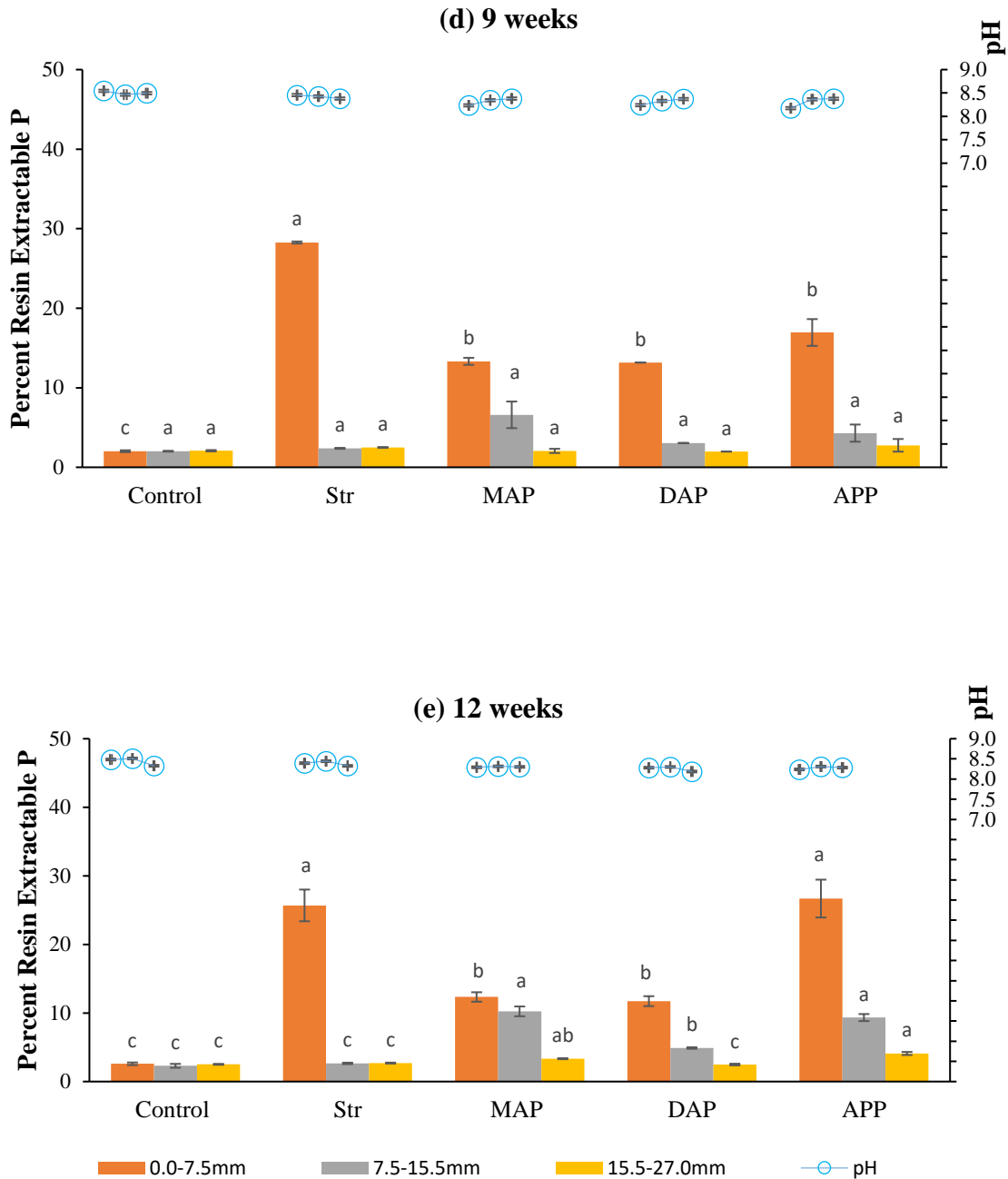
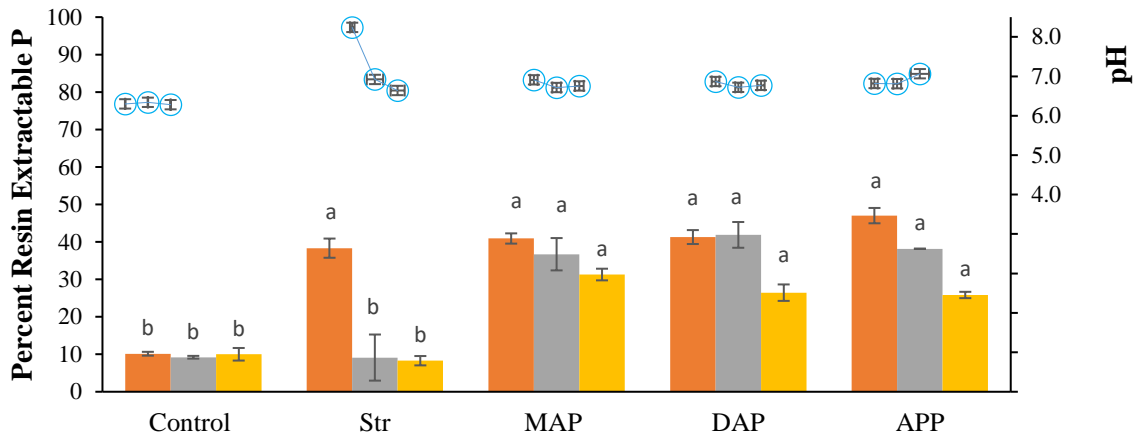


Figure 3.4. Percent resin extractable P (PRP) and pH for each dish section for all treatments after (a) 1 week, (b) 2 weeks, (c) 5 weeks, (d) 9 weeks, and (e) 12 weeks of incubation in calcareous soil. Means within each soil section for each treatment containing the same letters are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

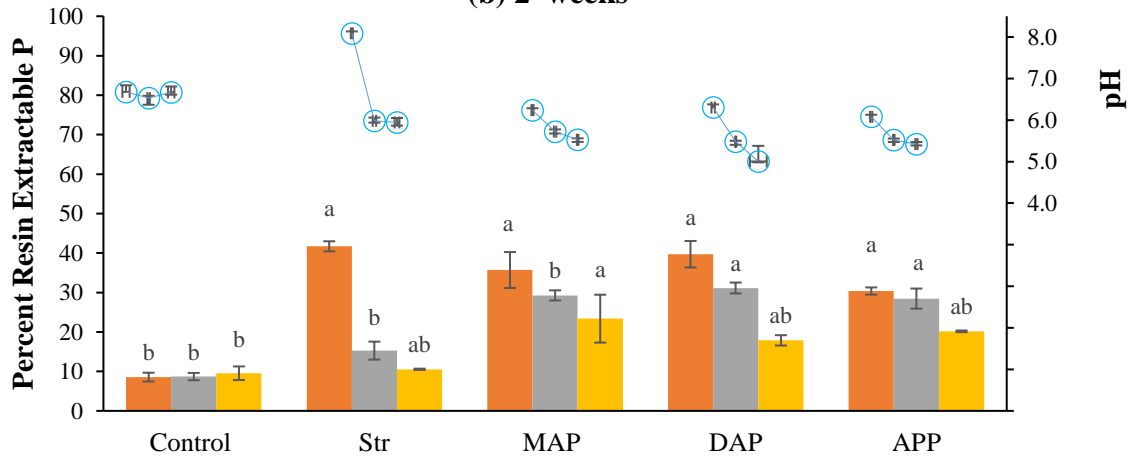
Based on the neutral soil results, after the first week of incubation, APP, MAP, and DAP showed significantly higher labile P in the second and third sections compared to struvite and control soil

(Figure 3.5). This could be due to the decreased pH effect due to the donation of protons when NH_4^+ ions nitrification and favoring the high P diffusion and extractable P amount. In the struvite samples, the first section had a higher pH compared to the control soil (Table 3.3). Similar findings were mentioned in Ahmed et al. (2018) as with the addition of struvite, the initial pH was increased and further increased with slow dissolution of struvite at the point of application. The alkaline effect of pH could be only seen in the first section and the second and third sections had lower pH since the dissolution rate is not becoming slower under neutral soil conditions. Struvite is considered most effective under moderately or low pH soils and under acidic and neutral soil conditions struvite solubility is in the range of 65-100%. That solubility range is similar to conventional P fertilizers (Kataki et al., 2016). The soil pH below 8.0 a sharp increment of struvite solubility can be seen with decreasing pH (Nelson et al., 2003). Hence higher soil solution P concentration can be seen due to fast dissolution (Degryse et al., 2017). However, in terms of resin extractable P, the highest P lability could be seen in the first section of struvite in all the time duration. The maximum labile P in the first sections was observed in struvite after 12 weeks of incubation. According to Hertzberger et al. (2020), struvite P availability is greatest under neutral pH conditions (pH around pH 6.00 to 7.00). Overall percent resin P (Figure 3.7b) ranged in all treatments from 15-48% while control soil showed around 11% in neutral soil. The overall P diffusion and resin extractable P in all treatments were higher in neutral soil since less precipitation and adsorption reactions might occur due to low pH conditions as well as sandy loam texture in neutral soil rather than high pH with free CaCO_3 and silt loam texture in calcareous soil. However, the soil solution P concentration depends on many factors such as soil and fertilizer characteristics, different struvite management practices, particle size, temperature, and granular size (Degryse et al., 2017; Martens et al., 2023).

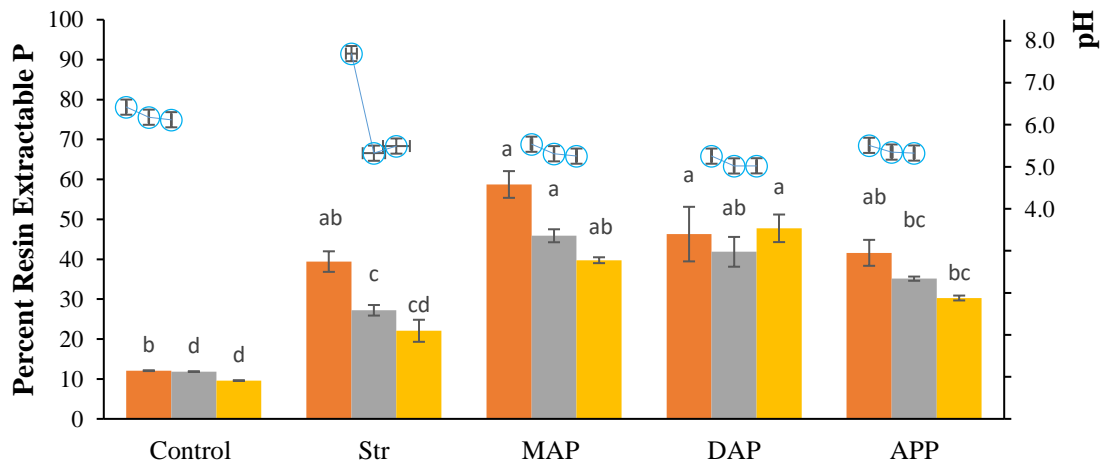
(a) 1 week



(b) 2 weeks



(c) 5 weeks



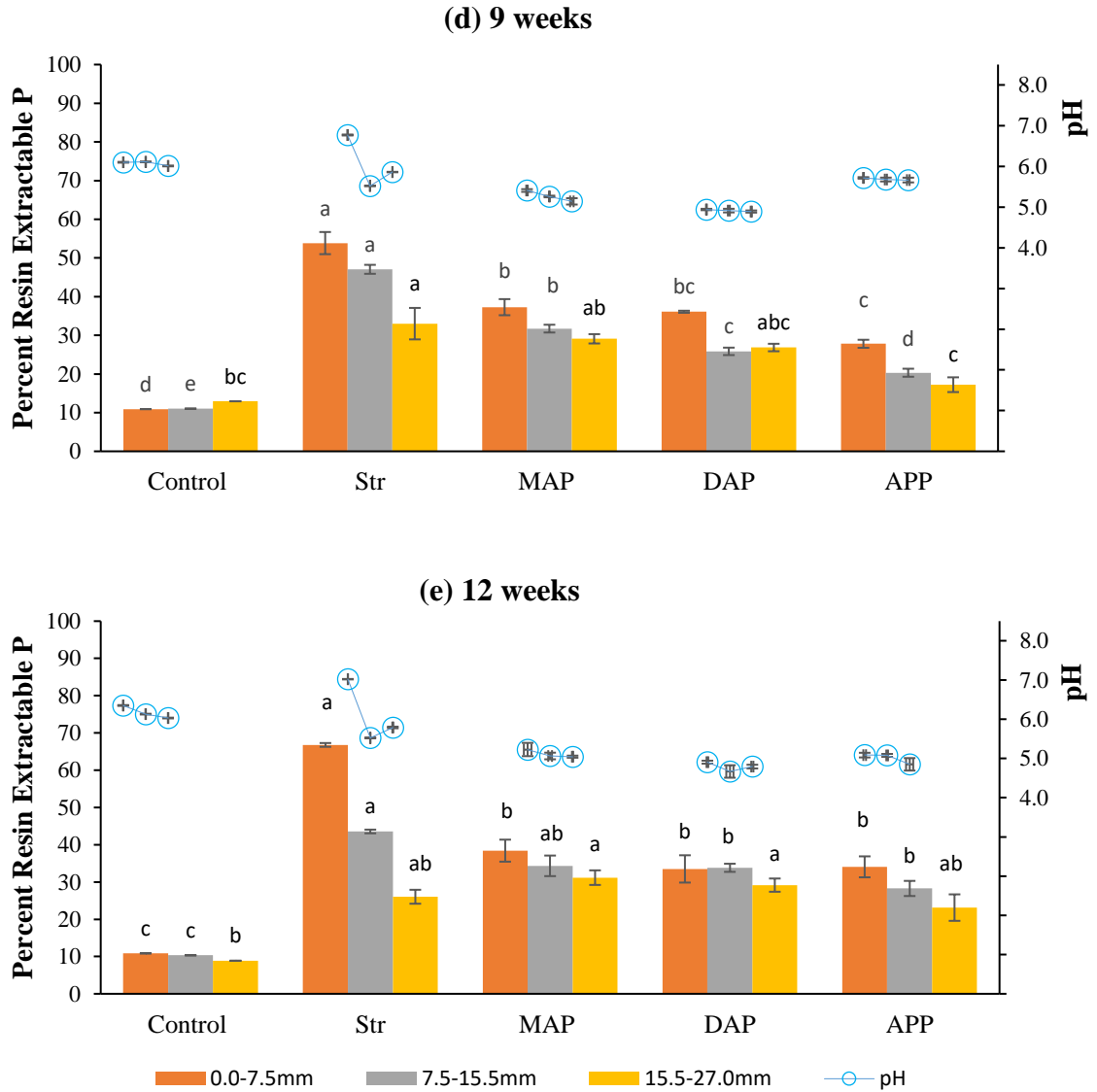
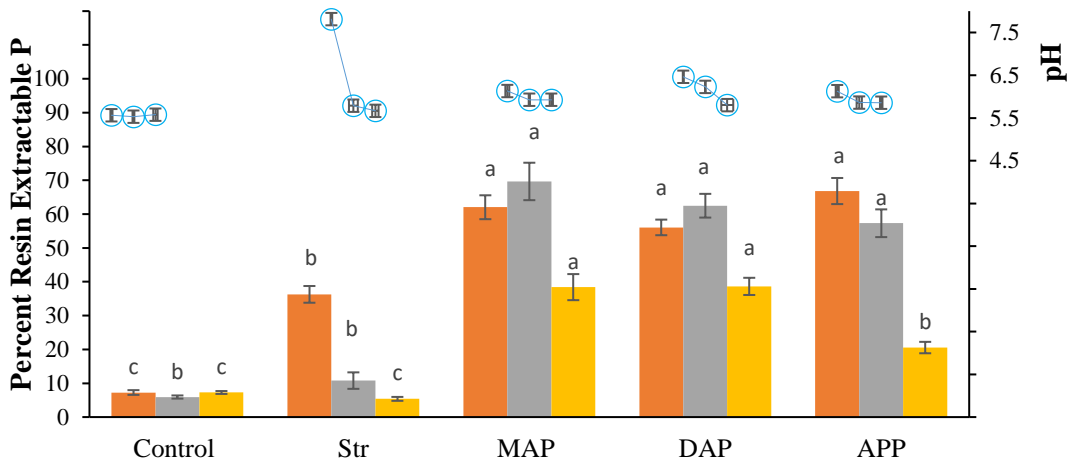


Figure 3.5. Percent resin extractable P (PRP) and pH for each dish section for all treatments after (a) 1 week, (b) 2 weeks, (c) 5 weeks, (d) 9 weeks, and (e) 12 weeks of incubation in neutral soil. Means within each soil section for each treatment containing the same letters are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

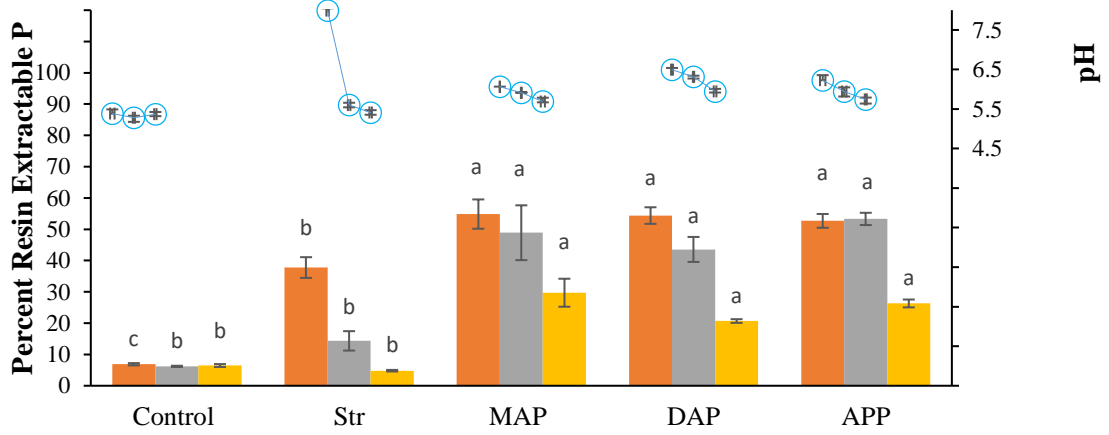
In acid soil incubation studies (Figure 3.6), after each incubation period, the pH of the first section of soil in struvite treatments increased from pH 5.00 to pH around 7.00. As discussed before, when struvite goes through a dissolution process, two protons are consumed, and pH is increased by two pH units under acidic soil conditions. Similar results were observed in (Talboys et al., 2016). However, soil pH was decreased when increasing the distance from the point of

application and all P treatments have relatively similar pH to the control soil. In conventional P fertilizers NH_4^+ nitrification is responsible for maintaining pH at lower values in the first section. In terms of resin extractability after 2 weeks of incubation, struvite showed significantly low P extractability in all three sections compared to all other conventional P fertilizers. However, after 5 weeks to 12 weeks of incubation periods, the extractability of the first and second sections of struvite was increased with time. The highest extractable P in the second section was shown in struvite treatment at 12 weeks. This is a piece of good evidence to show the slow-releasing nature of P by struvite. This might be due to the initial alkaline effect of struvite favoring the P diffusion into the second section and then enhancing the extractability in that section. At high pH, there is less chance of dissolving Fe and Al and forming precipitation with fertilizer P. Overall percent resin P ranged in all fertilizer treatments from 25-80% in acid soil while control soil showed 8% (Figure 3.7c). Especially struvite overall resin extractability has increased from 24% at the one week to 67% at the end of the 12-week incubation period. These results suggested that struvite can maintain a higher plant P availability for long periods.

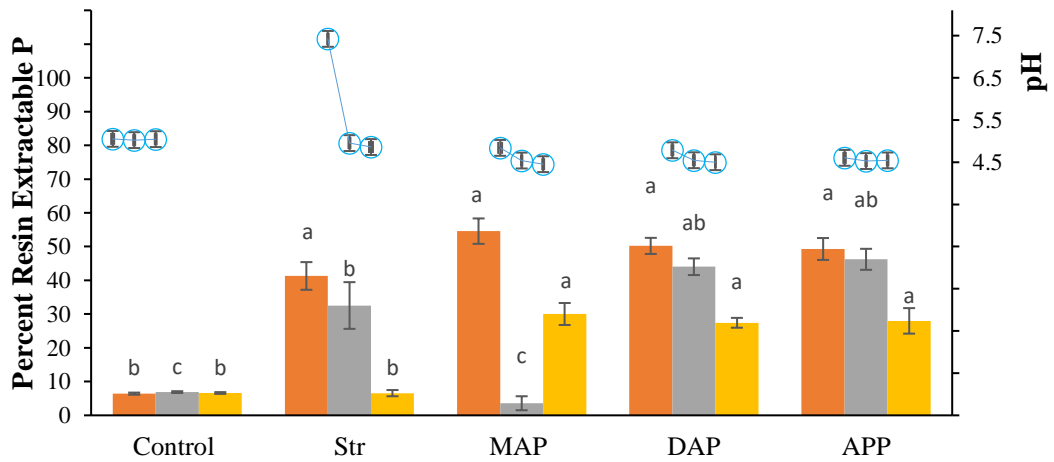
(a) 1 week



(b) 2 weeks



(c) 5 weeks



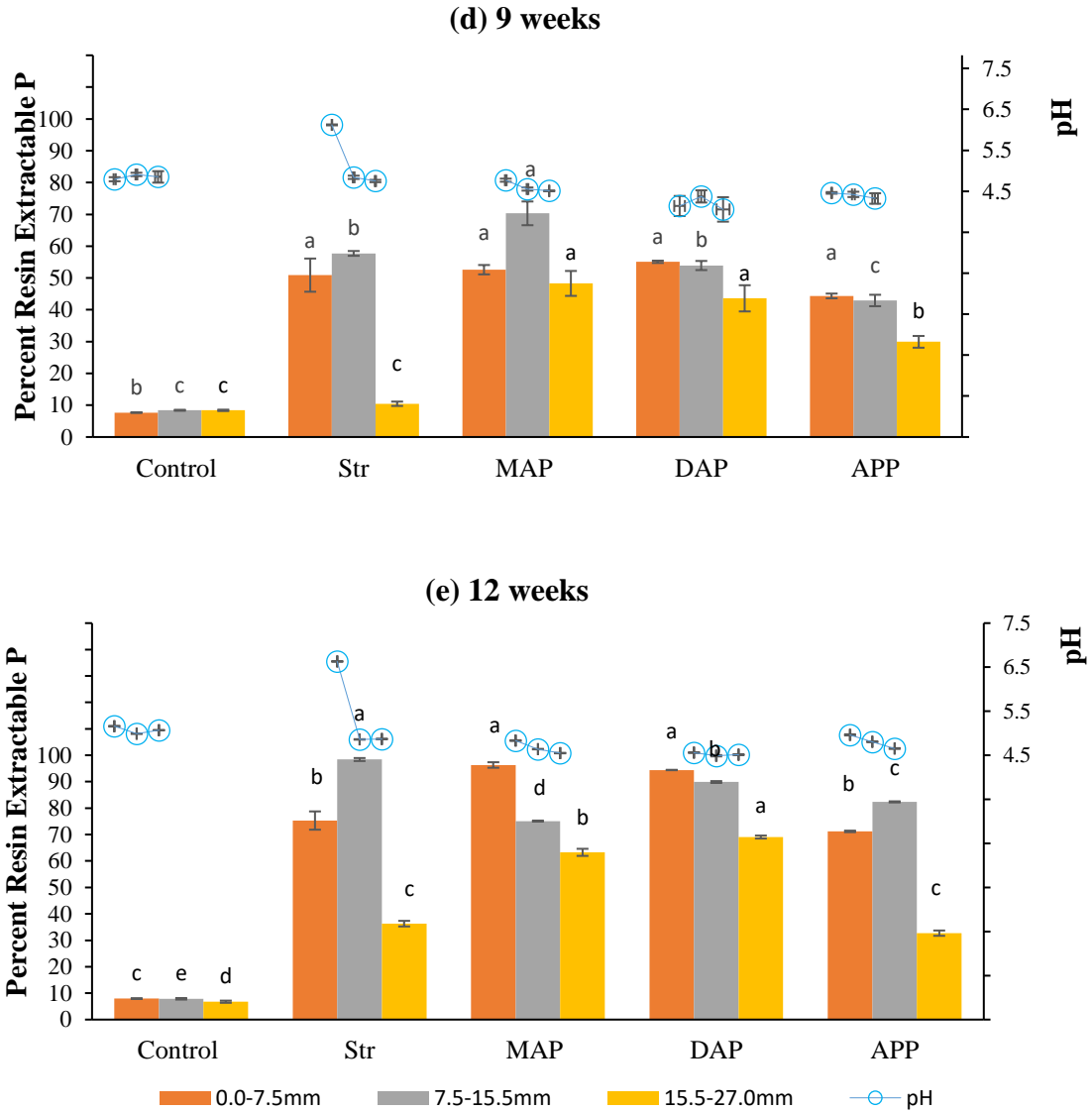


Figure 3.6. Percent resin extractable P (PRP) and pH for each dish section for all treatments after (a) 1 week, (b) 2 weeks, (c) 5 weeks, (d) 9 weeks and (e) 12 weeks of incubation in acid soil. Means within each soil section for each treatment containing the same letters are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

Percent resin P per Petri dish

The present resin P was calculated per petri dish after incubation period for each treatment in calcareous, neutral and acid soils.

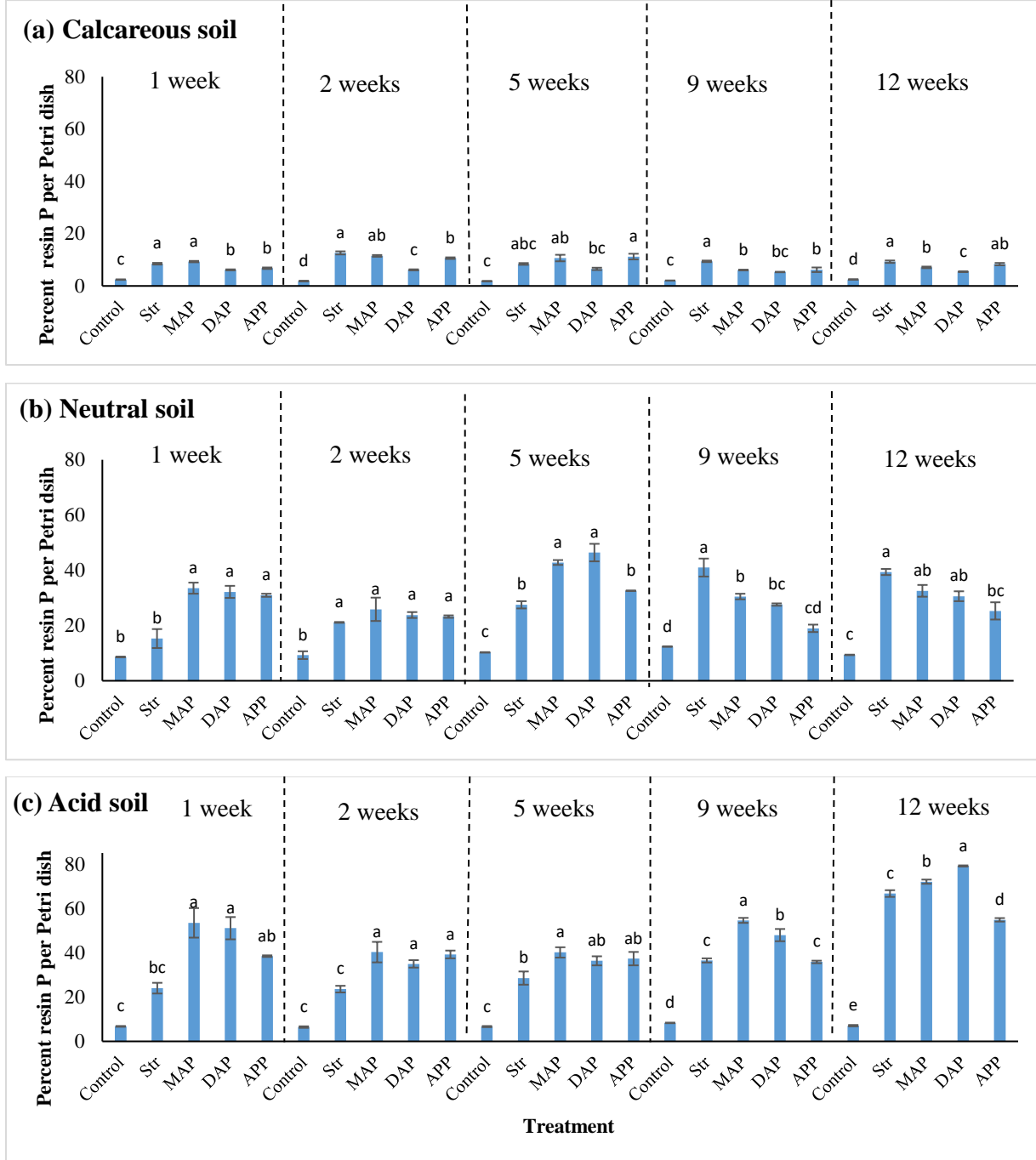


Figure 3.7. Percent resin extractable P (PRP) per Petri dish for all treatments after 1 week, 2 weeks, 5 weeks, 9 weeks and 12 weeks of incubation in (a) calcareous, (b) neutral and (c) acid soils. Means within each treatment for each time period containing the same letters are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

Reaction products of P

In calcareous soil, nonfertilized control soil was dominated by sparingly soluble ca-P species such as hydroxyapatite and calcium pyrophosphate (Figure 3.8). Struvite treated sample was dominated by reclaimed struvite. In addition, CaCO_3 sorbed P and hydroxyapatite minerals were observed in struvite samples. The reduced P diffusion can be explained by the speciation results. The DAP treatment was dominated by insoluble Ca-P species and CaCO_3 adsorbed P while MAP and APP treatments consisted of relatively more soluble Ca-P species (monocalcium phosphate monohydrate) in addition to insoluble Ca- P species. However, APP treatment contained a more soluble Ca-P mineral percentage than MAP, likely owing to differences in P species and their crystallinity. The P speciation results explained the reason behind higher P mobility (Figure 3.1) and extractability (Figure 3.4) in APP treatment was due to more soluble Ca-P minerals in APP treatment. The XANES speciation data gives a better insight into the reasons behind low P mobility and P extractability in calcareous soils. Analyses of XANES data for other soils are underway.

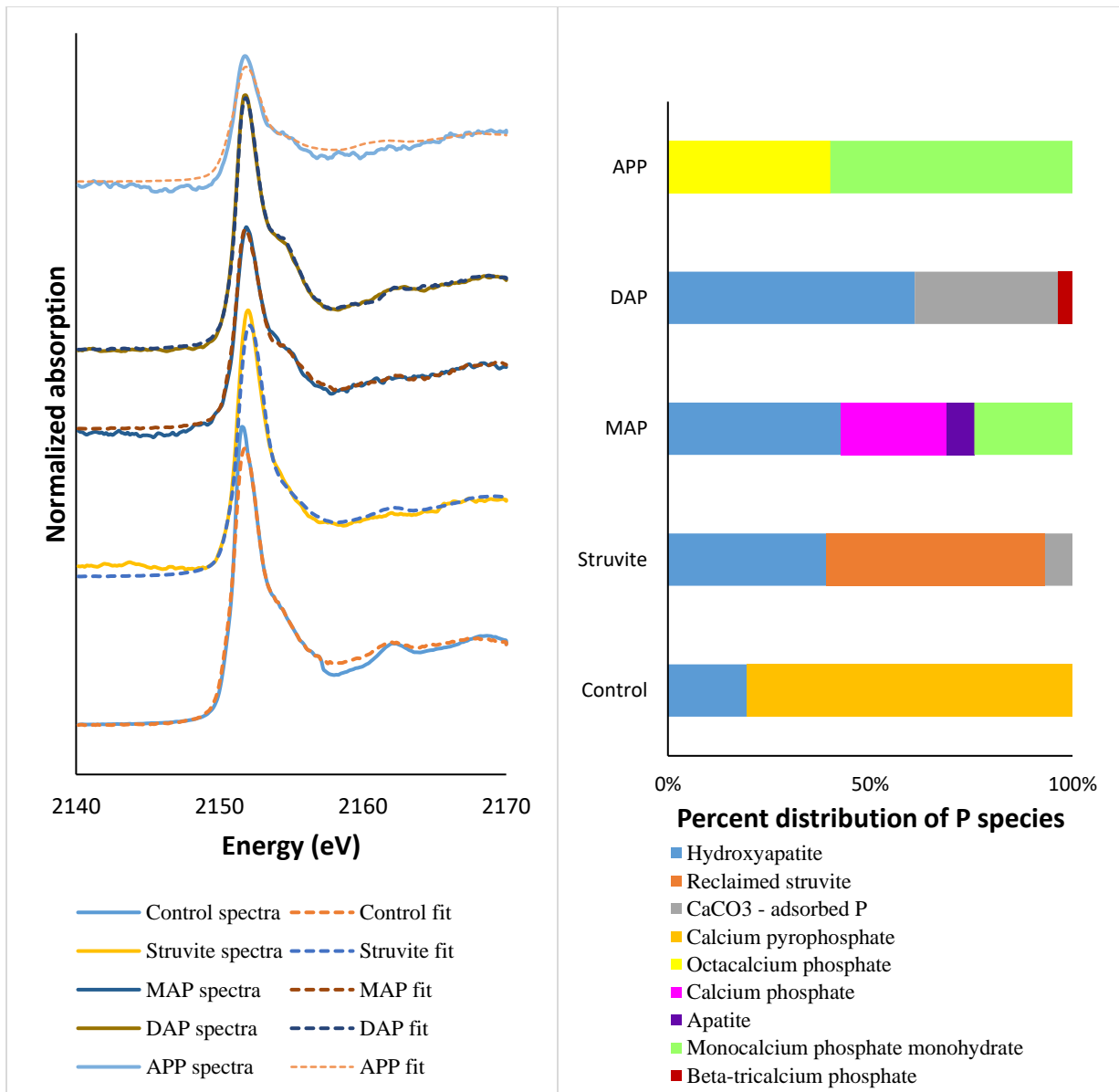


Figure 3.8. Normalized P K-edge X-ray absorption near edge structure spectra of center sections (0-7.5mm) of each treatment after 5 weeks incubation period with results of linear combination fitting as a percent distribution of P species in calcareous soil.

Summary

The findings of this study showed that reclaimed struvite is a potential P source for all three soils. Struvite especially acted better under neutral and acidic soil conditions than the mildly calcareous soils. The XANES data gives a better insight into the fate of P fertilizers in calcareous soils. A greater portion of struvite did not dissolve. Most of the added P transformed into insoluble Ca-P minerals and adsorbed into CaCO_3 in struvite and DAP treatments. In terms of P mobility and lability APP and MAP are best-suited fertilizers under calcareous soil conditions than DAP or struvite. Struvite showed comparable performance to all the conventional P fertilizers under neutral and acidic soil conditions. As a recycled P product, struvite may be a good alternative P fertilizer for sustainable agriculture due to its slow-releasing P nature under specific soil conditions. Other than the pH and soil texture, struvite granular size, plant species, and soil native P can influence struvite dissolution and potential plant uptake (Hedley & McLaughlin, 2015). Further research is needed to investigate how struvite solubility and P extractability change when blended with conventional P fertilizers with appropriate ratios to get the maximum plant growth.

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Chapter 4 - Impact of added rhizosphere- low molecular weight organic acids (LMWOAs) on struvite P dissolution, mobility, and potential plant bioavailability and fate of LMWOAS in three soils with contrasting pH

Abstract

Struvite is a reclaimed byproduct of wastewater treatment plants that has the potential to be used as an alternative P fertilizer for agricultural purposes. The low solubility of struvite is disadvantageous under certain soil conditions, especially in the early growing seasons of some crops. To overcome these issues, struvite can be co-applied with low molecular weight organic acids (LMWOAs), naturally exuded by plant roots into the rhizosphere, or rely solely on root exudates by crop plants. The main goals of this study were to investigate the impact of LMWOAs on struvite P solubility, mobility, and potential plant bioavailability relative to non-LMWOAs added struvite under mildly calcareous, neutral, and acidic soil. To gain better insight, we also determined the fate of LMWOAs in neutral and mildly calcareous soil using short-term laboratory incubation studies. Three different soils were incubated for 1, 5, 9, and 12 weeks in Petri dishes using struvite (Str) with and without LMWOAs addition with a no P control treatment. Citric acid (CA), oxalic acid (OA), malic acid (MA), and acetic acid (AA) were used as LMWOAs. Those were added as single organic acid at two rates (41mM & 82mM) or as an acid mixture (CA+ OA or CA + OA+ MA + AA) at one rate (82mM). At the end of the incubation, dishes were sectioned into three concentric rings at different distances from the point

of application (POA). Soil pH, resin extractable P to assess potential plant available P, and total P to determine P diffusion from the POA were analyzed. A complimentary short-term incubation study was designed to determine the fate of LMWOAs in mildly calcareous and neutral soils with similar treatment additions as mentioned above and incubated over one week. At the end of incubation, dissolved organic carbon (DOC) was extracted and analyzed. The X-ray absorption near edge structure spectroscopy was used to determine the reaction products of P in calcareous soil. Results showed the added organic acids in calcareous soil had less impact on struvite solubility, mobility (0- 10% of added P diffusion) into second and third sections, and P extractability (8-11% of PRP per Petri dish) due to microbial degradation and sorption reactions while neutral and acid soils showed the highest overall P diffusion (2-54% and 5-52%) into second and third sections and P extractability (15-57% and 25- 79% of PRP per Petri dish) respectively. Among organic acid treatments, Str + CA + OA + MA + AA High treatment showed superiority in P diffusion (20-40% vs 40-50% of added P movement into second and third sections) in neutral and acid soils respectively. This is because the mixture of ligands is more successful in chelation and ligand exchange-promoted P dissolution reactions. The overall P availability (PRP per Petri dish) of Str + OA High was high (25-57%) in neutral soil while Str + CA High was high in acid soils (30-71%). This is likely because CA promotes P release through the chelation of Al and Fe in acid soils while OA promotes P release in less acidic/neutral soils due to the high acidic strength of oxalic acid. The XANES data provided a clear insight that the added organic acids did not significantly change the P speciation in calcareous soil. Future studies with plants will give better insights into how these treatments impact crops.

Introduction

Phosphorus (P) is an essential element for all living organisms (Shim et al., 2020). The addition of P fertilizers is used for yield intensification in agriculture. Mineral P fertilizers (e.g., ammonium phosphates and superphosphates) are manufactured from non-renewable rock phosphate (Hertzberger et al., 2020) and they are highly soluble and increase potential environmental risks, such as eutrophication and water quality issues, due to the excess P addition into surface waters (Martens et al., 2023). To overcome the scarcity of natural rock phosphate reserves and limit the runoff losses of P (i.e., to close the P cycle), a wastewater-recovered nutrient product called struvite was investigated as a potential P fertilizer (Antonini et al., 2012; Talboys et al., 2016). It was proposed because of its low solubility compared to highly soluble phosphate mineral fertilizers (Hertzberger et al., 2020). Later, fertilizer-grade struvite came to the market under different trade names as slow-releasing fertilizer. Most of the struvite is reclaimed from wastewater treatment plants by precipitation (Talboys et al., 2016). Even though struvite has potential benefits such as high P content (generally remains 11-26%), ease of spread, and minimal potentially toxic trace element content, plants show some P deficiency, especially in the early growing seasons, due to their slow dissolution rates in soils (Antonini et al., 2012; Kataki et al., 2016). The struvite dissolution rate was in the range of 0.1 to 0.5 mM initial P /day (Talboys et al., 2016). However, it has been pointed out by the nutrient recovery industry and various researchers that organic acids secreted naturally by plants into the rhizosphere have the potential to increase struvite solubilization and P release compared to control soils without organic acids (Èm et al., 2001; Ström et al., 2005; Talboys et al., 2016).

Organic acids can be categorized into two main types based on their size: high molecular weight organic acids (HMWOAs) and low molecular weight organic acids (LMWOAs). The LMWOAs have significant importance in terms of favoring struvite solubility by modifying rhizosphere conditions (Oburger et al., 2009). Low molecular weight organic acids are identified as a natural modifier for increasing soil solution P concentration and hence limit the overapplication of fertilizer during agriculture production. Additionally, LMWOAs enhance the availability of soil phosphorus through various mechanisms. These include anionic organic acids competing with phosphate anions for P binding sites, increasing the solubility of P-containing compounds and minerals (thereby solubilizing recalcitrant P compounds), altering the surface of soil particles, and forming complexes with cations capable of forming P compounds (Thammaiah et al., 2023). Talboys et al. (2016) found that when a 1mM concentration of organic acids (acetic, oxalic, malic, and citric) was present in soil solution, the initial rate of dissolution and equilibrium P concentration significantly increased by up to 69% and 39%, respectively. The greenhouse experiments of Talboys et al. (2016) have discovered that plant P uptake from struvite was similar to diammonium phosphate (DAP) when growing buckwheat (*Fagopyrum esculentum*) plant species. Buckwheat has the potential to exude a large quantity of organic acids from its roots into the rhizosphere (Jian Zheng et al., 1998). The concentration of low molecular weight organic acids in plants depends on the microbial processes and plant species. Sometimes it ranges from micromoles to millimolar concentrations due to different root exudates (Fox & Comerford, 1990). The research found that no adverse effect occurred to soil properties even after the application of LMWOAs. This is because of the rapid microbial degradation of LMWOAs in soils (Adeleke et al., 2017). So, adding organic acids to the soil can be considered a sustainable agricultural practice. However, this scenario is ideal when the plant roots are there

since plant roots have the potential to exude organic acids continuously into the rhizosphere. Some studies have shown that LMWOAs are applied externally to enhance P solubilization, P fertilizer use efficiency, and plant growth. However, the efficiency of those processes declined due to the process of microbial-mediated decomposition of LMWOAs in soils (Thammaiah et al., 2023).

Dissolved organic carbon (DOC) is an important component of solutions in terrestrial and aquatic ecosystems. It influences acidity, nutrient availability, toxicity, and transport of metal contaminants in soils. Dissolved organic carbon contains a variety of organic compounds, from simple sugar molecules, and short chains of acids to complex organic compounds such as fulvic and humic acids (Dalva & Moore, 1991; Moore T.R et al, 1992). In streams and groundwater, DOC concentration in soil solution ranges from 2 to 10mg/L except where the water reservoir is dominated by wetlands (Dalva & Moore, 1991). Dissolved Organic carbon concentration is higher in rhizosphere soils relative to bulk soil (Jones, 1998). When organic acid concentration decreases DOC content is also decreased due to microbial decomposition of organic acids. During the decomposition process, a portion of carbon is released into the atmosphere as CO₂, and another portion is incorporated into new cell biomass (Jones et al., 1996; Jones & Willett, 2006).

However, due to a lack of information regarding the mechanistic understanding of how struvite behavior (solubility, P mobility, and extractability) changes under different soil conditions and under which circumstances struvite can be used as a potential P fertilizer, this study was designed under controlled laboratory conditions.

In this study, reclaimed struvite (Crystal Green: Ostara Nutrient Recovery Technologies) was used as the struvite source. The citric acid (CA), oxalic acid (OA), malic acid (MA), and acetic

acid (AA) were used as LMWOAs mainly found in the rhizosphere to understand the struvite solubility in a controlled environment with the addition of LMWOAs (without plants). The overall aims of this study were to investigate and compare the dissolution, transformations, and potential plant P availability of struvite with and without LMWOAs in mildly calcareous, neutral, and acid soils using short-term incubation studies with wet chemical analysis and synchrotron-based X-ray absorption near-edge structure spectroscopy technique. In addition, a complimentary short-term incubation study was designed to determine the fate of LMWOAs in mildly calcareous and neutral soils based on changing dissolved organic carbon (DOC) content in soils.

Materials and methods

Soil Collection

The same soils described in Chapter 3 were used for these studies. They were mildly calcareous soil (Ulysses silt loam) from Oakley City, KS, Neutral soil (sandy loam) from Ashland Bottom, KS, and Acid soil (loamy sand) from Piracicaba, São Paulo, Brazil. The surface soils (0- 20cm) were collected, carefully broken down the large aggregates, air dried, and sieved by 2mm by stainless steel mesh/sieve.

Soil characterization

Soils were characterized as described in Chapter 3. Sieved soils were used for incubation studies.

Main incubation study

Study design

The same setup of incubation study was designed for all three soils (mildly calcareous, neutral, and acid soil) for four different incubation periods (1 week, 5 weeks, 9 weeks, and 12 weeks) as separate incubation studies. The study design was a completely randomized design (CRD) with eight treatments including no P fertilizer (control) with four replicates. This study was composed

of a total of 32 Petri dishes per time duration, hence 128 dishes per soil and altogether 384 dishes (54mm diameter and 13.9 mm height).

Experimental setup and method

The same three soils were used for the study as in Chapter 3. The soil was evenly packed into the dishes to achieve a uniform bulk density of 1.05 kg m^{-3} , 1.05 kg m^{-3} , and 1.05 kg m^{-3} for mildly calcareous, neutral, and acid soils, respectively, after prewetting. After packing soil was brought to 55% of MWHC. Petri dishes were closed with the top covers, edges were wrapped using parafilm to mitigate moisture loss, and dishes were covered with aluminum foil to prevent light exposure and inverted to equilibrate overnight (24 hours) at room temperature ($\sim 25^\circ\text{C}$). The following day, six organic acids were added homogeneously to the soil surface of the relevant dish to mimic the rhizosphere conditions. The organic acid treatments were:

- i. Citric acid (41 mM or 12.5 mmol/kg of soil) – CA Low
- ii. Citric acid (82 mM or 25 mmol/kg of soil) – CA High
- iii. Oxalic acid (41 mM or 12.5 mmol/kg of soil) – OA Low
- iv. Oxalic acid (82 mM or 25 mmol/kg of soil) – OA High
- v. Citric and oxalic acid mixture (in 1:1 molar ratio) (82 mM or 25 mmol/kg of soil) – CA + OA High
- vi. Citric: oxalic: malic: acetic acid mixture (in 1:1:1:1 molar ratio) (82 mM or 25 mmol/kg of soil) – CA + OA + MA +AA High

Then the fertilizer treatments (struvite) were carefully introduced into the center of the dish except for the control soil. A single struvite granule ($37.64 \pm 0.05 \text{ mg}$) was added at a rate of 4.6mg P/Petri dish except for no P control. The eight treatments (with four replicates) used in this study were:

- i. Control soil (no P) – Control
- ii. Struvite without organic acids – Str
- iii. Struvite with citric acid (41 mM) - Str + CA Low
- iv. Struvite with citric acid (82 mM) – Str + CA High
- v. Struvite with oxalic acid (41 mM) – Str + OA Low
- vi. Struvite with oxalic acid (82 mM) – Str + OA High
- vii. Struvite with citric and oxalic (82 mM) – Str + CA + OA High
- viii. Struvite with the citric, oxalic, malic, and acetic acid mixture (82 mM) – Str + CA + OA + MA + AA High

After adding the treatment, dishes were closed and covered individually with parafilm and stacked. Stacked dishes were wrapped in aluminum foil and incubated for (1 week, 5 weeks, 9 weeks, and 12 weeks) inside the incubator (Precision Low Temp Incubator, Waltham, MA) in the dark at 25 °C. At the end of each incubation period, dishes were opened and sectioned into three concentric rings with distances from the point of application (application 0-7.5mm, 7.5-15.5mm, and 15.3-27.0 mm). Each sample (ring) was placed into a pre-weighed plastic specimen container (Fisher Scientific, Waltham, MA). Then the samples were oven-dried at 40 °C (Fisher Scientific drying oven, Waltham, MA). The final weight of the samples was recorded after drying. The soil was ground gently with a mortar and pestle and ground soil was used for future analysis.

Total P for all samples was determined by aqua regia digestion (without H₂O₂ pretreatment) and analyzed using ICP-OES (Agilent 5800) as described in Chapter 3.

Total P content data was normalized by calculating the percentage of P added (PPA) for each dish section for all treatments according to Hettiarachchi et al. (2010) as below:

$$PPA = \frac{(P_f)S_i \times M_i}{\sum_{i=1-3} [(P_f) S_i \times M_i]} \times 100$$

Where i is the dish section (1–3), $(P_f)S_i$ is the concentration of P fertilizer in each dish section, and M_i is the soil mass in each dish section. The $(P_f)S_i$ is calculated by subtracting the total P concentration of the unfertilized soil sample from the total P concentration in the fertilized dish section.

The potential plant available P was determined using anion exchange resin (AER) membranes according to Myers et al. (2005). Extracted orthophosphate ions were quantified by colorimetric method using a Beckman-Coulter DU-800 spectrophotometer (Brea, CA) (Murphy & Riley, 1962). Then the percentage of resin extractable P (PRP) for each section of the dish for all the treatments was calculated based on the equation described in Pierzynski and Hettiarachchi (2018) below according to Chapter 3:

$$PRP = \frac{REP_i}{Total P_i} \times 100$$

Where i is the dish section (1-3), REP_i is the resin P concentration and $Total P_i$ is the total P concentration in the i^{th} dish section.

The percent resin P was calculated per Petri dish after each incubation period for each treatment in calcareous, neutral and acid soils. Sum of resin P in each dish section (Section 1,2, and 3) was divided by sum of total P in each section and calculated the percentage.

The XANES analysis

The XANES analysis was performed for the composite samples of 0 – 7.5 mm section for five weeks incubated mildly calcareous soil (Oakley City, KS) samples. Five selected treatments were used to prepare composite samples (control soil, Str, Str + CA High, Str + OA High, and

Str + CA + OA + MA + AA High). The soil pellets were prepared using the composite samples (using equal masses of soil from four replicates) and carefully mounted on the sample holder with double-sided carbon tape (SPI Supplies, West Chester, PA) and placed into a helium-filled chamber for analysis. The P K-edge XANES spectra of soil samples were collected at the beamline NSLS – II- 7- ID-2, Brookhaven National Laboratory, Upton, NY. All K-edge P spectra were collected in partial electron yield (PEY) mode. Two to three scans were collected for each P-treated sample and control sample at a range of 2.1 to 2.3 keV.

Background correction, normalization and linear combination fitting of the spectra were performed using Athena (v.0.9.25) software as described in Chapter 3.

Complementary incubation study to determine the fate of LMWOAs using DOC content

Study design

To determine the fate of organic acids after bringing soils into the desired moisture condition, an additional petri dish incubation study was performed using two types of soils (mildly calcareous and neutral soils) and incubated over a one-week incubation period by adding organic acids in two different ways: one day after packing and one week after packing.

A short-term petri dish incubation study was performed based on a completely randomized design (CRD) with a 7 x 2 factorial design with seven fertilizer treatments with no P fertilizer (control) treatment with two ways of organic acids addition for 2 types of soil with 3 replicates. This study was composed of 24 dishes per one time of organic acid addition, 48 dishes per soil type, and altogether 96 dishes (54mm diameter and 13.9 mm height).

Experimental setup and method

The soil was packed to achieve a uniform bulk density of 1.05 kg m^{-3} and 1.05 kg m^{-3} for calcareous and neutral soils respectively. The soil was prewetted and packed evenly. After packing soils were brought to 55% of MWHC, the Petri dishes were closed with the top covers, edges were wrapped using parafilm to mitigate moisture loss, and the set of dishes was covered with aluminum foil to prevent light exposure and inverted to equilibrate overnight (24 hours) and one week at room temperature ($\sim 25^\circ\text{C}$).

The organic acids and fertilizer treatments were added into one set, one day after packing (one day after bringing to the desired moisture content). The organic acids and fertilizer treatments were added into another set, one week after packing with mildly calcareous soil. The same procedure was followed for the neutral soil.

For all the dishes, the fertilizer treatments (struvite) were carefully introduced into the center of the dish after the addition of organic acids into the soil surface homogeneously. Struvite granules were added at a rate of 4.6mg P/Petri dish. The eight treatments replicated four times consisted of no-P control, reclaimed struvite without organic acids (Str), reclaimed struvite with citric acid (41 mM) (Str + CA Low), reclaimed struvite with citric acid (82 mM) (Str + CA High), reclaimed struvite with oxalic acid (41mM) (Str + OA Low), reclaimed struvite with oxalic acid (82 mM) (Str +OA High), reclaimed struvite with citric + oxalic acids (82mM) (Str + CA +OA High) and reclaimed struvite with citric + oxalic +malic + acetic acids (82 mM) (Str + CA + OA +AA High).

After treatment was added dishes were closed and covered individually with the parafilm and stacked. Stacked dishes were wrapped in an aluminum foil and incubated over a one-week incubation period inside the incubator (Precision Low Temp Incubator, Waltham, MA) in the dark at 25°C . At the end of each incubation period, Petri dishes were opened. Moist soil (2.5 g)

was taken from the center of each petri dish and added to the Erlenmeyer flask (125 mL). Then MiliQ water (12.5 mL) was added into the flask and closed the top of the flask by parafilm to mitigate evaporation losses (Jones & Willett, 2006). Flasks were shaken at 200 rev pm for 60 minutes using a digital orbital shaker and after 10 minutes samples were filtered through Whatman No.42 filter paper and then 0.45 microns syringe filters. DOC content was analyzed using the direct method using TOC- L analyzer (Shimadzu).

Statistical analysis

The statistical analysis was performed using SAS version 9.4 software (SAS Institute Inc., Cary, NC, 2020). The experiment design of the main incubation study was completely randomized. Data were analyzed by ANOVA using PROC MIXED as a split-plot arrangement, the main plot as the fertilizer treatment, and the subplot as the dish section.

A complimentary incubation study was designed into a completely randomized design with 8 x 2 full factorial treatments. For both studies, Tukey's HSD test was performed to compare all the treatments at a $p = 0.05$ significance level.

Results and Discussion

Main incubation study

Soil pH

The pH of each soil section after each incubation studies are shown in the tables (4.1, 4.2 and 4.3). Soil pH changes are discussed below under diffusion and resin extractable P sections.

Diffusion of P

The P diffusion results in calcareous soil (Figure 4.1) showed that almost all added P was restricted at the point of application with struvite with and without organic acid-treated samples for all incubation periods. There was no significant P mobility among organic acid treatments relative to struvite without organic acid added alone even after 12 weeks of incubation. Even though LMWOAs have been added to favor the struvite solubility by reducing pH, the pH reduction effect was not observed with LMWOAs added struvite (Table 4.1). The acidity generated by organic acids was not adequate or strong enough to decrease the pH of alkaline (pH >8) soils. According to Ahmed et al. (2018), the initial struvite dissolution is strongly affected by initial pH as the initial dissolution of struvite is decreased with increasing pH. In addition to the pH of the soil, the struvite solubility and diffusion might be strongly affected by soil type, soil constituents, and soil properties. The soil used for this study contained 7.6% reactive CaCO₃ (Table 3.1), when CaCO₃ is dissolving it has the potential to neutralize the acidity generated by organic acids. So, the pH reduction might be negligible. In addition, P might precipitate with Ca²⁺ ions as calcium phosphate minerals or P fixation happens due to surface adsorption reactions with CaCO₃ (Leytem & Mikkelsen, 2005; McBeath et al., 2005). With the silt loam texture of this calcareous soil, there is a high chance of P sorption reactions with soil colloids and

CaCO₃. As a result, P might have gotten fixed in the first section right after the application. The XANES speciation data (Figure 4.9) proved that the struvite treatments were dominated by insoluble Ca-P minerals and CaCO₃ adsorb P in addition to insoluble reclaimed struvite. Kataki et al. (2016) indicated that the fertilizing effect of struvite varies with the soil type due to solubility differences and sorption properties of soils. Moreover, struvite is most effective in soils with moderate (neutral) or low pH (acidic) while the efficacy is limited in high pH soils (Kataki et al., 2016; Talboys et al., 2016). In addition to the above-mentioned explanations, organic acid decomposition in soil is also an unavoidable fact (Jones & Willett, 2006). Less amounts of organic acid might have remained in the soil to favor struvite solubility as a result of microbial-mediated decomposition or sorption reactions. However, in real-world scenarios, the effect may be different since plant roots can secrete organic acids into the rhizosphere under P-deficient conditions and increase the plant availability of P as observed in Talboys et al. (2016) with the presence of a live roots system. On the other hand, reactions between organic acids with CaCO₃ can determine exuded LMWOAs availability. When oxalate is present in calcareous soils, there is a great potential to form calcium oxalate in the root zone of the plants with Ca²⁺ ions by precipitation (Moradi et al., 2012). Calcium oxalate is a sparingly soluble stable precipitant. Oxalic acid can dissolve the calcium carbonate and reduce the active carbonates in calcareous soils by precipitating out of calcium oxalate and has the potential of releasing P primarily bound/held in calcium phosphate minerals. In contrast, citrate has a poor affinity for Ca²⁺ but a greater affinity for Al³⁺ and Fe³⁺ (Èm et al., 2002).

Table 4.1. The pH after 1, 5, 9, and 12 weeks of incubation in calcareous soil. Means within a soil section for each treatment in each time period containing the same letter are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

Incubation period	Treatment	pH		
		0.0-7.5 mm	7.5-15.5 mm	15.5-27.0 mm
1 week	Control	8.54 ± 0.03 bcd	8.56 ± 0.05 a	8.58 ± 0.04 a
	Str	8.38 ± 0.04 d	8.51 ± 0.05 a	8.62 ± 0.08 a
	Str + CA Low	8.57 ± 0.04 abcd	8.70 ± 0.06 a	8.76 ± 0.05 a
	Str + CA High	8.65 ± 0.04 ab	8.76 ± 0.04 a	8.78 ± 0.04 a
	Str + OX Low	8.43 ± 0.06 cd	8.58 ± 0.03 a	8.62 ± 0.03 a
	Str + OX High	8.50 ± 0.03 bcd	8.6 ± 0.01 a	8.57 ± 0.03 a
	Str + CA + OA High	8.61 ± 0.07 abc	8.63 ± 0.01 a	8.72 ± 0.05 a
	Str + CA + OA + MA + AA High	8.75 ± 0.04 a	8.76 ± 0.06 a	8.78 ± 0.05 a
5 weeks	Control	8.67 ± 0.01 a	8.65 ± 0.01 a	8.60 ± 0.01 a
	Str	8.63 ± 0.09 a	8.45 ± 0.01 a	8.52 ± 0.04 a
	Str + CA Low	8.78 ± 0.18 a	8.62 ± 0.02 a	8.56 ± 0.02 a
	Str + CA High	8.64 ± 0.16 a	8.62 ± 0.08 a	8.66 ± 0.08 a
	Str + OX Low	8.54 ± 0.23 a	8.48 ± 0.02 a	8.55 ± 0.01 a
	Str + OX High	8.46 ± 0.05 a	8.51 ± 0.06 a	8.57 ± 0.01 a
	Str + CA + OA High	8.69 ± 0.05 a	8.68 ± 0.01 a	8.66 ± 0.01 a
	Str + CA + OA + MA + AA High	8.54 ± 0.03 a	8.62 ± 0.01 a	8.61 ± 0.01 a
9 weeks	Control	8.55 ± 0.02 a	8.47 ± 0.07 a	8.50 ± 0.04 a
	Str	8.46 ± 0.02 a	8.43 ± 0.03 a	8.38 ± 0.01 a
	Str + CA Low	8.51 ± 0.01 a	8.55 ± 0.01 a	8.49 ± 0.01 a
	Str + CA High	8.41 ± 0.01 a	8.62 ± 0.01 a	8.63 ± 0.01 a
	Str + OX Low	8.4 ± 0.01 a	8.52 ± 0.01 a	8.5 ± 0.01 a
	Str + OX High	8.42 ± 0.01 a	8.46 ± 0.01 a	8.45 ± 0.01 a

	Str + CA + OA High	8.5 ± 0.01 a	8.53 ± 0.01 a	8.54 ± 0.01 a
	Str + CA + OA + MA + AA High	8.45 ± 0.01 a	8.5 ± 0.01 a	8.56 ± 0.02 a
12 weeks	Control	8.49 ± 0.05 a	8.51 ± 0.02 cd	8.33 ± 0.03 e
	Str	8.40 ± 0.03 ab	8.44 ± 0.02 d	8.33 ± 0.01 e
	Str + CA Low	8.26 ± 0.02 b	8.59 ± 0.02 ab	8.56 ± 0.01 ab
	Str + CA High	8.34 ± 0.05 ab	8.59 ± 0.02 a	8.58 ± 0.02 a
	Str + OX Low	8.29 ± 0.04 b	8.52 ± 0.06 bcd	8.41 ± 0.01 d
	Str + OX High	8.34 ± 0.04 ab	8.49 ± 0.01 d	8.43 ± 0.01 cd
	Str + CA + OA High	8.41 ± 0.01 ab	8.58 ± 0.01 abc	8.50 ± 0.01 bc
	Str + CA + OA + MA + AA High	8.35 ± 0.01 ab	8.58 ± 0.01 abc	8.52 ± 0.03 ab

Table 4.2. The pH after 1, 5, 9, and 12 weeks of incubation in neutral soil. Means within a soil section for each treatment in each time period containing the same letter are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

Incubation period	Treatment	pH		
		0.0-7.5 mm	7.5-15.5 mm	15.5-27.0 mm
1 week	Control	6.30 ± 0.01 d	6.34 ± 0.01 b	6.28 ± 0.01 a
	Str	8.24 ± 0.1 c	6.92 ± 0.36 ab	6.64 ± 0.31 a
	Str + CA Low	8.43 ± 0.005 abc	7.48 ± 0.02 ab	7.12 ± 0.25 a
	Str + CA High	8.40 ± 0.04 abc	7.36 ± 0.06 ab	7.24 ± 0.17 a
	Str + OX Low	8.27 ± 0.01 bc	6.77 ± 0.22 ab	6.55 ± 0.07 a
	Str + OX High	8.41 ± 0.04 abc	7.45 ± 0.10 a	7.30 ± 0.16 a
	Str + CA + OA High	8.55 ± 0.04 ab	7.37 ± 0.04 ab	7.22 ± 0.12 a
	Str + CA + OA + MA + AA High	8.54 ± 0.04 a	7.40 ± 0.11 ab	7.09 ± 0.21 a
5 weeks	Control	6.42 ± 0.01 b	6.18 ± 0.01 ab	6.12 ± 0.01 ab
	Str	7.69 ± 0.25 a	5.32 ± 0.50 b	5.49 ± 0.60 b
	Str + CA Low	7.89 ± 0.18 a	6.09 ± 0.38 ab	6.34 ± 0.05 ab
	Str + CA High	8.10 ± 0.11 a	6.79 ± 0.08 a	6.31 ± 0.33 ab
	Str + OX Low	7.93 ± 0.12 a	6.12 ± 0.07 ab	6.45 ± 0.05 ab
	Str + OX High	8.00 ± 0.17 a	6.76 ± 0.05 a	6.80 ± 0.02 ab
	Str + CA + OA High	8.15 ± 0.13 a	6.84 ± 0.05 a	6.98 ± 0.05 a
	Str + CA + OA + MA + AA High	8.24 ± 0.17 a	6.99 ± 0.07 a	6.80 ± 0.04 ab
9 weeks	Control	6.1 ± 0.01 h	6.11 ± 0.01 d	6.01 ± 0.01 de
	Str	6.77 ± 0.01 g	5.52 ± 0.006 g	5.86 ± 0.006 e
	Str + CA Low	7.28 ± 0.06 f	5.64 ± 0.06 f	6.10 ± 0.06 d
	Str + CA High	7.45 ± 0.003 d	6.34 ± 0.006 b	6.23 ± 0.003 c
	Str + OX Low	7.34 ± 0.003 e	5.74 ± 0.003 e	6.22 ± 0.003 c
	Str + OX High	7.51 ± 0.006 c	6.25 ± 0.006 c	6.61 ± 0.006 b
	Str + CA + OA High	8.01 ± 0.006 a	6.47 ± 0.004 a	6.72 ± 0.003 a

	Str + CA + OA + MA + AA High	7.78 ± 0.009 b	6.15 ± 0.006 d	6.24 ± 0.006 c
12 weeks	Control	6.35 ± 0.01 e	6.13 ± 0.01 a	6.03 ± 0.01 cd
	Str	7.02 ± 0.06 b	5.52 ± 0.003 d	5.79 ± 0.02 e
	Str + CA Low	6.78 ± 0.006 c	5.86 ± 0.003 b	6.04 ± 0.003 c
	Str + CA High	7.01 ± 0.006 b	6.17 ± 0.03 a	5.81 ± 0.04 e
	Str + OX Low	6.78 ± 0.006 c	5.45 ± 0.003 d	5.93 ± 0.007 d
	Str + OX High	7.33 ± 0.006 a	5.88 ± 0.006 b	6.54 ± 0.006 a
	Str + CA + OA High	7.32 ± 0.006 a	5.92 ± 0.004 b	6.35 ± 0.009 b
	Str + CA + OA + MA + AA High	6.55 ± 0.006 d	5.67 ± 0.006 c	6.01 ± 0.006 cd

Table 4.3. The pH after 1, 5, 9, and 12 weeks of incubation in acid soil. Means within a soil section for each treatment in each time period containing the same letter are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

Incubation Period	Treatment	pH		
		0.0-7.5 mm	7.5-15.5 mm	15.5-27.0 mm
1 week	Control	5.56 ± 0.05 c	5.53 ± 0.04 b	5.58 ± 0.01 c
	Str	7.81 ± 0.04 b	5.79 ± 0.2 ab	5.67 ± 0.14 bc
	Str + CA Low	7.95 ± 0.06 ab	6.05 ± 0.1 ab	5.94 ± 0.06 abc
	Str + CA High	7.97 ± 0.04 a	6.31 ± 0.15 a	5.95 ± 0.15 abc
	Str + OX Low	8.00 ± 0.05 ab	6.11 ± 0.25 ab	6.20 ± 0.04 a
	Str + OX High	8.13 ± 0.06 a	6.34 ± 0.09 a	6.3 ± 0.12 a
	Str + CA + OA High	8.06 ± 0.04 ab	6.21 ± 0.1 ab	6.16 ± 0.06 ab
	Str + CA + OA + MA + AA High	8.02 ± 0.09 ab	6.19 ± 0.08 ab	6.03 ± 0.11 abc
5 weeks	Control	5.05 ± 0.07 e	5.02 ± 0.04 cd	5.05 ± 0.04 d
	Str	7.42 ± 0.03 d	4.95 ± 0.04 d	4.86 ± 0.1 d
	Str + CA Low	7.57 ± 0.04 cd	5.28 ± 0.03 c	5.12 ± 0.03 bcd
	Str + CA High	7.63 ± 0.09 bcd	5.99 ± 0.08 a	5.69 ± 0.09 a
	Str + OX Low	7.72 ± 0.08 bc	5.21 ± 0.06 cd	5.08 ± 0.06 cd
	Str + OX High	7.82 ± 0.03 abc	5.66 ± 0.08 b	5.47 ± 0.01 abc
	Str + CA + OA High	7.89 ± 0.02 ab	5.78 ± 0.08 ab	5.51 ± 0.12 ab
	Str + CA + OA + MA + AA High	8.00 ± 0.06 a	5.99 ± 0.01 a	5.53 ± 0.08 a
9 weeks	Control	4.79 ± 0.05 d	4.91 ± 0.04 bc	4.85 ± 0.14 abc
	Str	6.12 ± 0.01 b	4.84 ± 0.04 bc	4.75 ± 0.03 abcd
	Str + CA Low	5.29 ± 0.03 c	4.44 ± 0.27 c	4.36 ± 0.19 d
	Str + CA High	6.39 ± 0.12 b	5.32 ± 0.12 ab	4.41 ± 0.09 cd
	Str + OX Low	7.27 ± 0.09 a	4.94 ± 0.10 bc	4.63 ± 0.02 bcd
	Str + OX High	7.30 ± 0.12 a	5.66 ± 0.11 a	4.98 ± 0.02 ab
	Str + CA + OA High	7.46 ± 0.04 a	5.39 ± 0.13 ab	4.47 ± 0.04 cd

	Str + CA + OA + MA + AA High	7.08 ± 0.16 a	5.92 ± 0.12 a	5.14 ± 0.07 a
12 weeks	Control	5.16 ± 0.09 e	4.99 ± 0.06 e	5.07 ± 0.06 d
	Str	6.63 ± 0.06 d	4.86 ± 0.06 f	4.87 ± 0.006 f
	Str + CA Low	7.15 ± 0.02 b	5.24 ± 0.05 d	5.14 ± 0.01 c
	Str + CA High	6.55 ± 0.05 d	5.26 ± 0.01 d	5.25 ± 0.02 b
	Str + OX Low	6.83 ± 0.009 c	4.92 ± 0.02 ef	4.96 ± 0.01 e
	Str + OX High	7.65 ± 0.009 a	5.87 ± 0.03 a	5.54 ± 0.003 a
	Str + CA + OA High	7.06 ± 0.006 b	5.72 ± 0.006 b	5.25 ± 0.01 b
	Str + CA + OA + MA + AA High	6.55 ± 0.006 d	5.50 ± 0.01 c	5.04 ± 0.02 d

In neutral soil (Figure 4.2), P diffusion after the first 5 weeks of incubation periods showed no significant increment in struvite in the presence of added organic acid treatments compared to struvite in the absence of added organic acid treatment. However, there was a trend of P movement in organic acid-treated struvite samples even though diffusion was not significantly different. After 9 weeks of incubation, Str + CA Low, Str + CA High, Str + OA High, and Str + CA + OA + MA + AA treatments showed a high percent P added in the second and third sections, while there was a significant reduction of added P in the first section. After 9 weeks of incubation, 16% of added P has moved towards the second section in Str + CA High treatment while 21% of P moved towards the second section in Str + CA + OA + MA + AA High sample. In Str + OA Low treatment and Str + OA High treatment showed more than 10% of added P in the third section. After 12 weeks of incubation, 37% of P diffused into the third section of Str + CA High treatment and 28% of P movement in Str + CA + OA + MA + AA High treatment. In str + OA low treatment, 21% of P moved towards the third section. In str + CA Low treatment, 20% of added P had moved towards the second section which was significantly higher than other treatments. So according to the overall diffusion trend, Str + CA Low, Str + CA High, Str + OA Low, and Str + CA + OA + MA + AA High treatments showed a significant enhancement of P movement compared to other treatments in neutral soil. Oxalic acid is a relatively (compared to other organic acids used in the study) strong weak acid ($pK_{a1} = 1.27$, $pK_{a2} = 4.28$), and this might be the reason for P mobilization in oxalic acid-treated struvite samples. After 9 weeks of incubation, P content in the oxalic acid-treated soils decreased with time compared to citric acid-treated soils. This might be because the microbial degradation of oxalic acid was faster than citric acid since citric is a tricarboxylic acid while oxalic acid is a dicarboxylic acid as observed

in (Figure 4.8 b). Menezes-Blackburn et al. (2016) mentioned that the degradation rate of citric acid was much lower than oxalic acid due to more C atoms consisting in citric acids and the greater binding capacity of citric acid with soil metals than oxalic acid. However, citric acid and oxalic acid contribute to P diffusion of struvite compared to no LMWOAs added struvite treatment. The reason for the organic acid mixture showing higher P mobility might be the combination effect of organic acids is more effective than the presence of a single type of organic acid since more organic ligands are consisted in an acid mixture. This is encouraging as real rhizosphere condition is a mixture of organic acids and more ligands contribute to more complexation reactions and more P mobilization.

In acid soil (Figure 4.3), a significant amount of added P in struvite with some organic acid-treated soils diffused into the second section even after 1 week of incubation. Out of these, Str + CA high treatment showed superior mobility by moving 14% of added P into the second section. A similar pattern of P diffusion was observed after 5 weeks of incubation as 20% of P moved to the second section in the same treatment. After 9 weeks, 31% moved into the second section, while 16% of added P diffused into the third section in Str + CA High treatment. This treatment showed a significantly higher P diffusion relative to all other treatments. At the end of the 12 weeks of incubation, 32% added P in the second section, and 17% moved towards the third section in Str + CA High treatment. In addition, in Str + CA + OA + MA + AA High treatment showed 37% of added P in the second and 13% in the third sections. These results can be explained using the ability to decrease P adsorption in soils when tricarboxylic acids (citric) are present (Bolan et al., 1994). According to Menezes-Blackburn et al. (2016), citrate was investigated as the most effective organic acid anion in terms of promoting P release through the chelation of Al in acidic soils. Oxalic is a dicarboxylic acid and has less stability constant for Al

($\log K_{Al}$) compared to tricarboxylic citric acid. However, oxalic acid-treated samples did not show a significant P movement. So citric acid has a higher stability constant for Al making more stable complexes and promoting P releasing by breaking down most common Al phosphate associations in acid soils (Bolan et al., 1994). However, in their studies, a lower rate of organic acid addition did not show a significant impact on P mobility enhancement. This could be that because lower doses of LMWOAs might have a higher chance of being degraded by soil microbes at 24 hours of incubation (Menezes-Blackburn et al., 2016).

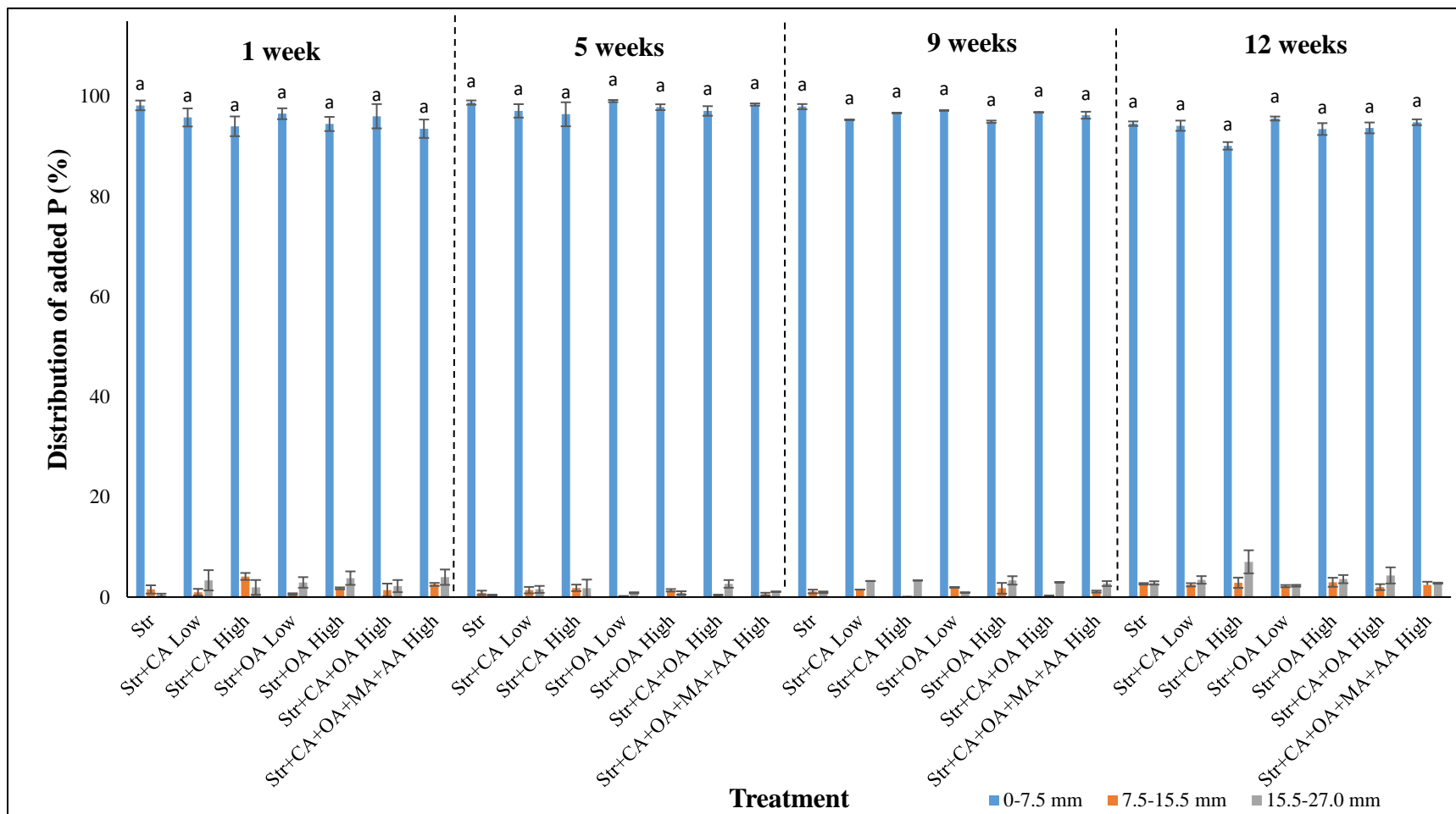


Figure 4.1. Distribution of added P (%) after 1 week, 5 weeks, 9 weeks, and 12 weeks of incubation in calcareous soil. Means within a soil section for each treatment in each time period containing the same letter are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

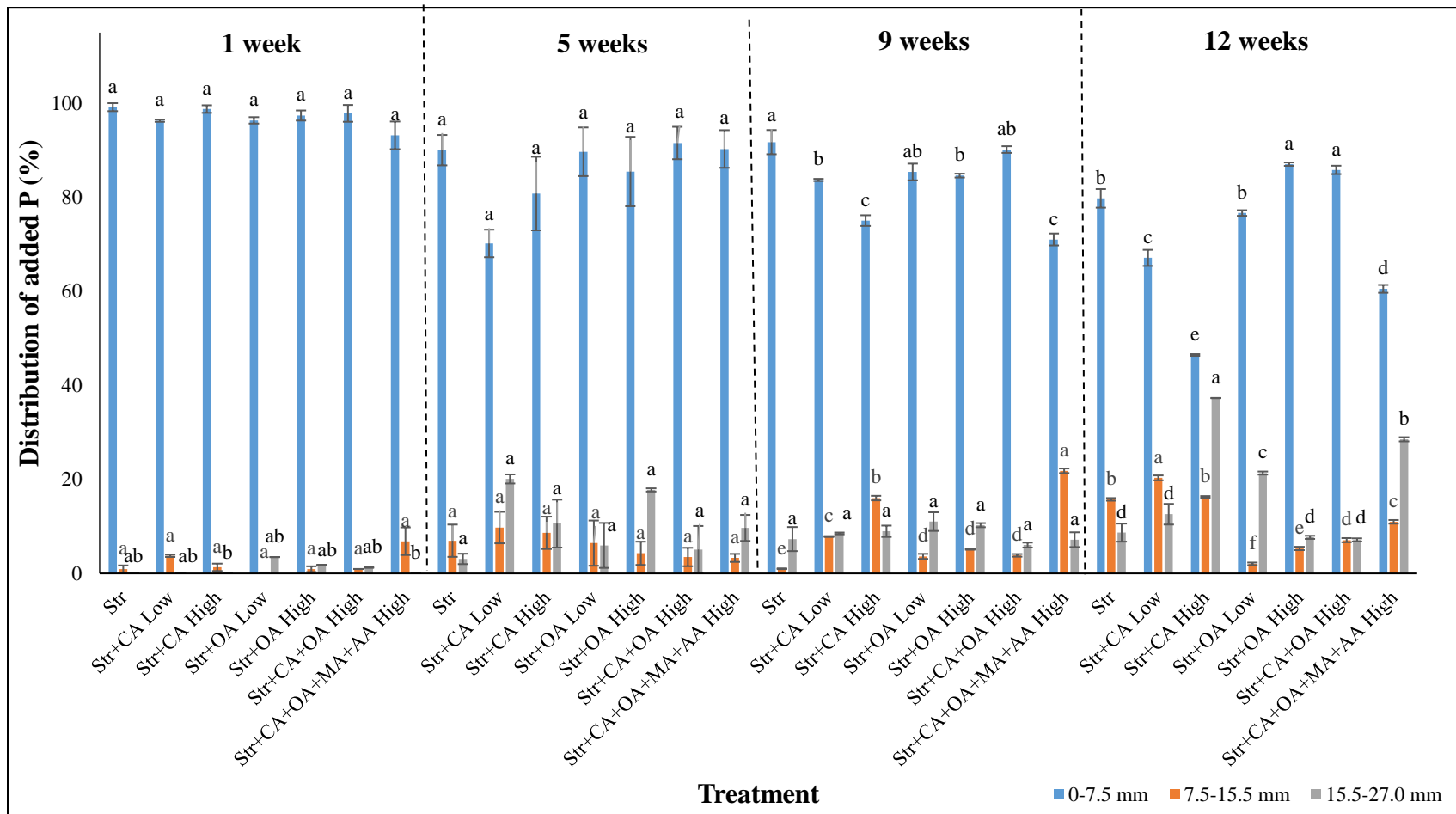


Figure 4.2. Distribution of added P (%) after 1 week, 5 weeks, 9 weeks, and 12 weeks of incubation in neutral soil. Means within a soil section for each treatment in each time period with the same letters are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

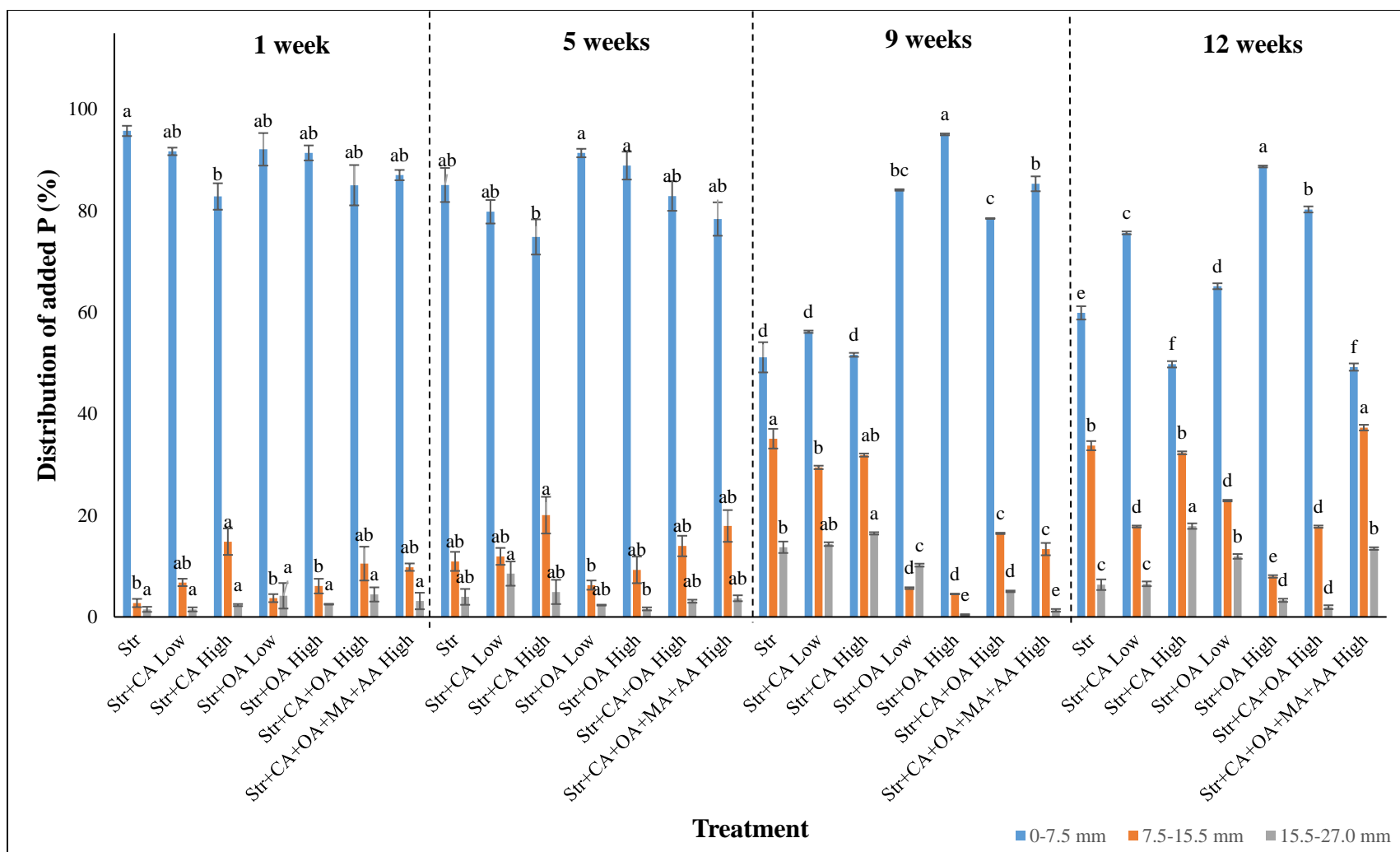


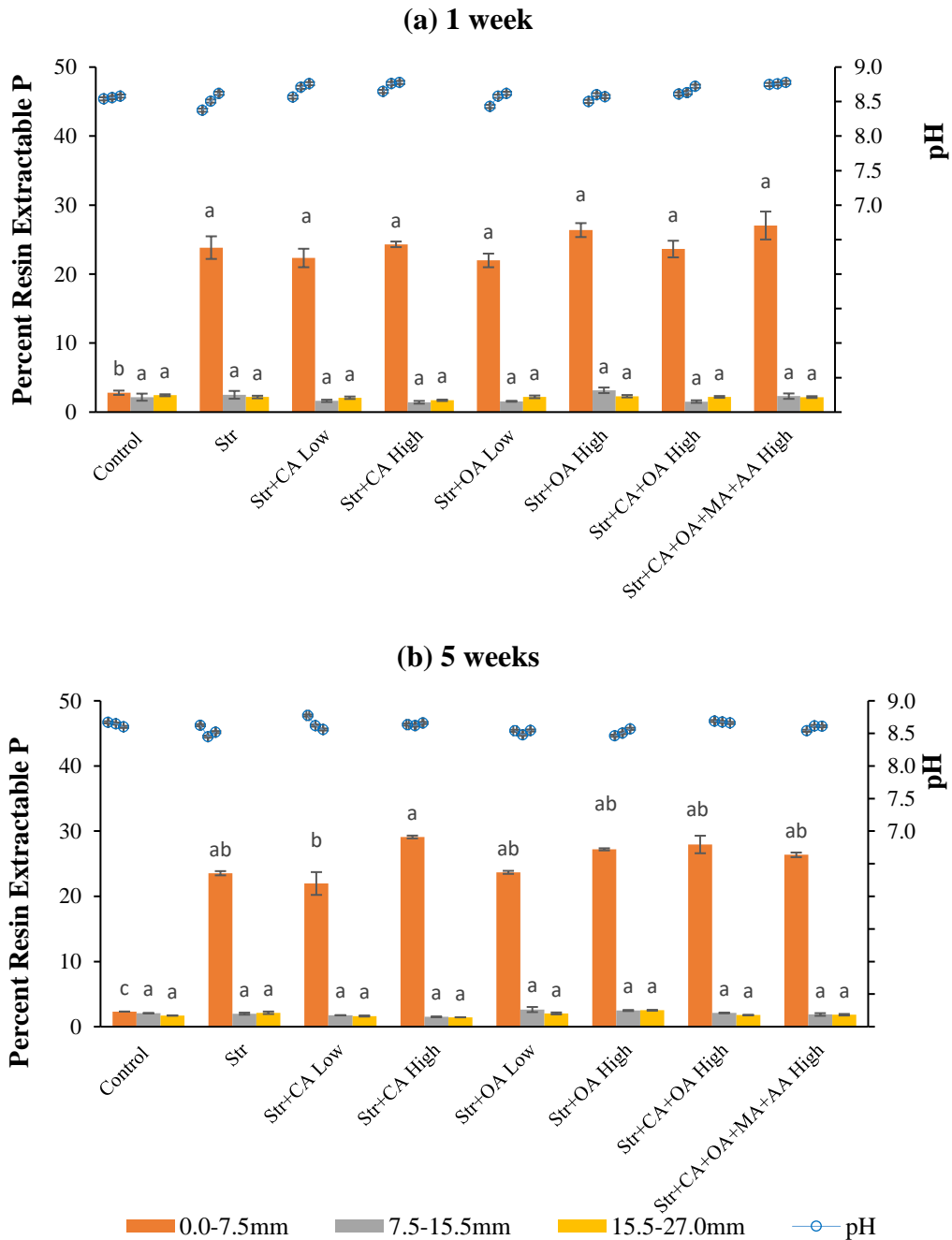
Figure 4.3. Distribution of added P (%) after 1 week, 5 weeks, 9 weeks, and 12 weeks of incubation in acid soil. Means within a soil section for each treatment each time period with the same letters are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

Potential plant available P and pH

Plant-available P species are orthophosphate anions and potential plant availability can be determined indirectly by extraction procedures. Labile P in soils is anion exchange resin extractable and the resin extractable P method mimics the soil solution/plant root model (Myers et al., 2005). The soil pH has a significant impact on P concentration, aboveground biomass, and P uptake response ratios (Hertzberger et al., 2020).

In calcareous soil (Figure 4.4), even though organic acids have been added with struvite at two rates (41 mM and 82 mM) a significant pH reduction could not be seen in organic acid-treated soils relative to control soil (Table 4.1). Some studies showed that 1mM of organic acid addition can potentially increase struvite dissolution by 39-69% compared with the control without organic acids added struvite in an aqueous mixture (Ahmed et al., 2018). However, soil is a complex matrix, so the observation might be contrasting with soils. The reason for the different observations in this study might be due to sorption reactions of organic acid anions into soil colloids (Earl et al., 1979; Jones et al., 1996; Jones & Kochian, 1996) or due to microbial degradation of LMWOAs within a short period as discussed in Menezes-Blackburn et al. (2016). In terms of resin extractable P, the first section showed the higher P extractability in all the treatments in all time durations except in the control soil. However, the overall P resin extractability (PRP per Petri dish) in native soil and struvite treatments ranged from (1.8-2.5% vs 8-12%) respectively in calcareous soil (Figure 4.7a). These were lower than neutral (8-12% vs 15-60%) and acid (6-8% vs 24-80%) soils (Figure 4.7a and Figure 4.7.b). This could be due to P fixation in calcareous soil by forming secondary minerals by precipitation with Ca^{2+} ions and surface adsorption reactions with CaCO_3 (Leytem & Mikkelsen, 2005). However, with the presence of real plant roots, the pH reduction and P availability are different due to the continuous secretion of organic acids and phosphate-solubilizing bacteria in the rhizosphere

promoting the dissolution of P and changing the P availability (De Freitas et al., 1997; Illmer & Schinner, 1995).



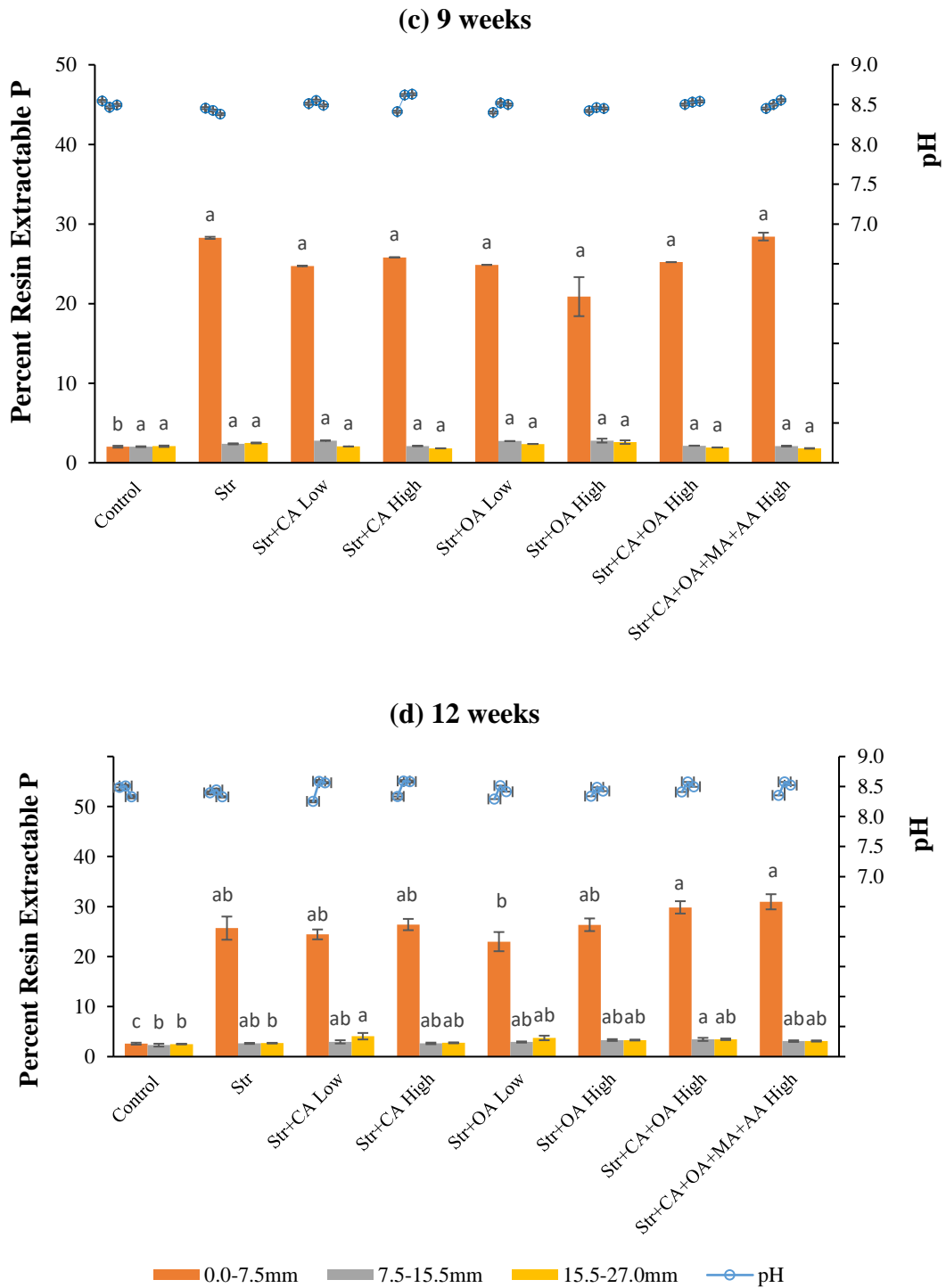


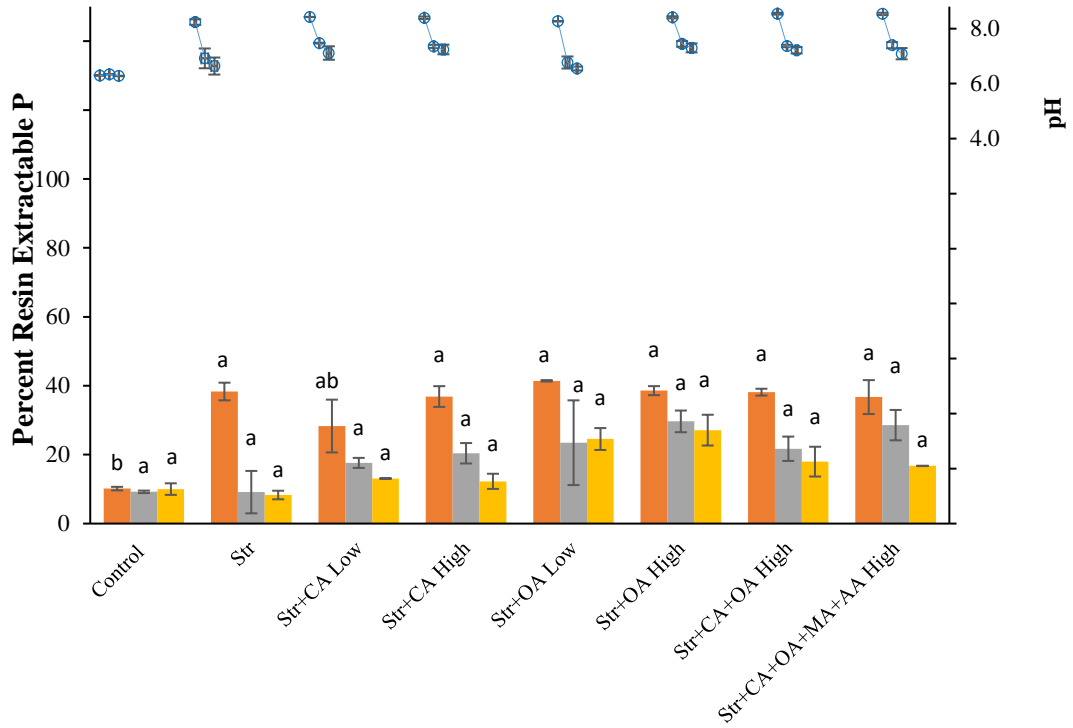
Figure 4.4. Percent resin extractable P (PRP) and pH for each dish section for all treatments after (a) 1 week, (b) 5 weeks, (c) 9 weeks, and (d) 12 weeks of incubation in calcareous soil. Means within each soil section for each treatment containing the same letters are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

In neutral soil, in the first section of struvite-treated samples with and without LMWOAs, pH values were relatively higher (Table 4.2) compared to the control soil since the initial pH was increased with the struvite addition and the slow dissolution of struvite increased the pH further at the point of application (Ahmed et al., 2018).

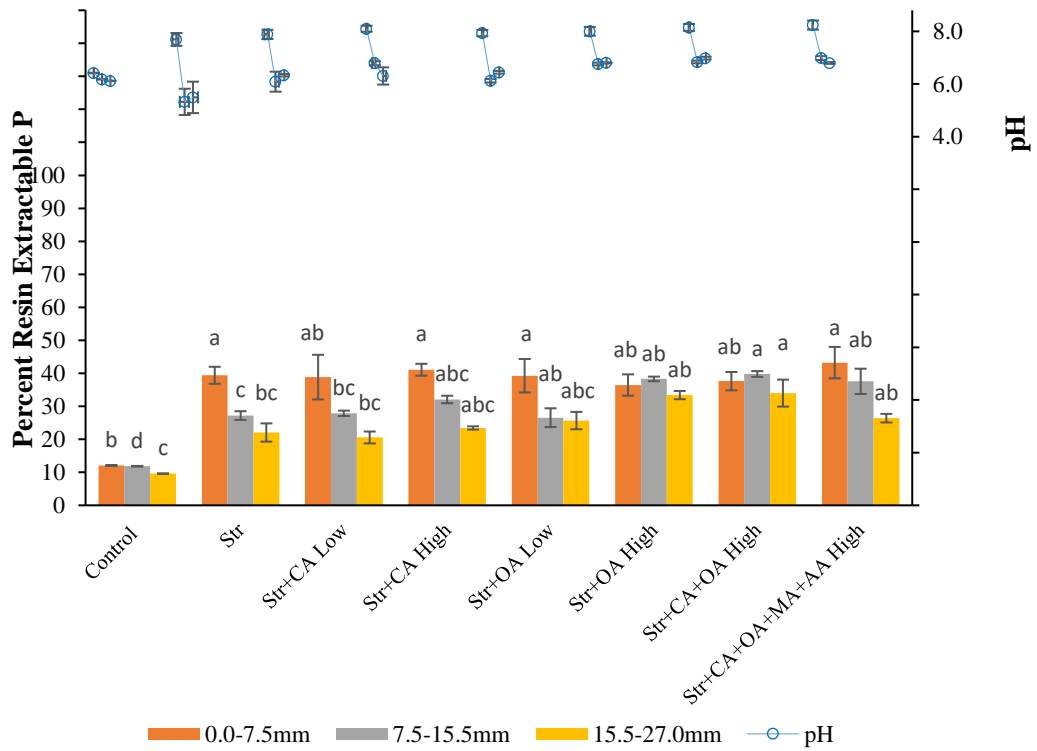
After 1 week of incubation, the highest labile P was shown in the first section of all the treatments in neutral soil except for the control treatment (Figure 4.5a). The percent resin extractable P in neutral soil ranged from 28 to 80%, while in general percent resin P (PRP) increased with incubation time. After 5 weeks of incubation (Figure 4.5b), around 40% of PRP could be seen in the second section of Str + OA High, Str + OA Low, and Str + CA + OA High treatments, while 32% of PRP was seen in Str + CA High treatment. In terms of P availability, 33%, 25%, 34%, and 23% of PRP were seen in the third section of the above-mentioned treatments, respectively. After 9 weeks of incubation, (Figure 4.5 c) resin P extractability of some treatments with LMWOAs increased in the first section compared to struvite without adding organic acid treatments. Those treatments are Str + CA Low, Str + CA High, Str +OA Low, Str +CA + OA + MA, and Str + OA High. Moradi et al. (2012) stated that the applied LMWOAs (malic, citric and oxalic acid) effectively increase the plant available P in soil (calcareous) solution by decreasing P sorption. Using the acidification, chelation, and exchange reaction mechanisms, LMWOAs have the potential to extract more P into the soil solution from adsorbed P species (Chen et al., 2008). After 9 weeks Str + CA High treatment and Str + OA Low treatments showed higher labile P in the second section. In the third section, the highest P availability could be seen in Str + OA Low and Str + OA High and high treatments. After 12 weeks, the highest resin extractability could be seen in the third section of Str + OA High treatment. This result can be explained using the relatively strong acidic nature of oxalic acid compared to other organic acids. With the presence of LMWOAs, the degree of P sorption by soil is reduced. However, the efficacy of the organic ligand

depends on the acid strength and the number of carboxyl groups. So, the relatively higher acid strength of oxalic acid could be the reason for more P desorption by ligand exchange reactions and more labile P extraction (Moradi et al., 2012). Some studies discussed that oxalate anion is dominant and most effective in promoting P release through chelation in less acidic (neutral) or calcareous soils (Menezes-Blackburn et al., 2016). Generally, resin extractability (Figure 4.5) indicated that more P was resin extractable in the second section relative to the third section even though the pH of both sections was similar. It could be because relatively more added P remained in the second section than in the third section.

(a) 1 week



(b) 5 weeks



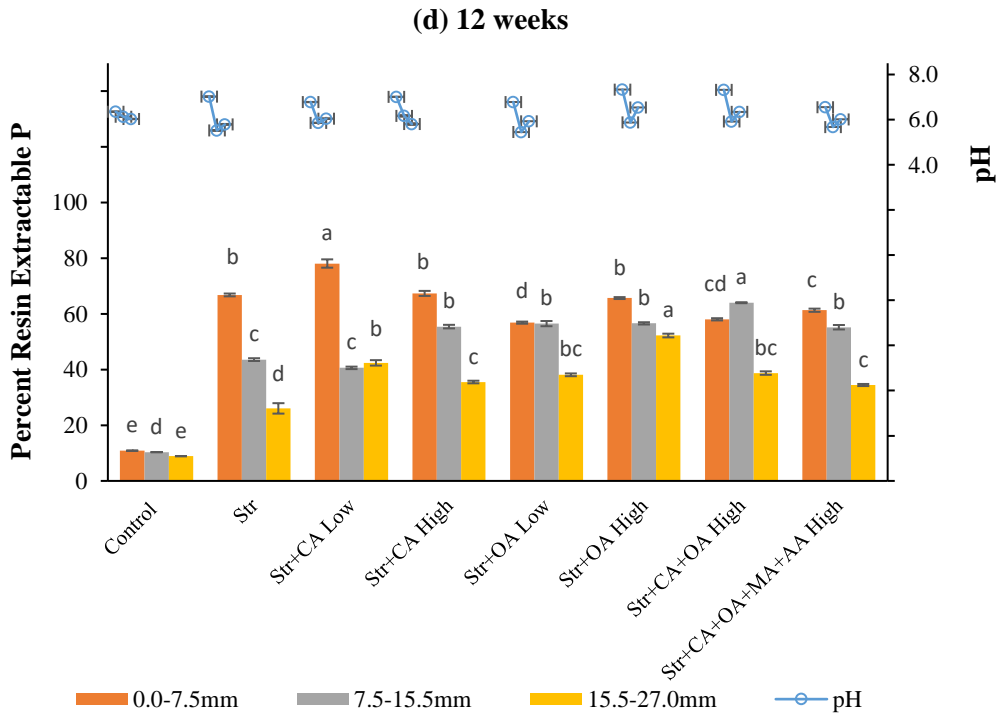
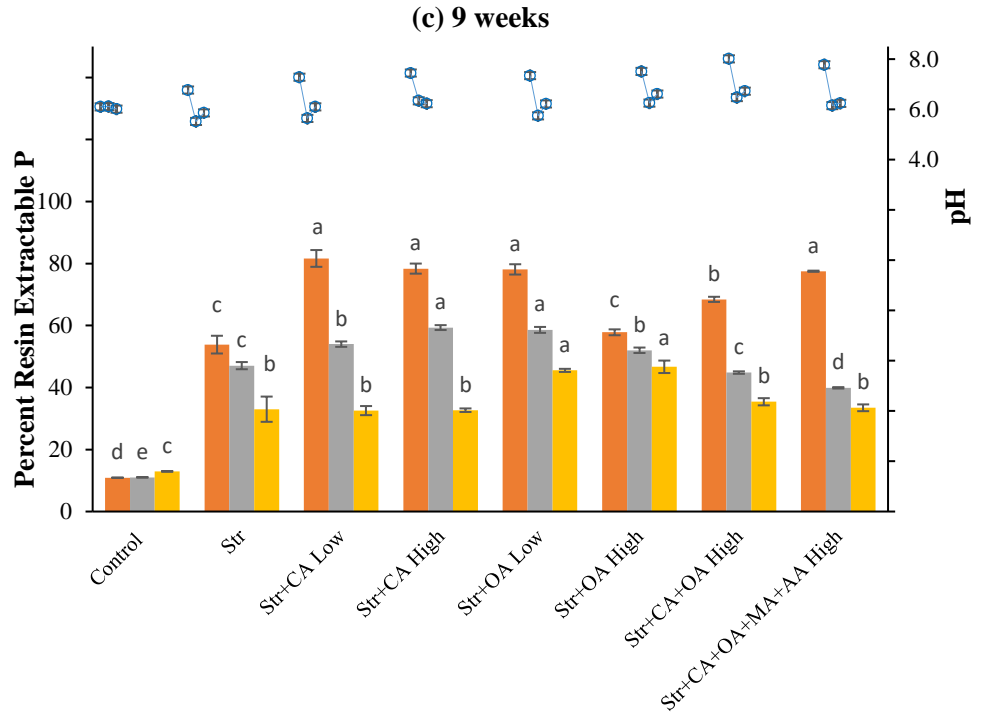


Figure 4.5. Percent resin extractable P (PRP) and pH for each dish section for all treatments after (a) 1 week, (b) 5 weeks, (c) 9 weeks and (d) 12 weeks of incubation in neutral soil. Means within each soil section for each treatment containing the same letters are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

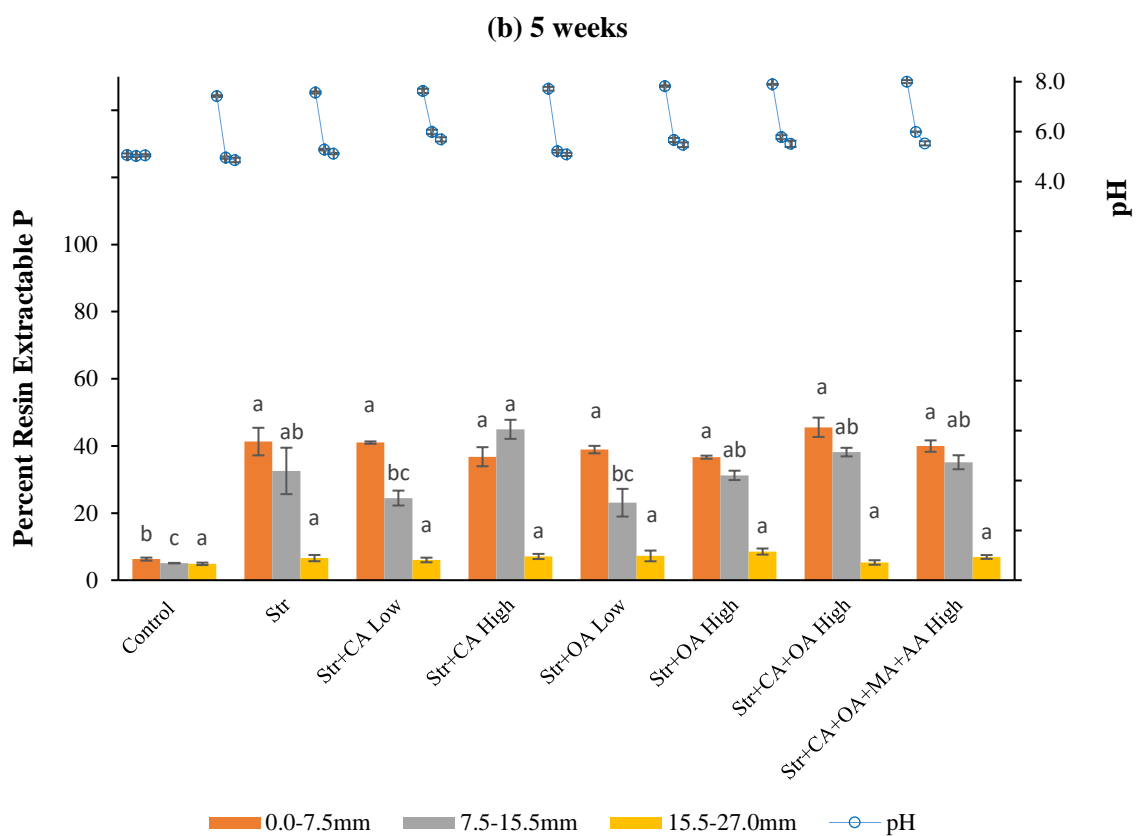
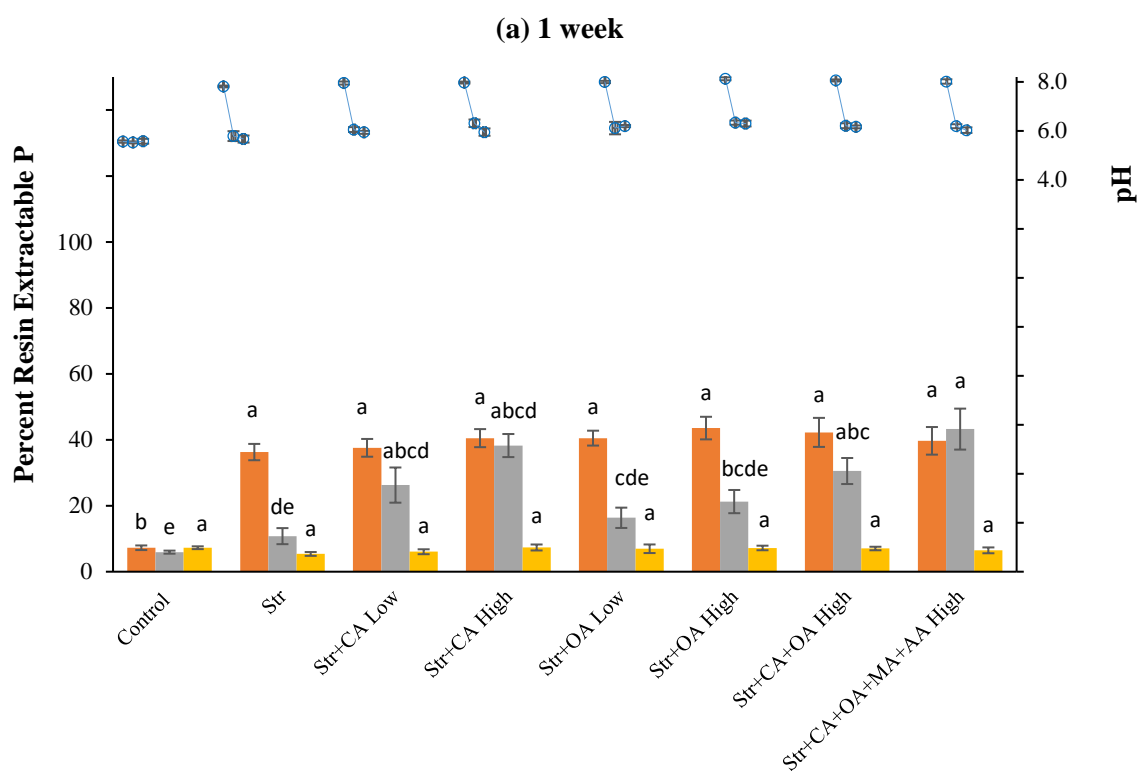
In acid soil (Figure 4.6 & Table 4.3), the overall pH of the struvite treatments was higher in the first section compared to the control soil. As discussed before, the protons consumed for the dissolution of struvite in acid soil were the reason for this increased pH. Similar results were seen before by other researchers (Talboys et al., 2016a). However, when the distance from the point of application was increased, the pH values were similar to native soils. Acid soil consists of reactive metals such as Al and Fe, and these metals capability to make complexes with organic ligands. These reactions release protons and decrease pH (Menezes-Blackburn et al., 2016). Overall, the percentage of resin P in acid soil varied from 35 to 98% in fertilizer treatments.

From 1 week to 12 weeks, overall resin extractability increased in acid soil (Figure 4.7 c). After 1 week of incubation, the highest resin extractability of P could be seen in the first section in all the fertilizer treatments except Str + CA + OA + MA + AA High treatment, in which the highest labile P was seen in the second (Figure 4.6 a). At the end of 5 weeks, the highest PRP (45%) was in Str + CA High treatment (Figure 4.6 b).

Based on the overall resin extractability (percent resin P per Petri dish) (Figure 4.7c), acid soil (PRP of 25-80%) was higher than neutral (PRP of 15-40%) and calcareous soil (9-11%) with fertilizer treatments. The percent resin P increased from 1 week to 12 weeks in all the fertilizer treatments with time. The Str + CA High (30-70% of PRP) and Str + CA + OA + MA + AA (31-80% of PRP) treatments showed higher extractability in all the periods.

Oxalic acid-treated struvite samples did not show extractability enhancement after 9 weeks of incubation. According to Menezes-Blackburn et al. (2016), citrate ligand is most effective in promoting P release through the chelation of Al in acid soils. Citrate has greater affinity to Fe^{3+} and Al^{3+} and has the potential to release P from Fe-phosphate and Al-phosphate minerals (Èm et al., 2002). So, the chelation potential of citrate with Al and Fe and releasing P from recalcitrant Fe and Al P minerals is good evidence for higher labile P in citric acid-treated

struvite samples. The organic acid mixture showed the highest P extractability since the mixture of ligands is always more effective in chelation and ligand exchange reactions compared to a single type of ligand.



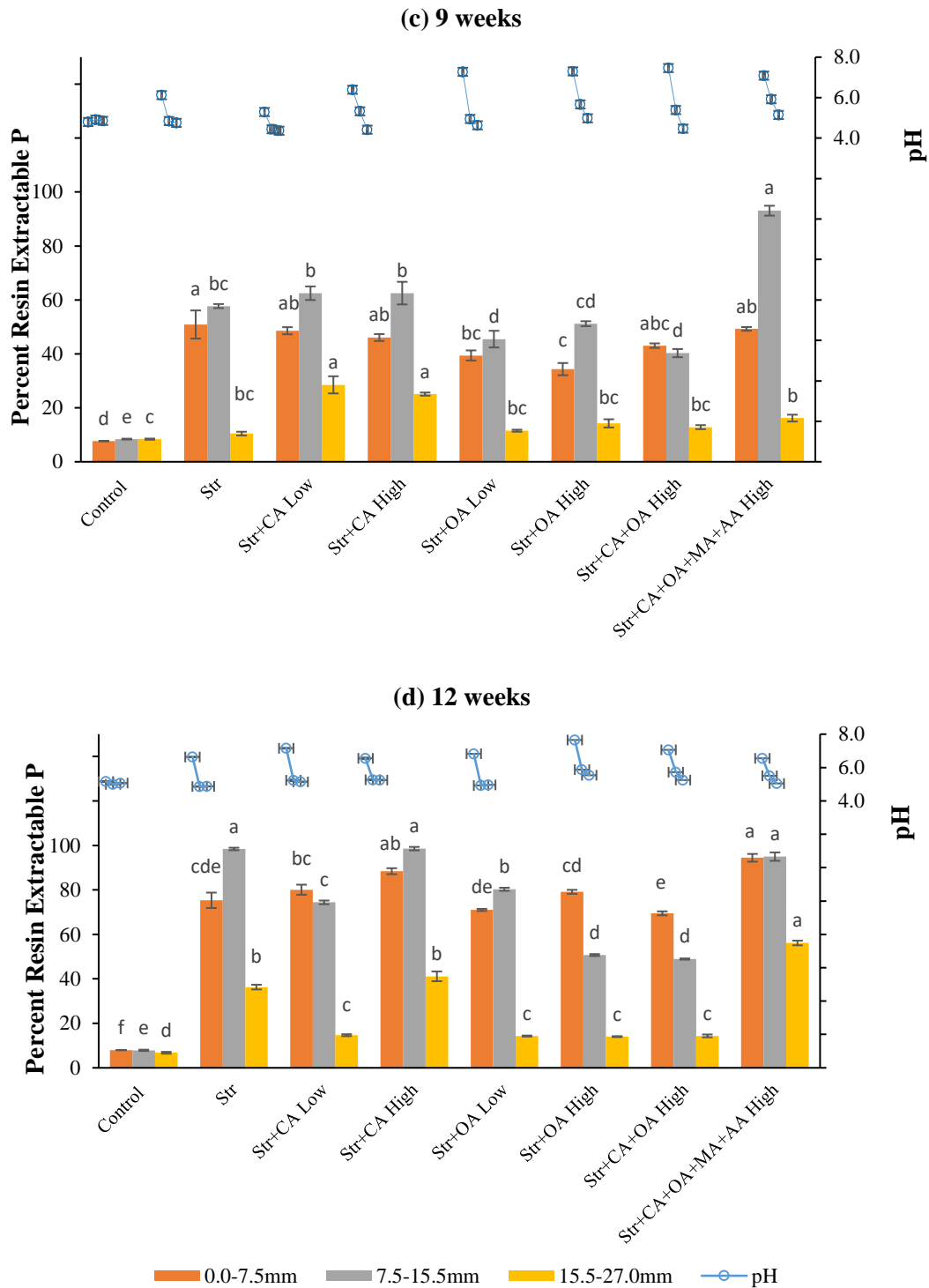
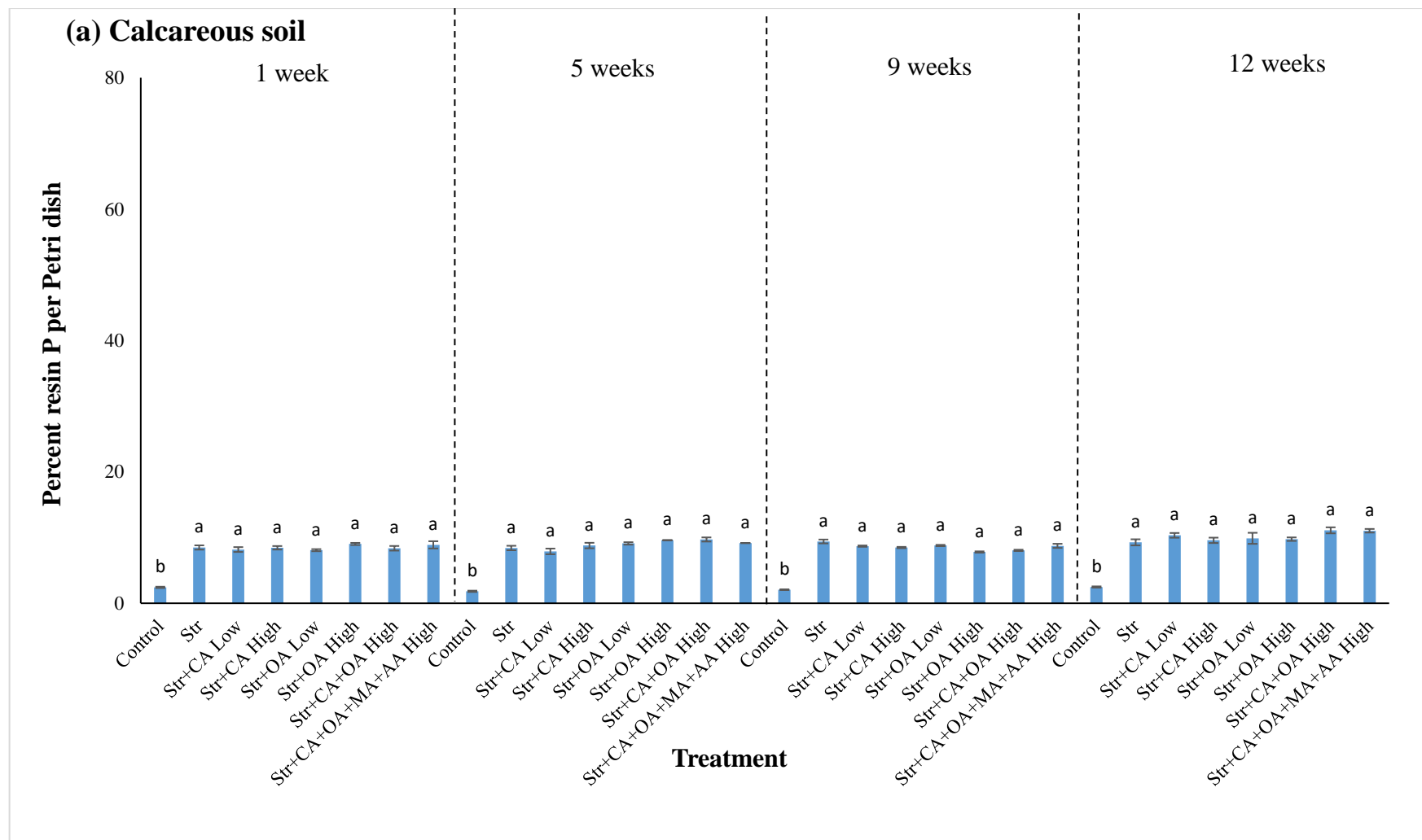
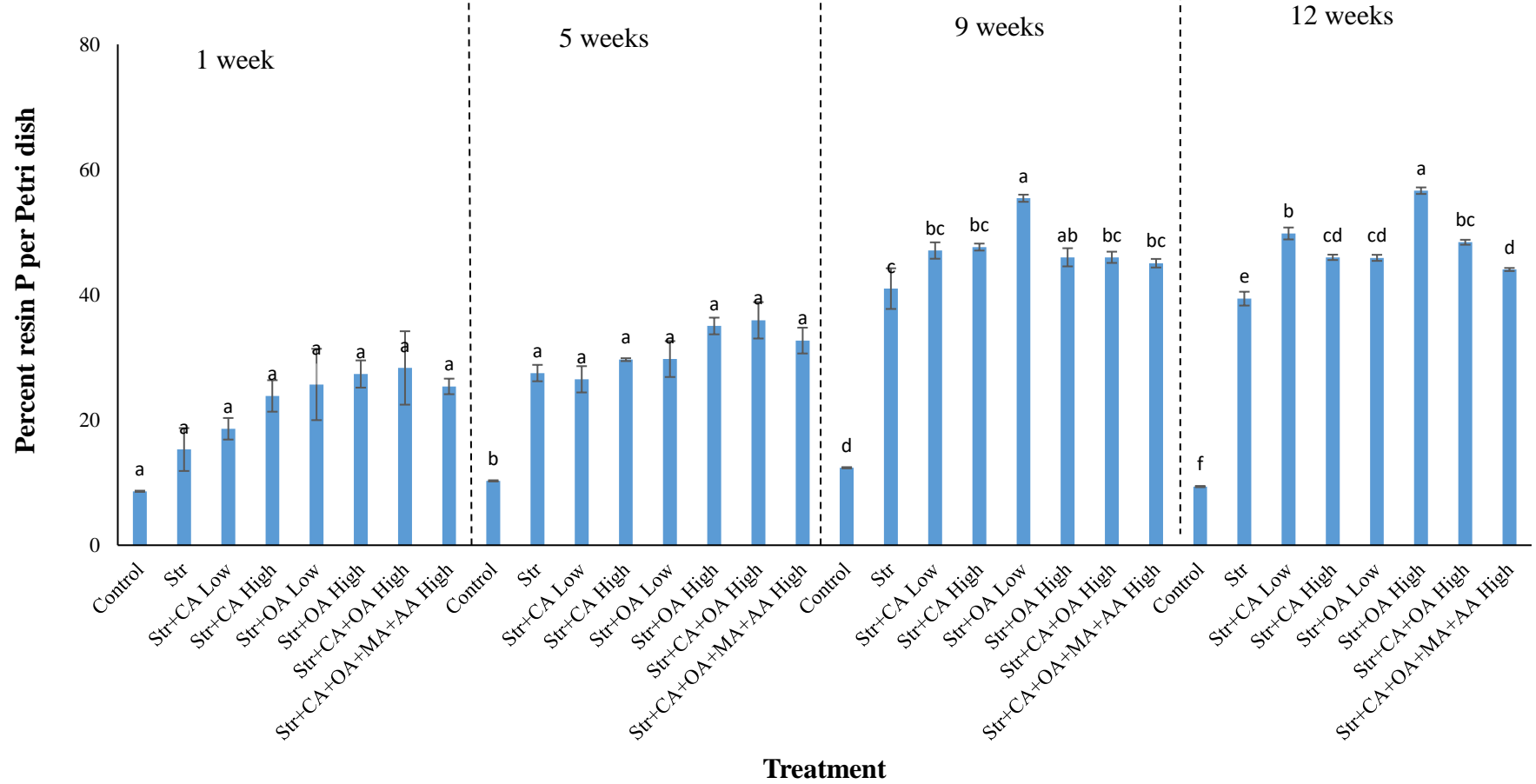


Figure 4.6. Percent resin extractable P (PRP) and pH for each dish section for all treatments after (a) 1 week, (b) 5 weeks, (c) 9 weeks, and (d) 12 weeks of incubation in acid soil. Means within each soil section for each treatment containing the same letters are not significantly different at $p = 0.05$ according to Tukey's pairwise method.



(b) Neutral soil



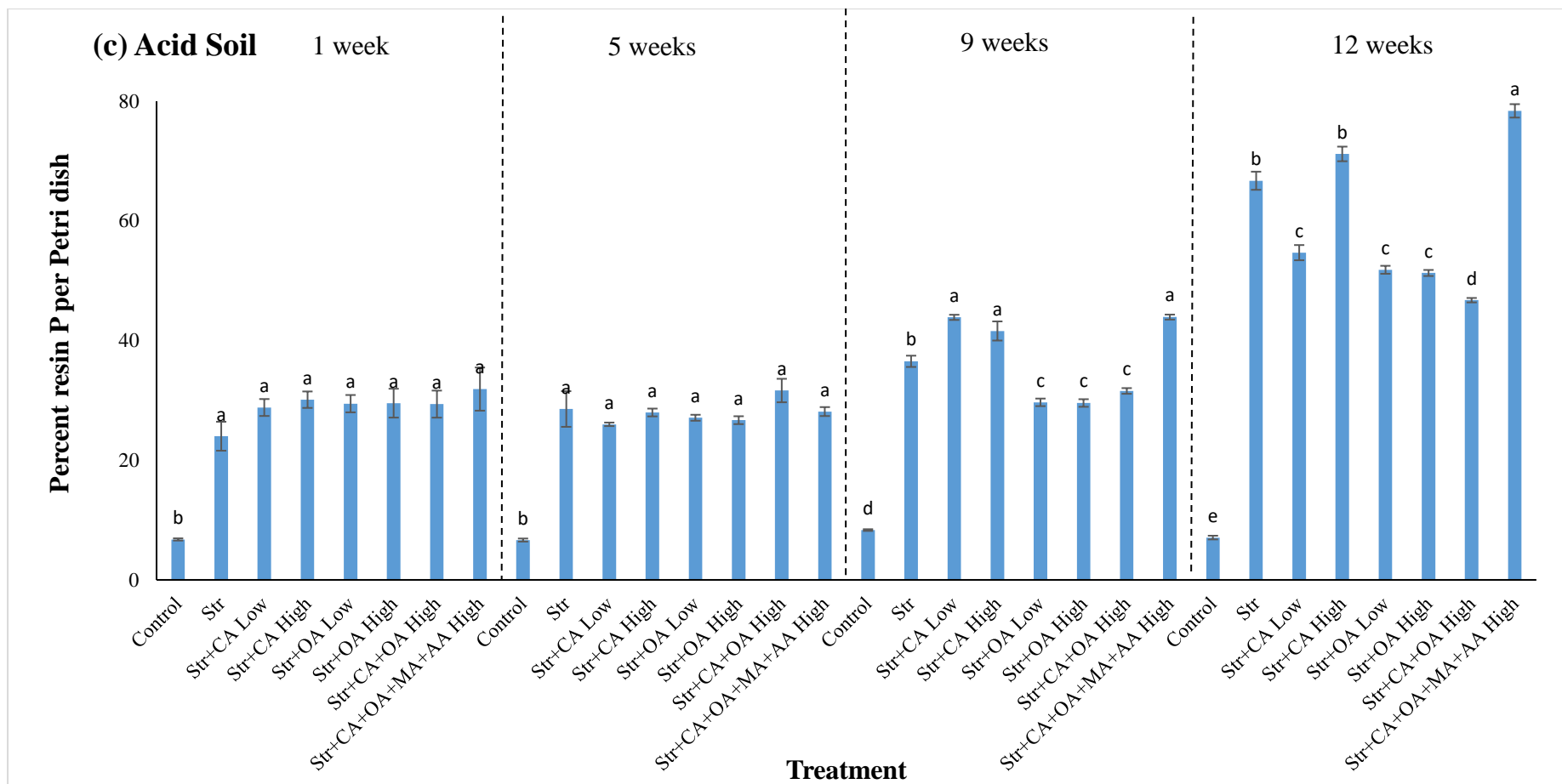


Figure 4.7. Percent resin extractable P (PRP) per Petri dish for all treatments after 1 week, 5 weeks, 9 weeks, and 12 weeks of incubation in (a) calcareous, (b) neutral, and (c) acid soils. Means within each treatment for each time period containing the same letters are not significantly different at $p = 0.05$ according to Tukey's pairwise method

Complimentary Incubation study

Dissolved organic carbon (DOC) concentration did not depend on the treatment addition time (i.e., whether it was added 1 day or 1 week after bringing soils to the desired moisture condition) (Figure 4.8). However, it greatly depends on the soil type. The study of Moradi et al. (2012) discussed the effect of organic acids is dependent on the soil mineralogy, soil physical and chemical properties, and the type of organic acids. Even though organic acids have been added as treatment it did not contribute to increasing the DOC in the mildly calcareous soil. It seems like almost all the added organic acids had decomposed or sorbed into the soil colloids or surface of CaCO_3 in calcareous soils. The calcareous soil was silt loam textured soil while neutral soil was sandy loam textured, and the clay % is 21.8% and 5.5%, respectively in both soils. Organic acids contain carboxyl groups which carry negative charges and can easily sorb into the soil colloids. The general sorption trend is oxalate > citrate > malate > acetate (Earl et al., 1979; Jones et al., 1996; Jones & Kochian, 1996). When the clay content is higher, the sorption probability is higher in the calcareous soil. Soils with high clay content provide more binding sites for binding agents (phosphates and anions) due to higher surface area per unit volume. In addition, organic acid is the most labile C source in soils, when organic acids are added with NH_4^+ fertilizers (struvite), the C: N ratio changes, and microbial decomposition is facilitated. Some studies indicated that organic acid biodegradability is higher in the rhizosphere of P-deficient soils (Jones et al., 2003). In calcareous soil, P was deficient due to fixation with calcium by precipitation as Ca-P minerals or sorbed into CaCO_3 . Similar observations were seen by XANES speciation data in calcareous soils (Figure 4.9). So, it can be considered a possible explanation for the observed short half-lives of added LMWOAs in calcareous soil. In contrast to the calcareous soil, neutral soil showed the preservation of added organic acids. This could be due to the lower

sorption capacity for organic acids in neutral soil. Less microbial activity in neutral soil may be another reason, based on low organic C content and the textural differences (Table 3.1). In sandy loam textured soil nutrient retention and organic matter content are less relative to silt loam soils, so neutral soil may lack nutrients and organic matter natively for microbial activity. The highest DOC content indicates Str + CA High treatment. This is because the tricarboxylic nature of citric acid's degradation is much lower than oxalic acid since citric acid contains 3-fold more C atoms than oxalic acids. So, it takes time to degrade by microbes when citric acid is there. A similar discussion was found in Menezes-Blackburn et al., (2016). Some studies showed that citric acid had higher persistence capacity (at least 10 weeks in the rhizosphere soil system) (Hocking, 2001). However, Str + CA Low treatment did not persist in this soil relative to Str + CA High. It is because the lower dose of LMWOAs may have been degraded by soil microbes at 24 hrs incubation. So, most fractions of low doses might have decomposed over time. Similar results were reported by other researchers (Menezes-Blackburn et al., 2016).

This complementary incubation experiment gave a clear insight into the reason behind the low effectiveness of LMWOAs on struvite solubility in calcareous soils in terms of pH reduction, P diffusion, and low resin P extractability.

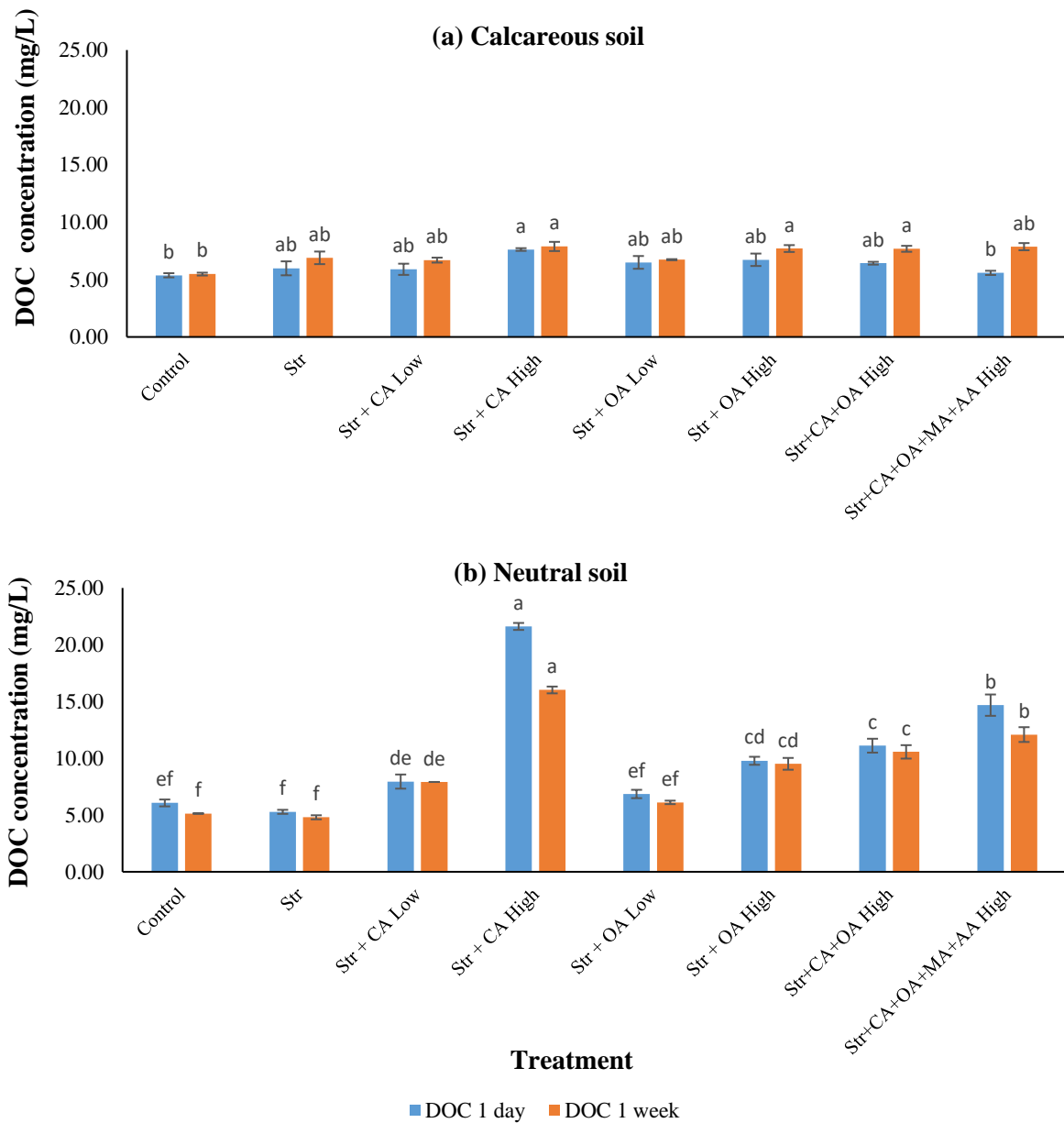


Figure 4.8. Dissolved Organic Carbon (DOC) for each treatment after addition of organic acids with treatment 1 day and 1 week after packing in (a) calcareous soil and (b) neutral soil. Means within each time after packing for each treatment containing the same letters are not significantly different at $p = 0.05$ according to Tukey's pairwise method.

Reaction products of P

In calcareous soil, the non-fertilized control sample was dominated by insoluble Ca-P mineral species (hydroxyapatite and calcium pyrophosphate) while all the struvite treated samples were dominated by reclaimed struvite in addition to calcium carbonate adsorbed P, octacalcium phosphate and hydroxyapatite (figure 4.9). However, none of these P species were soluble. Due to formation of insoluble Ca-P minerals and CaCO_3 adsorbed P, at the point of application, P mobility in the struvite treatments was restricted. Therefore, it is not surprising to see, lower percentage of added P and low P extractability in the second and third sections in each struvite treated samples. Even after the co-addition of LMWOAs, more soluble species were not observed. However, there were some differences. For example, Str + OA High treatment showed a lower fraction of insoluble hydroxyapatite and the absence of CaCO_3 adsorbed P fraction compared to other struvite treatments. Moreover, compared to hydroxyapatite, soluble octacalcium phosphate fraction was observed in this oxalic acid-treated struvite sample. The citric acid-treated sample showed a greater fraction of CaCO_3 adsorbed P (Figure 4.9). According to Èm et al. (2002), oxalic acid can dissolve the calcium carbonate and reduce the active carbonates in calcareous soils by precipitating out of calcium oxalate and has the potential of releasing P primarily bound held in calcium phosphate minerals. In contrast, citrate has a poor affinity for Ca^{2+} ions and less ability to dissolve calcium carbonate. Different effects of organic acids on CaCO_3 might have caused some differences in speciation, although the overall effect on P speciation, mobility and resin extractability were same in calcareous soils.

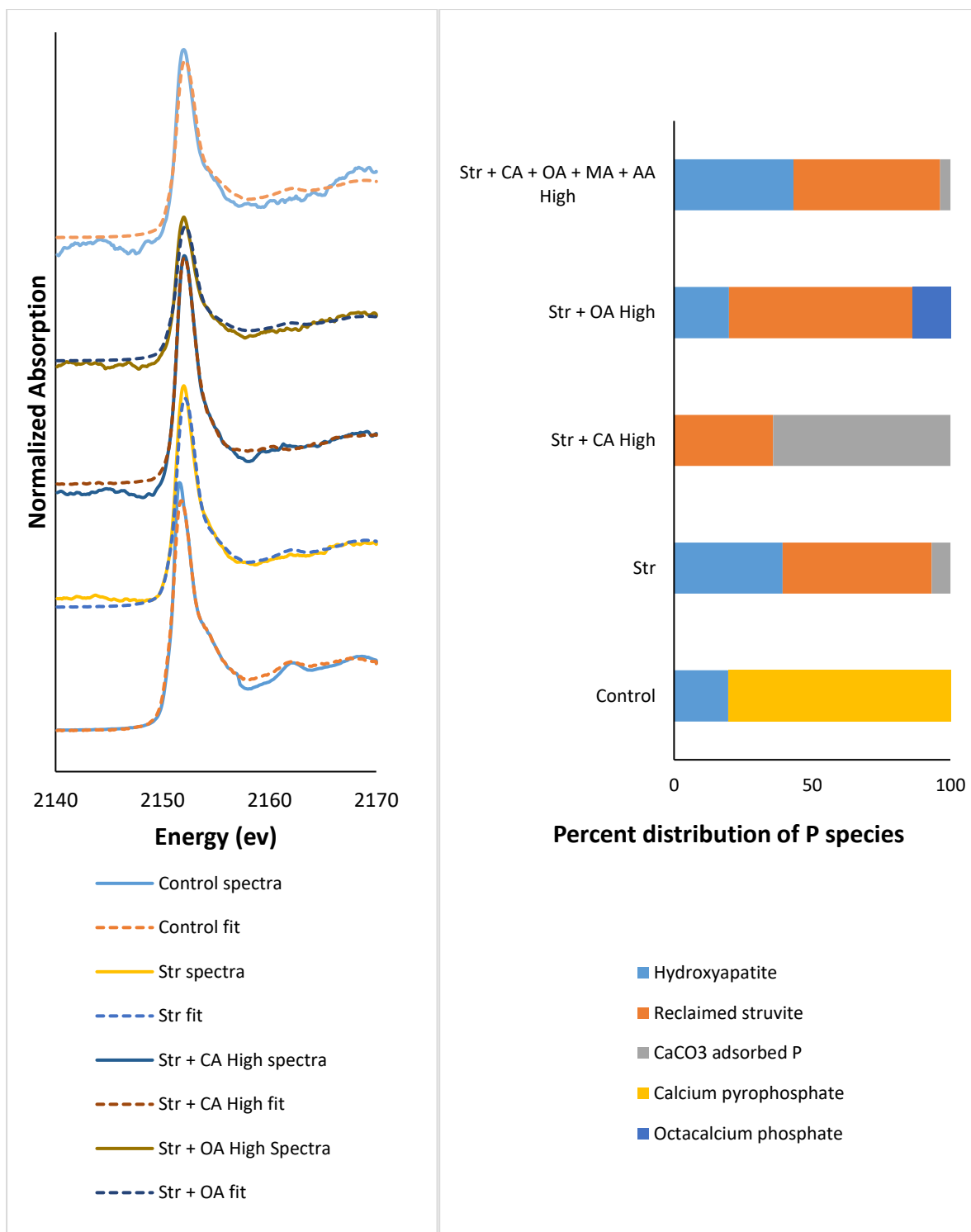


Figure 4.9. Normalized P K-edge X-ray absorption near edge structure spectra of center sections (0-7.5mm) of each treatment after 5-week incubation period with results of linear combination fitting as a percent distribution of P species in calcareous soil.

Summary

The findings of this study showed that in general the addition of LMWOAs was effective in neutral and acid soils in terms of enhancing P availability from struvite under laboratory conditions. In neutral soil, oxalic acid treatment and four organic acid mixture treatments showed higher P diffusion and higher P availability, while citric acid treatment and four organic acid mixture treatments were better performed in acid soil. Adding LMWOAs with struvite enhances P diffusion and resin P extractability by enhancing the solubility of struvite, likely through mechanisms such as pH reduction, ligand exchange reactions, chelation reactions, and P desorption in neutral and acid soils. Organic acids added to the calcareous soils disappear within a short period likely due to sorption by CaCO_3 . The XANES speciation data confirms that the addition of LMWOAs was not effective in forming more soluble P minerals. Further, some studies suggested that applying organic acids externally will be useful to overcome the reduction of LMWOAs efficiency on P solubilization due to microbial-mediated decomposition. It may promote struvite phosphorus use efficiency and plant growth in most soils other than alkaline calcareous soils (Thammaiah et al., 2023). Further research is needed to investigate struvite as a potential, effective P fertilizer in the presence of plants.

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Chapter 5 - Overall summary and implications

As rock phosphate is a non-renewable source, P fertilizer demand is increasing over time.

The recycling and reusing of recovered P fertilizer concepts are focused on finding alternative P fertilizer sources during agriculture production. This could also provide opportunities for preserving surface water quality by limiting the addition of nutrients into waterbodies due to readily soluble mineral fertilizers. Struvite is reclaimed from wastewater treatment plants, a good source of nutrients available for plants consisting of PO_4^{3-} , NH_4^+ , and Mg^{2+} ions. However, the slow dissolution of struvite under some soil conditions (alkaline pH) limits crop growth in early growing seasons. Scientists suggested that LMWOAs secreted from the rhizosphere can potentially overcome P deficiency and solubility issues of struvite. Since the limitation of fundamental information available regarding struvite performances under different soil conditions with varying pHs and impaction of the rhizosphere of LMWOAs on struvite dissolution under different soils, two studies were designed to carefully assess the dissolution, transformation, and potential plant P bioavailability of reclaimed struvite compared to conventional P fertilizers and co-addition of LMWOAs effect on struvite under mildly calcareous, neutral and acid soils using short term laboratory incubation studies with wet chemical analysis.

Based on the diffusion of P and percent resin extractable P results from the first study, the performance of struvite was less compared to conventional P fertilizers under mildly calcareous soil conditions. The XANES data gave us a clear insight that most of the added P was restricted at the point of application (POA) in calcareous soils due to precipitation and sorption reactions with Ca^{2+} and CaCO_3 . Under neutral soil conditions, more than 20% of added P in struvite have diffused into further sections from the POA, while around 40% have diffused in acid soil at the end of the 12-week incubation period. Overall, resin P

extractability in calcareous soil is lower than that of neutral and acid soils. The highest resin P extractability could be seen in struvite at the end of the 12-week incubation period in all three sections in acid soil, followed by neutral soil. In terms of diffusion and potential plant availability, MAP and APP performance was superior in calcareous soil, while struvite showed comparable performance with MAP, DAP, and APP in neutral and acid soils. However, the struvite performance may be altered when struvite is blended with conventional P fertilizers for calcareous soils. When Struvite was added into all three soils, the initial pH increment was observed at the POA due to the slow dissolution of struvite using protons from the surrounding soil solution. This effect is beneficial under acidic soil conditions since the reduction of Fe and Al phosphate formation occurs due to less dissolved Al and Fe in high pHs.

Percent P added and percent resin extractable P results for the co-addition of LMWOAs with struvite study showed that the organic acid addition impacted positively on struvite dissolution, P diffusion, and potential P availability under neutral and acidic conditions, but not under calcareous soil conditions. The complementary incubation study results (DOC results) proved that microbial degradation and sorption reactions of LMWOAs in calcareous soil might be the culprits for less efficacy of organic acids in calcareous soils. However, under neutral and acidic soil conditions, struvite with four mixtures of LMWOAs treatment (Str + CA + OA + MA + AA High) showed superiority in terms of P mobility in both acid and neutral soil. The overall P availability of oxalic acid was higher in neutral soil while citric acid in acid soil. Overall results suggested that the addition of LMWOAs with struvite facilitated the P diffusion and resin P extractability by enhanced dissolution of struvite, likely due to the mechanism, such as pH reduction, ligand exchange reactions, chelation reaction, and P desorption in neutral and acid soils. However, the struvite performance is expected to be enhanced when the plant roots are present in the real field situation since the continuous

secretion of organic acids into the rhizosphere can overcome the degradation and sorption issues.

Although the solubility, P transformation, and availability results of fertilizer may be changed in practical situations due to changing environmental conditions compared to controlled laboratory conditions such as these are needed, for understanding fundamental processes, controlled experiments.