# Influence of pH, phosphorus addition and soil water content on phosphorus pools in acid sulfate soils

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Dedicated to my beloved family

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# Abstract

Acid sulfate soils (ASS) are common in wetlands and important for crop production. However, little is known about P availability in ASS which is constrained due to high Fe content and changing redox potential. This thesis aimed to investigate the effect of P addition, pH and soil water content on P pools in ASS. A sandy sulfuric ASS (pH 3.2) was used in the experiments.

In the first experiment, the ASS was amended with NaOH or $Ca(OH)_2$) to achieve soil pH 4 or 5.5, without or with addition of inorganic P equivalent to 20 kg $ha^{-1}$. Soils were incubated in either submerged or moist conditions (100% of maximum water holding capacity). After two weeks, P pools and Fe /Al oxides were determined. P pools were higher with P addition than without. The increase in pH enhanced Fe oxides but had little effect on labile P. Added soluble P was available after two weeks of addition under submerged conditions.

In Experiment 2, the ASS (pH adjusted to 5.5) was amended with phosphate at 0, 96, 385, 578 and 770 mg P $kg^{-1}$ and incubated under submerged conditions. P pools were measured after two and four weeks. Sorption and release of P were determined in incubated soils and jarosite, separately. The maximum sorption capacity of the soil was 350 mg P $kg^{-1}$. Jarosite had a high capacity to bind P and contributed about 50% to P binding in this soil. Labile P represented more than 50% of added P at P rates above 350 mg P $kg^{-1}$.

The third chapter included two experiments. In the first experiment, the ASS (pH adjusted to 5.5) was amended with 400 mg P $kg^{-1}$ as inorganic P, manure or combinations of inorganic P/manure and was incubated submerged. After two and four weeks, labile P was measured. Labile P in treatments with 50% of P added as manure was about half of that in treatments with a greater proportion of inorganic P. In the second experiment, treatments (inorganic P alone: 100F, manure alone: 100M, combination of manure and inorganic P: 50M/50F and unamended control) were kept submerged, then moist, followed by resubmergence. At the end of first submergence, labile P was two-fold higher in 100F than in manure treatments. But in the moist period and after resubmergence, labile P did not differ between 100F and 100M. Oxalate extractable Fe was higher in 100M than 100F and remained unchanged until the end of the experiment.

In experiment 4, the ASS (without or with pH increase to 5.5) was amended with mangrove roots to achieve an organic carbon (OC) addition of 50% or 150% of native soil OC and incubated under submerged, moist and then resubmerged conditions. At the end of each four-week period, P pools, amorphous Fe/Al and potential P sorption were measured. Mangrove roots addition decreased the redox potential and increased labile P under submerged conditions, but only at pH 5.5. P binding was enhanced by root addition during the first 8 weeks, but not after re-submergence.

# Declaration

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Sonia Samanthi Mayakaduwage

Date 10/09/20

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## Publications arising from this thesis

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2. Mayakaduwage, S., Mosley, L.M, Marschner, P., 2020. Threshold of labile phosphate in a sandy acid sulfate soil. Geoderma 371, 114359 (doi: https://doi.org/10.1016/j.geoderma.2020.114359)
3. Mayakaduwage, S., Mosley, L.M., Marschner, P., 2020. Phosphorus pools in acid sulfate soil are influenced by soil water content and form in which P is added**.** Geoderma 381, 114692 (doi: https://doi.org/10.1016/j.geoderma.2020.114692)
4. Mayakaduwage, S., Mosley, L.M., Marschner, P., 2020. Phosphorus pools in acidic acid sulfate soil are influenced by pH, water content and addition of organic matter. **Submitted to Journal of Soil Science and Plant Nutrition**

# Chapter 1

## Introduction and literature Review

## 1.1 Introduction

Wetlands are one of the three ecosystems in the world, and have important functions in global food production and as centres of biodiversity (Kirk, 2004). The total global area of inland natural wetlands is estimated about 1300 km$^2$ (Davidson and Finlayson, 2018). Wetland soils which are flooded or submerged at least a part of the year, play a crucial role in nutrient retention and cycling (Moreau et al., 2013). When a soil is submerged, depletion of oxygen typically results in reduction of other oxidised soil components ($NO_3^-$, $MnO_2$, $Fe^{III}$, $SO_4^{2-}$ and $CO_2$) by anaerobic microbes (Dent, 1986). Reaction of $Fe^{2+}$ formed following $Fe^{III}$ reduction with reduced inorganic sulfur results in formation of iron sulfide minerals such as iron monosulfide (FeS) and pyrite ($FeS_2$). Under anoxic conditions, aqueous FeS can react with $H_2S$ forming pyrite ($FeS_2$) (Rickard and Luther, 1997). Many wetland soils have suitable conditions for the formation of pyrite (saturated, high organic matter, dissolved iron and sulfate present). These are defined as acid sulfate soils (ASS) if, due to pyrite oxidation, sulfuric acid may be produced, is being produced, or has been produced in amounts that have a lasting effect on the main soil characteristics (Pons, 1973). Globally, ASS cover over 17 million ha, of which there are 7.5 million ha in the tropics and more than 5 million ha in Australia (Andriesse and Van Mensvoort, 2006; Fitzpatrick and Shand, 2008). ASS are chemically stable under flooded/reducing conditions. However during dry periods, ASS may become oxidized and release sulfuric acid leading to pH 4 or less in soil and leachate.

The macronutrient phosphorus (P) is important in plants for photosynthesis, respiration, enzyme reactions, and carbohydrate metabolism (Vance, 2011b). Plant P availability is very low because only < 1% of total soil P is immediately available as $H_2PO_4^-$ or $HPO_4^{2-}$ (orthophosphate) in the soil solution. In acidic soils, Fe minerals play a crucial role in P cycling. Under reducing conditions, the less soluble ferric ($Fe^{III}$) compounds are reduced to more soluble $Fe^{2+}$, releasing P bound to ferric compounds. In aerobic conditions, $Fe^{2+}$ is oxidized to hydrated ferric oxide minerals (or to Fe oxyhydroxy sulfate minerals in acid sulfate soils) which can bind P. Most studies on P in submerged and aerated soils have been carried out with non-ASS soils. Little is known about P availability in ASS. Due to the high Fe concentration and very low pH under aerated conditions, P pools in these soils may be different to those in non-ASS soils. ASS contain a variety of amorphous Fe oxides (e.g. pyrite under submerged conditions and jarosite, schwertmannite and goethite when they are oxidised). When P fertiliser is added to ASS, plant available P concentration remain low due to adsorption of P to those Fe oxides. This may lead to limit the crop growth in ASS. The following literature review will provide an overview about submerged soils, ASS (including distribution and biogeochemistry) and forms of P in the soil.

## 1.2 Submerged soils

Submerged or flooded soils are widespread, covering 5-7% of the global land surface (Kirk, 2004). They are important for global food production (e.g. rice), nutrient cycling and biodiversity (Kirk, 2004). When a soil is submerged, oxygen is depleted because dissolved oxygen in soil pores is utilized by aerobic microorganisms and oxygen diffusion rates in water are four orders of magnitude lower than in air (Kihara et al., 2014). Therefore the soil becomes anoxic (Boivin et al., 2002). According to Ponnamperuma (1972), there are four types of submerged soils: waterlogged soils, marsh soils, paddy soils and subaqueous soils.

Reduction processes often result in distinctly different layers within submerged soil (a) a partially oxidized A horizon, (b) a mottled zone in which either oxidation and reduction can occur and (c) a permanently reduced bluish green zone (Ponnamperuma, 1972). In oxygenated water, submerged soil may have an oxygen rich surface layer (few millimetres to centimetres thick). Below this layer, the oxygen concentration decreases quickly (Ponnamperuma, 1972). If the soil is permanently or periodically saturated, organic matter decomposition is slow causing its accumulation in the A horizon. Ferrous compounds in the permanent waterlogged give it its bluish green colour. This zone may have secondary minerals present such magnetite, pyrite, marcasite, siderite, vivianite etc (Ponnamperuma, 1972).

The most distinct chemical characteristic of submerged soils is their reduced state. Oxidation-reduction are chemical reactions in which electrons are transferred donor to acceptor. The electron donor loses electrons through oxidation number whilst the acceptor gains electrons is reduced (Fiedler et al., 2007). The redox potential (Eh) can be measured in volts and is considered as an indicator for electron activity or tendency of the medium to be reduced after acquiring electrons (Fiedler et al., 2007). In well oxygenated soils, Eh can reach > 0.4 V, submergence usually results in Eh < -0.2 V (Yu et al., 2007). In soils, organic matter is decomposed by microorganisms and acts as the source of electrons. During aerobic microbial respiration, oxygen is the prime acceptor of the electrons. When oxygen is depleted under submerged conditions, aerobic microbial processes are replaced by anaerobic processes, where microbes use a variety of compounds to replace oxygen as terminal electron acceptor thereby reducing them (Faulkner and Richardson, 1989). Reduction processes in submerged soil follow the sequence $O_2$, $NO_3^-$, $MnO_2$, $Fe^{3+}$, $SO_4^{2-}$ and $CO_2$. Each reduction stage is associated with a lower Eh at pH 7 (Islam and Islam, 1973; Takeda and Furusaka, 1970; Zehnder and Stumm, 1988) as shown below where organic matter is represented by $CH_2O$.

Aerobic respiration (by facultative anaerobes and aerobes) at Eh >0.4 V:

$$CH_2O + O_2 \rightarrow CO_2 + H_2O \tag{1}$$

Denitrification at Eh <0.4 V:

$$CH_2O + 0.8\,NO_3^- + 0.8H^+ \rightarrow CO_2 + 0.4N_2 + 1.4\,H_2O \tag{2}$$

Manganese reduction (by Mn reducing bacteria) at Eh <0.38 V:

$$CH_2O + 2\,MnO_2 + 4H^+ \rightarrow CO_2 + 2Mn^{2+} + 3\,H_2O \tag{3}$$

Iron reduction (by iron reducing bacteria) at Eh between 0.3V and -0.1 V:

$$CH_2O + 2FeOOH + 4H^+ \rightarrow CO_2 + 2Fe^{2+} + H_2O \tag{4}$$

Sulfate reduction (by sulfate reducing bacteria) at Eh between -0.1 V and -0.15 V:

$$CH_2O + 0.5SO_4^{2-} + 0.5H^+ \rightarrow CO_2 + 0.5HS^- + H_2O \tag{5}$$

Methane formation (by methanogenic bacteria) at Eh between -0.15 and -0.22 V:

$$CH_2O + 0.5CO_2 \rightarrow CO_2 + 0.5\,CH_4 \tag{6}$$

Since easily reducible species are reduced first at higher Eh values, their presence can inhibit the reduction of other species that are reduced at lower Eh values either by depletion of electron donors (organic matter) or oxidizing the reduction products (Carlson et al., 2013; Hubert and Voordouw, 2007). For example, Yuan et al. (2015b) showed that nitrate inhibits sulfate reduction by adding nitrate (up to 200 mg nitrate-N kg$^{-1}$) to an acid sulfate soil along with organic matter at different rates and keeping them for two weeks under flooded conditions. They found that acid volatile sulfur concentrations (attributed to FeS) were lower when nitrate was added and particularly at the highest nitrate addition rate, compared to the treatment without nitrate. This indicates that organic matter was utilized by nitrate reducing microorganisms leading to less available electron donors for sulfate reduction. Earlier, Chidthaisong and Conrad (2000) found that addition of nitrate and sulfate to anaerobic soil inhibited methane formation since microbes utilizing these electron acceptors outcompeted methanogens for organic matter.

The processes represented in Equations 1 to 6 influence soil pH. If decomposable organic matter is present, the soil pH may increase in acidic soils or decrease in alkaline soils under submerged conditions (Ponnamperuma, 1984; Yuan et al., 2015b). The pH increase in acidic soils during submergence can be attributed to proton consumption during reduction processes (Narteh and Sahrawat, 1999; Ponnamperuma, 1972). For example, lowland acid rice soils with initial pH 4.3 were kept under flooded conditions for 15 weeks. During the first week of submergence, the pH increased to 5.6 and by week 15 it was 6.8-7.4 (Narteh and Sahrawat, 1999). In alkaline soils, the accumulated $CO_2$ from the above reactions causes the soil pH to decrease during the first phase of submergence, as $CO_2$ reacts with water to form weak carbonic acid which consumes alkalinity (Sahrawat, 1998). In alkaline soil, Włodarczyk et al. (2007) found that the pH had decreased from 7.5 to 6.5 in 35 days of flooded conditions.

Redox potential is important for formation and dissolution of Fe minerals. Reduction of Fe and Mn oxides and the decrease in soil pH results in production of soluble $Fe^{2+}$ and $Mn^{2+}$ species in submerged soil (Fageria et al., 2008; Phillips and Greenway, 1998). Due to Fe reduction, CEC of submerged rice soil increased more than two fold (Favre et al., 2002). This can be explained by the role of Fe in clay CEC. Positively charged iron oxyhydroxides bound on clay surfaces neutralize the negative charge of the clay. Removal of iron oxyhydroxides on clay surfaces by reduction exposes the cation exchange sites on the clay (Favre et al., 2002). Poorly crystalline iron oxyhydroxides have a high surface area and therefore provide sorption sites for metals and metalloids such as As. These metalloids can be released into the environment as a result of iron reduction in paddy soils (Takahashi et al., 2004; Weber et al., 2009). For example, paddy soils contaminated with As were studied under laboratory conditions and it was found that concentration of both $Fe^{2+}$ and As ions in soil pore water increased during a 14 day incubation under submerged conditions (Xu et al., 2017). On the other hand, sulfate reduction to sulfide under flooded conditions may lead to formation of insoluble metal-sulfide complexes which decreases the availability of such metals in soil (Kashem and Singh, 2001; Van den Berg et al., 1998).

## 1.3 Acid Sulfate Soils (ASS)

### 1.3.1 Distribution and general properties of ASS

ASS are defined as soils which sulfuric acid may be produced, is being produced or has been produced in amounts that have a lasting effect on main soil characteristics (Fanning et al., 2017). The distribution of ASS is related to the rate of sedimentation and alterations in relative sea levels, climate, hydrology and chemical composition of the flood water (Fitzpatrick et al., 2008). ASS are commonly found in coastal areas (where sulfate is abundant for sulfate reduction reactions), particularly in South and Southeast Asia, West and Southern Africa, South America, Caribbean and Australia some of which may be densely populated and economically important. They also occur inland in wetlands and along rivers or lakes (Fitzpatrick et al., 2008). ASS cover over 17 million ha in the world, of which there are 7.5 million ha in the tropics and more than 5 million ha in Australia (Andriesse and Van Mensvoort, 2006; Fitzpatrick et al., 2008; Shamshuddin et al., 2004). In Australia, ASS occupy about 4 million ha in coastal areas and 1.5 million ha inland (Fitzpatrick et al., 2008).

ASS materials are usually comprised of iron sulfides (FeS) /pyrite ($FeS_2$), resulting from reactions of hydrogen sulfide and dissolved $Fe^{2+}$ under anaerobic conditions (Blunden, 2000). Monosulfidic (FeS/ detectable inorganic sulfides), sulfuric ($pH < 4$) or hypersulfidic materials (which oxidise to produce sulfuric acid: $pH < 4$) may also be present (Australian Soil Classification). Under reducing conditions, ASS are chemically stable. However, when ASS are aerated, pyrite oxidation occurs and sulfuric acid is produced, which can lead to $pH<4$ or less in soil and leachate when the acid neutralising capacity is insufficient (Andriesse and Van Mensvoort, 2006). In Australia, ASS contain about 1 billion tonnes of iron sulfides (Fitzpatrick et al., 2009). When oxidised, one tonne of iron sulfides can produce about 1.5 tonnes of sulfuric acid (Fitzpatrick et al., 2009). In South Australia, coastal ASS occupying 241,000 ha, have an acid reservoir of nearly two metric tonnes (Poch et al., 2009).

### 1.3.2 Processes in submerged conditions

In waterlogged/anoxic conditions and with sufficient available organic matter, sulfate is reduced to sulfide by microorganisms (Dent, 1986). Simultaneously, $Fe^{III}$ oxides (e.g. FeOOH) are reduced to $Fe^{2+}$. This process includes a number of steps that can be outlined in the following equations:

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + HCO_3^{2-} \tag{7}$$

$$2FeOOH + S^{2-} + 6H^+ \rightarrow S^0 + 2Fe^{2+} + 4H_2O \tag{8}$$

where $CH_2O$ stands for organic matter (Berner, 1984). Reduction of $Fe^{III}$ and $SO_4^{2-}$ is mainly carried out by heterotrophic ferric and sulfate reducing bacteria, respectively, which require organic matter as an energy source (Wendt-Potthoff et al., 2010). They hydrolyse complex organic matter including carbohydrates, lipids and proteins and use $Fe^{+3}$ and $SO_4^{2-}$ as electron acceptors (Wilkin and Barnes, 1996). Sulfate reduction is maximal at a redox potential of -150 mV and pH 6.5-8.5 (Norton et al., 2012). Pyrite formation occurs via either the hydrogen sulfide or the polysulfide pathway (Luther, 1991; Rickard and Luther, 1997). In the polysulfide pathway, $S^0$ from Eq. (2) reacts with sulfide to produce polysulfide ions.

$$HS^- + nS^0 \rightarrow S_{n+1} + H^+ \tag{9}$$

The polysulfide ions ($S_{n+1}$) then react with aqueous FeS which results from the reactions between $H_2S/HS^-$ and iron species, to form pyrite (Luther, 1991).

$$FeS + S_{n+1} \rightarrow FeS_2 + S_n^{2-} \tag{10}$$

In the hydrogen sulfide pathway, aqueous FeS is oxidized by $H_2S$, forming pyrite (Rickard and Luther, 1997).

$$FeS + H_2S \rightarrow FeS_2 + H_2 \tag{11}$$

As long as ASS containing pyrite and other iron sulfides remain under reducing conditions, only minor geochemical changes may occur aside from sulfide mineral transformations and recrystallization. In these conditions, ASS may have a steely blue-grey colour, which can be attributed to $Fe^{2+}$ (Ponnamperuma, 1972). In long-term anoxic conditions different colour combinations may occur from accumulation of secondary Fe oxides, sulfides, carbonates and salt (Ponnamperuma, 1972; Rankama and Sahama, 1950). The other noticeable characteristic of sulfate reduction is release of hydrogen sulfide ($H_2S$). If heavy metals are present in ASS, $H_2S$ may react with them to form insoluble sulfides (Sheoran and Sheoran, 2006). Soil pH may increase to between 6.5 and 7, however pH values may fluctuate depending on the $Fe^{III}$ reduction rate (Kirk, 2004). Reduction of $Fe^{III}$, and other electron acceptors, results in the consumption of $H^+$ (Eq.2) and therefore increases soil pH (Jayalath et al., 2016; Yuan et al., 2015a). Iron reduction can also increase soil P availability because phosphate anions can be bound to iron III oxides (Ponnamperuma, 1972). When the oxides are reduced, P is released (Baldwin and Mitchell, 2000). Hydrolysis of Fe and Al phosphates under reduced conditions may also lead to increased P availability. These reactions will be discussed further below.

### 1.3.3 Processes in oxidized conditions

Anthropogenic activities such as excavation, drainage or disturbance, tidal variations in low lying coastal areas, decreasing water table level and wetting and drying processes in wetlands can cause ASS to be drained or exposed to air. Then pyrite in ASS is oxidized releasing sulfuric acid. Pyrite oxidation process can be described in the following equations (Johnson and Hallberg, 2005):

$$4FeS_2 + 15O_2 + 14H_2O \rightarrow 4Fe(OH)_3 + 8SO_4^{2-} + 16H^+ \qquad (12)$$

In more detail, pyrite reacts with oxygen:

$$FeS_2 + \frac{7}{2}O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \qquad (13)$$

The rate of pyrite oxidation is often highest at low pH (< 4). Main oxidants of pyrite in natural systems are oxygen and $Fe^{3+}$, the rate limiting step is $Fe^{2+}$ oxidation which is catalysed by acidophilic iron oxidizing bacteria. Since these microorganisms are chemolithotrophic, organic matter is not required as the energy source (Moses and Herman, 1991; Moses et al., 1987).

Oxidized ASS generally have a pH < 4 (Demas et al., 2004; Fitzpatrick et al., 2009). The low pH of oxidized ASS can cause release of $Fe^{3+}$, $Al^{3+}$ and sometimes $Mn^{2+}$ from clay and other minerals (Fanning et al., 2010; Kaksonen et al., 2017). Then, concentrations of these ions in the soil solution can be high, in some cases reach toxic concentrations (Mosley et al., 2014a; Mosley et al., 2014b). In addition, $Ca^{2+}$ in the soil solution may react with $SO_4^{2-}$ released from pyrite oxidation resulting in precipitation of $CaSO_4$ (gypsum), and reducing $Ca^{2+}$ availability (Ritsema and Groenenberg, 1993).

Hydrolysis of $Fe^{3+}$ results in formation of amorphous hydroxides as ferrihydrite, jarosite, schwertmannite or crystalline Fe oxides like goethite and lepidocrocite depending on the pH (Ahern et al., 2006; Fitzpatrick and Shand, 2008). Dissolution of these secondary Fe minerals can result in release of acidity (Fitzpatrick et al., 2009; Trueman et al., 2020). Amorphous Fe (hydr)oxides are reduced more easily than crystalline Fe (hydr)oxides. In well drained ASS, jarosite ($KFe_3(OH)_6(SO_4)_2$) precipitates as yellow mottles in strongly oxidising and acidic conditions (Dent, 1986). Although jarosite is stable under oxidising conditions, drop in redox potential can destabilise jarosite, releasing protons (Lin et al., 1998). Chu et al. (2006) found that organic matter addition caused depletion of dissolved oxygen and enhanced jarosite dissolution. Jarosite may be important for phosphate binding through two mechanisms: anion exchange between sulfate and phosphate or ligand exchange mechanism where surface hydroxyl groups of jarosite are replaced by phosphate (Wang et al., 2013; Zhong et al., 2007). In addition, jarosite can scavenge metals, and therefore influences fate and transport of metals in ASS environments (Accornero et al., 2005).

### 1.4 Phosphorus in soils

Phosphorus (P) is essential for plants. Being a component of nucleic acids, P is critical for storage of genetic information, cell division and plant development (Marschner, 2012). Moreover, P is vital for all plant processes which require energy transfer, since it is a part of ATP (Adenosine triphosphate) and ADP (Adenosine diphosphate) (Oberson and Joner, 2005; Vance, 2011a). Although soil total P concentration is high, plants can only take up P from the soil solution where it is present as $H_2PO_4^-$ and $HPO_4^{2-}$ Phosphate ions quickly bind with soil organic matter and mineral components, therefore their concentration in soil solution is lower than that of other nutrient anions (such as sulfate, nitrate) even in highly fertilized soils (<20µM) (Marschner, 2012; Warren, 1992). Thus, P is considered as one of the most immobile and unavailable plant nutrients. Limitation of plant available P in soil reduces yield and thereby can negatively impact global food security.

### 1.4.1 Phosphorus in aerated soils

Soil P comes from sedimentary or igneous rocks as the primary mineral apatite and its different forms including fluoroapatite $Ca_5(PO_4)_3F$ or hydroxyl apatite $Ca_5(PO_4)_3(OH)$ (Willard, 1979). Over time, insoluble apatite is converted into different P forms through thermal and chemical processes generating different types of inorganic and organic P pools which vary in lability/solubility. P pools in soil may consist of labile, moderately labile, relatively insoluble, and resistant P forms (Alamgir and Marschner, 2016).

In the soil solution, soil $P_i$ is present as orthophosphate ($H_2PO_4^-$ and $HPO_4^{2-}$) and may be derived from fertiliser addition, weathering of apatite, or mineralisation of organic matter. The form of orthophosphate depends on soil pH, both $H_2PO_4^-$ and $HPO_4^{2-}$ are dominant at pH<7 while the latter is the major species pH>7 (Willard, 1979). Orthophosphate ions can form complexes with metal cations (Al, Fe), organic matter or clay minerals reducing their concentration in the soil solution. In acidic soils, trivalent Fe and Al cations in the soil solution bind with orthophosphates through electrostatic and covalent bonding leading to the formation of various minerals such as vivianite [$Fe_3(PO_4)_2.8H_2O$] and variscite [$AlPO_4.2H_2O$] (Dunham-Cheatham, 2012). In alkaline or neutral soils, Ca and Mg, bind to orthophosphate forming dicalcium or octacalcium phosphates, hydroxyapatite, Mg phosphates and eventually poorly soluble apatites (Nriagu and Moore, 2012). Between 85 and 90% of added $P_i$ can be adsorbed to or precipitated with Fe, Al and Ca (Gichangi et al., 2009; Khan and Joergensen, 2009). Therefore P fertiliser use efficiency (proportion of applied nutrient taken up by the crop) is very low compared to fertiliser efficiency of other nutrients, e.g. N.

Soil organic P (Soil $P_o$) which is positively correlated to organic C, derives from plant residues, animal waste and soil microbial biomass and accounts to 20%- 80% of total P (Condron et al., 2005; Nash and Halliwell, 1999; Richardson et al., 2001). Soil $P_o$ is bound to minerals or organic carbon and is released into the soil solution by enzymes such as phosphatase that can be secreted by microbes or plant roots (Richardson, 2007). Inositol (Phytate- a low molecular weight monoester) is a stable form of $P_o$ in the soil (Turner et al., 2005). Phytate is the main P storage form in plant seeds and other parts of plants (Noack et al., 2012). After addition to the soil, phytate can precipitate with Fe, Al or Ca in the soil forming very stable salts (Ognalaga et al., 1994; Turner et al., 2005). Further, phytate can associate with clay minerals, fulvic/humic acid, proteins or metals, forming complex structures. Thus phytate is more resistant to mineralization than other types of $P_o$ (Rojo et al., 1990; Turner et al., 2005).

Microbial biomass P (MBP) contains nucleic acids, polyphosphates and phospholipids and accounts for 3% of total P in arable soils (Magid et al., 1996). Although it represents a small proportion of total P, MBP is significant as it is a labile pool.

The solubility and availability of soil P forms depend on soil chemical/biological properties such as soil pH, organic matter /clay content and microbial activity (Alamgir and Marschner, 2016; Holford, 1997). Soil pH plays an important role on the size of labile P pool because P is released into the soil solution from Fe phosphates when the pH increases, whilst Ca phosphates release P with decreasing pH, at pH values < 8 (Hinsinger, 2001). Ch'ng et al. (2014) found that amendment of organic matter to acid soils increased soluble P associated with Fe/Al by increasing the soil pH. Further, organic matter can act as a source of P which can be utilised by plants after mineralisation (Tiessen et al., 1994). On the other hand, several studies have reported that organic matter may decrease labile P and increase less available pools by providing binding sites (Huang et al., 2012; Schlesinger et al., 1998).

### 1.4.2 Phosphorus in submerged and drained soils

Submergence can influence P availability. In alkaline soils, P can be bound with Ca minerals such as hydroxyl-apatite. Solubility of P can increase if the pH decreases after submergence since the solubility of hydroxyl-apatite ($Ca_5(PO_4)_3(OH)$) increases with decreasing pH (Ponnamperuma, 1972). In acid soils P forms salts with Fe or Al such as strengite ($FePO_4.2H_2O$) or variscite ($AlPO_4.2H_2O$) or is bound to Fe and Al oxides (Iuliano et al., 2007; Iuliano et al., 2008). As a result of submergence and reduction of $Fe^{III}$ to more soluble $Fe^{2+}$, P that was adsorbed to or co-precipitated with the Fe is released. For example, there was a positive correlation between $Fe^{2+}$ and P release in valley soil under flooded conditions (Peretyazhko and Sposito, 2005). From 1 to 11 days of submergence, $Fe^{2+}$ increased from 0 to 800 mg $kg^{-1}$ and available P, from 1 to nearly 12 mg $kg^{-1}$. Similarly, Zhang et al. (2003) reported that P availability in paddy soils was related to Fe reduction. Both, soluble $Fe^{2+}$ and available P increased two-fold after 14 days of incubation under flooded conditions. On the other hand, the concentration of diothionite soluble Fe (crystalline iron oxide) decreased during that period by nearly 50%. These results show that reductive dissolution of Fe oxides releases Fe and bound P (Zhang et al., 2003). Soluble P in acid soils may also increase after submergence as $Fe^{3+}$ and Al phosphates release P when pH increases as $HPO_4^{2-}$ is replaced with $OH^-$ (Von Uexküll and Mutert, 1995). Furthermore, organic anions ($COO^-$) that are released during decomposition of organic matter by microorganisms (Ponnamperuma, 1972) can replace phosphate bound to clay increasing P availability in submerged soils. However, P availability may increase only temporally. Tian et al. (2017) observed that during the first five days after flooding reactive P concentration remained at 12 mg $L^{-1}$ and decreased to 2 mg $L^{-1}$ by day 35. Similarly, Amarawansha et al. (2015) found that dissolved reactive P in manure amended soil under experimental flooded conditions increased from 0.5 to 1.6 mg $L^{-1}$ during the first four weeks of submergence and then started to decrease with time. The decrease in available P in flooded soil with time may be due to adsorption and re-precipitation of available P with amorphous $Fe(OH)_2$-$Fe(OH)_3$ or formation of ferrous phosphate compounds such as vivianite, variscite, stregnite etc ($Fe_3(PO_4)_2.8H_2O$) (Iuliano et al., 2007; Iuliano et al., 2008; Khalid et al., 1977; Ponnamperuma, 1972).

When previously submerged soils dry, dehydration and re-oxidation of reduced components results in binding of P to poorly crystalline hydrous ferric oxide ($FeOOH.0.4H_2O$ or $Fe_2O_3{\cdot}2FeO(OH){\cdot}2.6H_2O$) surfaces. These amorphous iron oxides have a high specific surface area and therefore a high P adsorption capacity. Twinch (1987) reported that soluble reactive P decreased from 6.4 to 0.5 µg $g^{-1}$ as the water content decreased from 85% to 32%. Similarly, Forber et al. (2017) found that soluble reactive P in the soil solution decreased from 275 to 175 µg $L^{-1}$ during 25 days of dry period. Under persisting dry/oxidizing conditions, poorly crystalline ferric hydroxide forms can be converted to more stable crystalline iron minerals such as hematite (Lynch et al., 2014). The increase in crystallinity of Fe oxyhydroxide during dry periods may result in loss of P binding capacity of sediments and more available P upon reflooding than before drying because crystalline Fe oxyhydroxides have a smaller surface area than amorphous Fe minerals (Cornell et al., 1989). For example, Qiu and

McComb (2002) studied wetland sediments amended by P at a rate of 30 µg $g^{-1}$. During the wet period, P release from soil was 10 µg $g^{-1}$, but after air drying and subsequent rewetting it was 25 µg $g^{-1}$ (Qiu and McComb, 2002). Furthermore, a negative correlation was observed between iron crystallinity upon drying (measured as a ratio between ammonium oxalate extractable Fe to citrate-dithionite-bicarbonate extractable Fe) and soil P sorption capacity. Olila et al. (1997) conducted a greenhouse study of peat soil cores under simulated flooding and dry conditions. They found that the amount of P released during reflooding from previously dry soil was 2-4 fold higher than the amount released in continuously flooded conditions. Further, P availability in ASS after submergence of dry soil can be increased due to acidification during the dry period. Acidification caused by oxidation of pyrite (FeS) can release P associated with Ca minerals after rewetting (Kraal et al., 2009).

## 1.5 Research gaps and aims

The effects of submergence and oxidation of soils on P availability and P forms have been in studied in acidic soils, but not specifically in ASS. Given the importance of ASS for crop production (e.g. paddy rice cultivation) and the generally low soil P availability, more studies on P forms in ASS are needed. Bioremediation of acid sulfate soils using plants and organic matter is also gaining more prominence as a management strategy but it is unclear whether P is a limiting factor that also needs to be addressed. Neutralizing agents (e.g. NaOH or $Ca(OH)_2$) have been found to increase P availability in acid soils by either by exchanging $OH^-$ with $PO_4^{3-}$ from Fe oxy-hydroxides or by reducing the surface charge of Fe minerals which retain P (Oburger et al., 2011). However little is known about how neutralizing agents and inorganic P addition influence P availability in ASS under submerged or oxidising conditions.

The typically predominant jarosite $KFe_3(OH)_6(SO_4)_2)$ mineral in ASS may be important because it can bind soluble P through anion/ligand exchange mechanisms which can be released by reductive dissolution of Fe oxyhydroxides (Peretyazhko and Sposito, 2005; Zhang et al., 2003). However, its role in P binding and release is not clear. The role of organic matter addition to ASS has been studied in relation to redox potential and pH (Gale, 2017; Kölbl et al., 2019; Yuan et al., 2015b) but not how it may affect P forms in ASS. Organic matter could influence P availability directly by adding P and indirectly by influencing redox potential and competing with phosphate at binding sites.

This study has the following aims.

1. Determine the influence of pH increase and addition of inorganic P on P pools and Fe oxides in ASS under submerged and moist conditions (Chapter 2)
2. Assess changes in P pools in ASS over time after inorganic P addition under submerged conditions and the capacity of the soil and jarosite to bind and subsequently release P (Chapter 3)
3. Investigate the effect of P added as manure, inorganic P or combinations of manure and inorganic P on P pools in ASS under changing water regime (Chapter 4)
4. Determine the effect of organic matter addition rate, pH increase, and changes in water content on P pools and P sorption in ASS (Chapter 5)

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# Chapter 2

# Phosphorus pools in sulfuric acid sulfate soils. Influence of water content, pH increase and P addition

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# Statement of Authorship

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# Phosphorus pools in sulfuric acid sulfate soils: influence of water content, pH increase and P addition

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## Abstract

**Purpose** Acid soils often have low P availability limiting plant growth, which is addressed by application of inorganic fertilizers and neutralizing agents. However, little is known about the effect of addition of inorganic P and neutralizing agents on P pools in acid sulfate soils under submerged or moist conditions.

**Materials and methods** Sulfuric acid sulfate soil ($pH < 4$) was amended with two neutralizing agents (NaOH or $Ca(OH)_2$) to achieve soil pH 4 or 5.5, without or with addition of inorganic P equivalent to 20 kg $ha^{-1}$. Soils were incubated at 25 °C in either submerged or moist conditions (100% of maximum water holding capacity). After 2 weeks, soil P pools (labile P, moderately labile P, non-labile P and residual P) and Fe and Al oxides were determined.

**Results and discussion** Adjustment of pH had little effect on the measured parameters. Labile, moderately labile and non-labile P pools were higher with P addition than without P addition. With P addition, labile and non-labile P pools were up to twofold higher in submerged incubation than in moist incubation. Labile P, non-labile P and residual P represented 70%, 15% and 15% in submerged incubation and 40%, 40% and 30% in moist incubation, respectively. In submerged incubation, Fe oxides were higher in soils amended with neutralizing agents than in the original soil which can be explained by the higher pH.

**Conclusions** A high proportion of added P was available after 2 weeks of application particularly in submerged incubation. The pH increase had little effect on P availability.

**Keywords** Acid sulfate soil · Neutralizing agent · Phosphorus addition · Phosphorus pools · Water content

## 1 Introduction

Acid sulfate soils (ASS) are defined as soils in which sulfuric acid may be produced, is being produced or has been produced in amounts that have a lasting effect on main soil characteristics (Fanning et al. 2017). Acid sulfate soils are commonly found where conditions are conducive for sulfate reduction such as water logged, sulfate and organic matter–rich coastal and inland wetlands or floodplain areas, and rivers or lakes (Fitzpatrick et al. 2008). In Australia, it has been estimated that ASS occupy about 4 million ha in coastal areas and 1.5 million ha in inland areas (Fitzpatrick et al. 2008). Areas containing ASS are often used for paddy rice cultivation where they may be under oxidized and reduced conditions (Fanning et al. 2010). ASS materials typically contain iron sulfides such as FeS or pyrite ($FeS_2$) formed in reactions of hydrogen sulfide with dissolved $Fe^{2+}$ under anaerobic conditions (Blunden 2000). Acid sulfate soils are chemically stable under reducing conditions. However, entry of air such as upon drainage can lead to pyrite oxidation which can result in formation of sulfuric acid and $pH < 4$ or less in soil and leachate (Andriesse and Van Mensvoort 2006).

As a macronutrient, phosphorus (P) plays a critical role in plants for processes such as photosynthesis, respiration and carbohydrate metabolism (Hawkesford and Barraclough 2011). In soil, P is often poorly available to plants because only a very small proportion (< 1%) of total soil P is

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immediately available in the soil solution. The majority of soil P is in the form of poorly available inorganic (e.g. precipitated with Al, Fe or Ca) or organic compounds. In ASS, a large proportion of soil P is likely to be bound with Fe or Al hydroxides (Iuliano et al. 2007). Fe and Al minerals are sensitive to changes in redox potential and soil pH. Changes in redox potential during flooding and draining/oxidation influence Fe forms and may therefore also affect P availability. When less soluble ferric ($Fe^{III}$) compounds are reduced to more soluble $Fe^{2+}$ under anaerobic/submerged conditions, P associated with ferric compounds is released into the soil solution (Peretyazhko and Sposito 2005). Under oxidizing/dry conditions on the other hand, $Fe^{2+}$ is converted to hydrated ferric oxide minerals (or in acid sulfate soils, Fe oxyhydroxy sulfate minerals) which can occlude P (Ponnamperuma 1972; Fitzpatrick et al. 2017).

P availability in acid soils can be enhanced by application of P fertilizer or pH increase (Antoniadis et al. 2015). However, due to the binding of P on Fe minerals in acid soils, even soluble P fertilizer can become insoluble and less available to plants shortly after application (Ryan et al. 1985). It has been suggested that neutralizing agents (e.g. NaOH or $Ca(OH)_2$) increase P availability by exchange of $OH^-$ with $PO_4^{3-}$ from Fe hydroxide compounds and by reducing the positive surface charge of Fe minerals, thereby weakening P retention (Oburger et al. 2011).

However, the pH increase induced by neutralizing agents in acid soils can also influence Fe forms. At pH > 3.5, hydrolysis of $Fe^{3+}$ results in precipitation of hydroxyl-iron species such as $Fe(OH)^{2+}$, which bind P in the soil solution (Penn and Camberato 2019). In addition, it has been reported that $Ca(OH)_2$ may limit P availability by precipitation of relatively insoluble calcium phosphates (Curtin and Syers 2001). Van Mensvoort et al. (1985) suggested that liming of flooded ASS could reduce P availability by formation of insoluble Al phosphate compounds.

Although low P availability in acid soils is commonly addressed by adding neutralizing agents (e.g. lime) and application of inorganic P, little is known about the interactions between neutralizing agents and P availability in sulfuric soils under submerged or drained conditions. This limits the ability to understand how to promote plant growth in sulfuric soils which may provide benefits to remediation (Gardner et al. 2018). Further, due to the high amounts of Fe and dramatic changes in pH in submerged and moist ASS, P availability in ASS may be affected by soil water content to a greater extent than in other acid soils. This study aimed to (i) determine the influence of pH increase by two different neutralizing agents (NaOH and $Ca(OH)_2$) on P pools and Fe oxides in ASS under submerged and moist conditions and to (ii) investigate the effect of inorganic P addition on P pools and Fe oxides with or without pH adjustment. We hypothesized that (i) the effect of pH increase on soluble P and Fe will be greater in soil amended with inorganic P than without P addition; (ii) without P addition, available P will be lower in moist incubation than in submerged incubation, due to oxidation and precipitation of $Fe^{3+}$; and (iii) P availability will be lower in soils amended with $Ca(OH)_2$ compared to NaOH due to formation of calcium phosphate minerals.

## 2 Materials and methods

Sulfuric acid sulfate soil (pH < 4) was collected from Gillman in the Barker Inlet, South Australia (34° 49′ 47.25″ S; 138° 32′ 40.24″ E). Properties of the soil were as follows: pH 3.16 (1:1 soil:water), EC1 5123 mS $cm^{-1}$, sand 89%, silt 7%, clay 4%, total P 0.30 mg $g^{-1}$, total Fe 21.9 mg $g^{-1}$, total organic carbon 18.9 mg $g^{-1}$ and maximum water holding capacity (WHC) 140 mg $g^{-1}$. The soil had the following properties used for acid sulfate soil characterization: acid neutralizing capacity 0% $CaCO_3$, actual acidity 51 mmol H+ $kg^{-1}$, HCl-soluble sulfur 11.5 mg $g^{-1}$ and chromium reducible sulfur 17 mg $g^{-1}$ (for further details and methods, see Thomas 2011). The soil was stored at room temperature (water content 0.01 g $g^{-1}$) before starting the experiment. For details of the site and soil classification, see Kölbl et al. (2019).

### 2.1 Experimental design

The soil was sieved to < 2 mm and mixed with reverse osmosis (RO) water at a 1:2 ratio to form a slurry. There were two incubation conditions: submerged or 100% WHC, referred to as submerged and moist, respectively. The ten amendment treatments differed in soil pH (4 or 5.5), neutralizing agent (NaOH or $Ca(OH)_2$) and the addition of inorganic P equivalent to 20 kg P $ha^{-1}$. This P rate was used because it is commonly applied in cropped soil. The amendment treatments were as follows (Table 1): control without or with P addition (ControlP0, Control+P), pH increase to 4 with NaOH without or with P addition (Na4P0, Na4+P), pH increase to 5.5 with

**Table 1** Amendment treatments

| Treatment name | pH adjustment to | Neutralizing agent | P addition |
|---|---|---|---|
| ControlP0 | – | – | – |
| Control+P | – | – | – |
| Na4P0 | 4 | NaOH | – |
| Na4+P | 4 | NaOH | + |
| Na5.5P0 | 5.5 | NaOH | – |
| Na5.5+P | 5.5 | NaOH | + |
| Ca4P0 | 4 | $Ca(OH)_2$ | – |
| Ca4+P | 4 | $Ca(OH)_2$ | + |
| Ca5.5P0 | 5.5 | $Ca(OH)_2$ | – |
| Ca5.5+P | 5.5 | $Ca(OH)_2$ | + |

**Table 2** Parameters at 0 week of sampling in treatments with different soil amendments (for treatment names, see Table 1) ($n = 4$, ± standard error)

| | ControlP0 | Control+P | Na4P0 | Na4+P | Na5.5P0 | Na5.5+P | Ca4 P0 | Ca4+P | Ca5.5P0 | Ca5.5+P |
|---|---|---|---|---|---|---|---|---|---|---|
| Soil pH | 3.2 ± 0.03 | 3.2 ± 0.03 | 4.6 ± 0.04 | 4.3 ± 0.03 | 5.7 ± 0.02 | 5.6 ± 0.01 | 4.5 ± 0.02 | 4.1 ± 0.01 | 6.0 ± 0.04 | 5.9 ± 0.03 |
| Redox potential (mV) | 644 ± 2.4 | 588 ± 3.6 | 468 ± 4.3 | 466 ± 2.5 | 403 ± 5.2 | 419 ± 4.0 | 504 ± 3.8 | 506 ± 2.7 | 435 ± 3.7 | 447 ± 3.4 |
| Labile P (mg kg$^{-1}$) | 4 ± 0.1 | 284 ± 2.6 | 7 ± 0.2 | 380 ± 5.8 | 6 ± 0.1 | 324 ± 5.0 | 6 ± 0.2 | 308 ± 6.0 | 5 ± 0.3 | 307 ± 4.5 |
| MBP (mg kg$^{-1}$) | 6 ± 0.2 | 14 ± 1.6 | 3 ± 0.3 | 6 ± 0.4 | 4 ± 0.2 | 146 ± 13.7 | 4 ± 0.2 | 16 ± 6.8 | 2 ± 0.1 | 260 ± 3.4 |
| MLP (mg kg$^{-1}$) | 5 ± 0.2 | 34 ± 0.9 | 6 ± 0.0 | 21 ± 1.7 | 8 ± 0.2 | 28 ± 0.6 | 8 ± 0.8 | 31 ± 2.4 | 13 ± 0.4 | 40 ± 2.0 |
| NLP (mg kg$^{-1}$) | 25 ± 0.7 | 120 ± 0.7 | 28 ± 0.5 | 109 ± 4.3 | 32 ± 0.7 | 125 ± 1.8 | 40 ± 0.7 | 114 ± 1.4 | 28 ± 0.7 | 110 ± 0.9 |
| RP (mg kg$^{-1}$) | 67 ± 0.4 | 58 ± 2.3 | 108 ± 1.5 | 9 ± 0.5 | 136 ± 2.2 | 133 ± 2.0 | 64 ± 0.4 | 46 ± 0.8 | 59 ± 0.4 | 68 ± 1.0 |

NaOH without or with P addition (Na5.5P0, Na5.5+P) (pH increase to 5.5 with NaOH, P added), pH increase to 4 with Ca(OH)$_2$ without P or with P addition (Ca4P0, Ca4+P) and pH increase to 5.5 with Ca(OH)$_2$ without P or with P addition (Ca5.5P0, Ca5.5+P). Soil pH was increased to 4.0 ± 0.5 or 5.5 ± 0.5 by drop-wise addition of 0.5 N NaOH or Ca(OH)$_2$ and thorough mixing. Soil pH was measured 24 h after adjustment, and if the target pH was not reached, NaOH or Ca(OH)$_2$ was added again. After reaching the target pH, soils were dried in a fan-forced oven at 30 °C for 36 h until the water content was 100% WHC. Then, soil pH was measured again in a 1:1 soil:water slurry. Final pH values were 4.0 ± 0.1 or 5.5 ± 0.1. Soil equivalent to 20 g dry weight was placed in 70-mL plastic containers. In treatments with P addition, inorganic P (as KH$_2$PO$_4$) was thoroughly mixed at 20 kg P ha$^{-1}$ (equivalent to 769 mg P kg$^{-1}$ soil$^{-1}$). Soil was incubated at 25 °C in the dark for 2 weeks, at two different water contents. The 2-week incubation was chosen because in a preliminary experiment with this soil which was conducted over 10 weeks, P pools changed little after 2 weeks. For submerged incubation, RO water was added so that the soil surface was covered by a 2-cm layer of water which was maintained by adding water throughout the following 2 weeks. The vials were closed tightly to minimize entry of air. For moist incubation, soil was incubated at 100% WHC. This water content was selected based on Jayalath et al. (2016) who found that oxidation/acidification in ASS was maximal at 100% WHC. Throughout the moist incubation, 100% WHC was maintained by checking the water content by weight regularly and adding water if required. All treatments were sampled after 2 weeks. There were four replicates per treatment.

## 2.2 Analyses

Soil pH was measured in a 1:1 soil:water slurry. Soil maximum water holding capacity was measured using a sintered glass funnel connected to a 1-m water column (Wilke 2005). Soil texture was determined by the hydrometer method (Gee and Or 2002). To determine total P, soil was digested with nitric acid-perchloric acid at a 4:1 ratio and total P in the digest was determined by the phosphovanado-molybdate method (Hanson 1950). Total Fe was determined after concentrated nitric acid dissolution (Zarcinas et al. 1996). The extracts were filtered and analysed for Fe by inductively coupled plasma optical emission spectroscopy (ICP-OES; Agilent, Mulgrave, Australia). In the submerged treatment, soil pH and redox potential were measured by inserting the probes into the soil. In the moist treatments, the electrode was inserted into the soil to measure redox potential; soil pH was determined in a 1:1 soil:water slurry ratio. A chemical fractionation scheme developed by Ivanoff et al. (1998) was used to determine soil P pools, with a few modifications. Briefly, soil (1 g dry soil equivalent) was sequentially extracted with 0.5 M NaHCO$_3$, 1 M HCl and 0.5 M NaOH to separate labile, moderately labile P (MLP) and non-labile P (NLP) pools, respectively. After shaking (16 h, 4 h and 16 h with 0.5 M NaHCO$_3$, 1 M HCl and 0.5 M NaOH, respectively), centrifugation and filtration, P in the extracts was determined by the malachite green method (Ohno and Zibilske 1991). To determine residual P, the remaining soil pellet was digested with nitric acid-perchloric acid at a 4:1 ratio and residual P in the digest was determined by the phosphovanado-molybdate method (Hanson 1950). The Fe and Al oxides in soil were determined using the extraction procedure of Holmgren (1967). To 0.5 g dry soil equivalent, 0.5 g sodium dithionite and 25 mL of 0.75 M sodium citrate solution was added and shaken for 16 h at 25 °C. After shaking, 25 ml RO water containing three drops of Superfloc solution was added. Then, the mixture was shaken vigorously for 5 s and centrifuged to obtain extracts free of soil particles. The supernatant was filtered and diluted 10-fold and acidified with 0.2% v/v nitric acid. Then, Fe, Ca, Al and Na concentrations in the extracts were determined using ICP-OES.

## 2.3 Data analysis

There were four replicates per treatment. Before conducting analysis of variance (ANOVA), the data was checked for normality (*W* test) and was log-transformed to achieve normal distribution. Data of submerged and moist treatments was analysed separately by two-way ANOVA with P addition and pH adjustment as factors (GenStat 15th edition; VSN

Int., Ltd., UK). Tukey's multiple comparison tests at 95% confidence interval were used to determine significant differences among treatments (P addition × pH adjustment) separately for submerged and moist incubations. To compare submerged and moist treatments, paired *t* test was carried out for each amendment treatment separately (GenStat 15th edition; VSN Int., Ltd., UK).

## 3 Results

The initial soil pH of ControlP0 was < 3.3, indicating pyrite oxidation had occurred leading to production of sulfuric (pH < 4) materials (Table 2). Redox potential values ranged from 400 mV to around 650 mV in all samples at the start of the incubation. Labile P, MLP and NLP were higher with P addition than without P addition.

After 2 weeks, soil pH or redox potential values were not or only slightly influenced by incubation water content (Table 3). Labile P was 10- to 100-fold higher in +P treatments than 0P treatments, and it was higher in submerged than in moist incubation except in Ca5.5P0 where it was 20% higher in moist incubation (Fig. 1a, b). In submerged incubation, labile P in 0P treatments was 30% higher than ControlP0 in Na4P0 and Ca4P0. In +P treatments, labile P was higher than Control+P only in Ca4+P and Ca5.5+P where it was about 70% higher. In moist incubation, labile P differed little among 0P treatments except in Ca5.5P0 where it was about 40% higher than ControlP0. With P addition, labile P was about 30% lower than Control+P in Na5.5+P, Ca4+P and Ca5.5+P.

MLP in submerged incubation (Fig. 1c) was four to sevenfold higher in +P treatments than in 0P treatments with the greatest difference in Ca5.5. It differed little among 0P treatments. Compared to Control+P, MLP was 30% lower in Na4+P and 20% higher in Ca5.5+P. MLP was not detectable in moist incubation treatments.

NLP in submerged incubation was about fourfold higher in +P than in 0P treatments (Fig. 2a). In general, NLP differed a little between pH-adjusted treatments and their respective control. In +P treatments, NLP was about twofold higher in submerged than in moist incubation (Fig. 2b). Without P addition, NLP was not affected by incubation water content except in ControlP0 and Ca5.5P0 where it was higher in moist than in submerged incubation. In moist incubation, NLP was higher than ControlP0 only in Ca5.5P0 where it was 30% higher. NLP differed a little among +P treatments.

Residual P (RP) in submerged incubation without P addition compared to ControlP0 was about 20% higher in pH-adjusted treatments, except in Ca4P0 where it was twofold higher (Fig. 2c). Compared to 0P treatments, RP with P addition was lower in Na4P+ and Ca4P+, but higher in Na5.5P+ and Ca5.5P+. With P addition, RP was about 30% higher in Na5.5P+, Ca4P+ and Ca5.5P+ than in Control+P. Incubation

**Table 3** pH and redox potential values at the end of the incubation in treatments with different soil amendments (for treatment names, see Table 1) ($n = 4$, ± standard error)

| Parameter | Incubation | ControlP0 | Control+P | Na4P0 | Na4+P | Na5.5P0 | Na5.5+P | Ca4P0 | Ca4+P | Ca5.5P0 | Ca5.5+P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | Submerged | 3.2 ± 0.02 | 3.1 ± 0.01 | 4.5 ± 0.03 | 4.2 ± 0.03 | 5.3 ± 0.05 | 5.1 ± 0.03 | 4.5 ± 0.03 | 4.3 ± 0.03 | 6.1 ± 0.04 | 5.4 ± 0.04 |
|  | Moist | 3.0 ± 0.01 | 3.0 ± 0.03 | 4.4 ± 0.02 | 4.0 ± 0.04 | 5.2 ± 0.02 | 5.0 ± 0.05 | 4.6 ± 0.05 | 3.9 ± 0.05 | 4.6 ± 0.03 | 5.3 ± 0.04 |
| Redo× potential (mV) | Submerged | 621 ± 3.68 | 576 ± 3.84 | 531 ± 3.97 | 504 ± 5.86 | 451 ± 1.80 | 534 ± 3.03 | 534 ± 2.69 | 501 ± 4.06 | 451 ± 4.06 | 406 ± 4.38 |
|  | Moist | 669 ± 2.66 | 532 ± 3.42 | 541 ± 3.86 | 504 ± 3.64 | 463 ± 1.89 | 493 ± 2.45 | 463 ± 5.17 | 499 ± 2.93 | 480 ± 2.93 | 454 ± 3.57 |

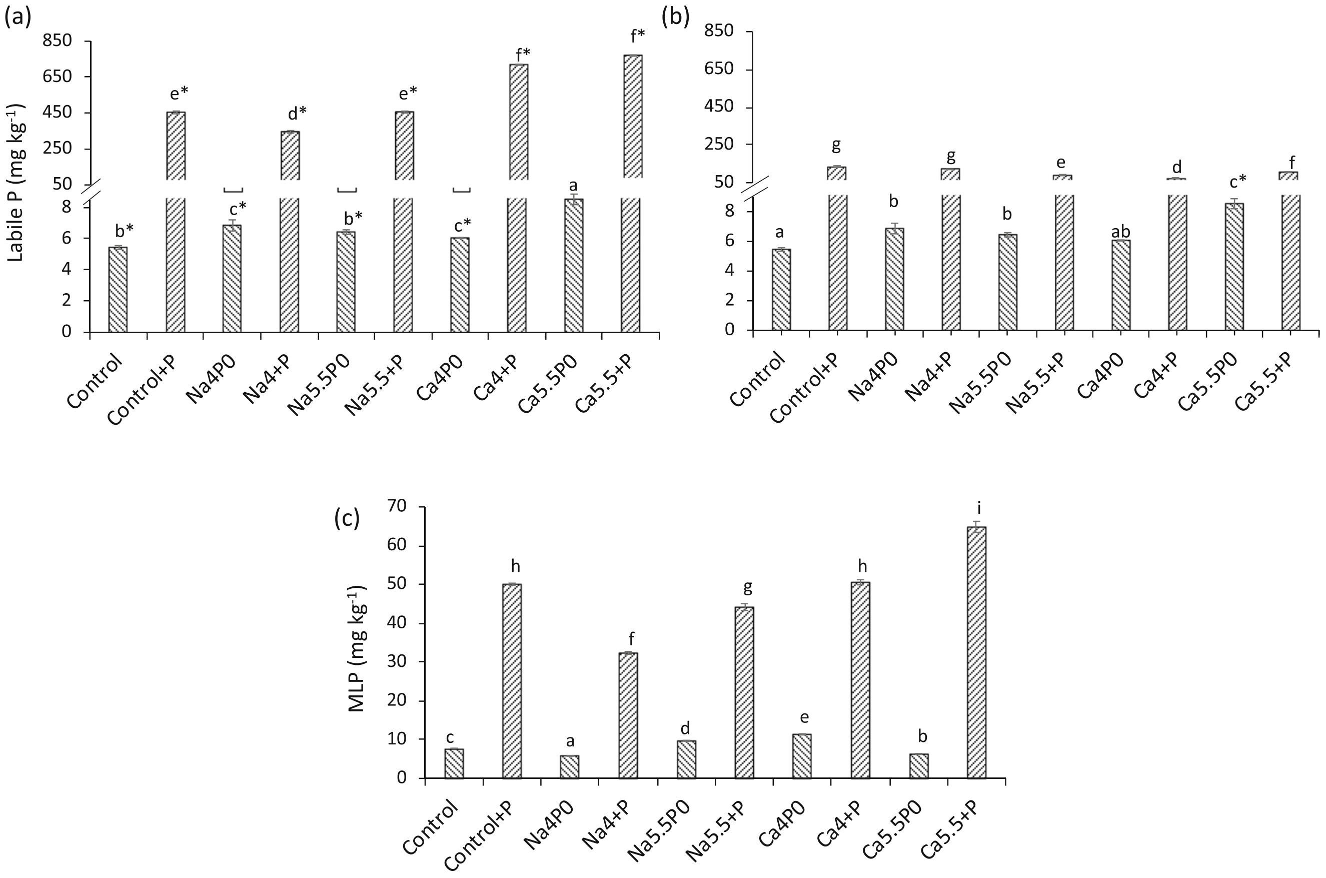

**Fig. 1** Labile P (**a**, **b**) and moderately labile P (**c**) pools in treatments with different amendments (for treatment names, see Table 1) after 2 weeks of submerged (**a**, **c**) or moist incubation (**b**). Columns with different letters are significantly different ($n = 4$, ± standard error, $P \leq 0.05$). Means with asterisk are significantly higher than the incubation with the other water content. Moderately labile P was detected only in submerged incubation

water content had an effect on RP only in Ca treatments where it was higher in submerged than in moist incubation in Ca4, but lower in Ca5.5. In moist incubation, RP differed a little between pH-adjusted treatments and the respective controls in except in Ca5.5P0 where it was 30–80% higher (Fig. 2d).

Iron oxides were up to threefold higher in submerged than in moist incubation except in ControlP0 where it was twofold higher in moist than in submerged incubation (Fig. 3a, b). P addition had little effect on Fe oxides. Only in submerged incubation compared to the respective treatment without P, Fe oxides were twofold higher in Na4+P and 50% lower in Ca4+P. In submerged incubation, compared to the controls, Fe oxides were about threefold higher in pH-adjusted treatments. In moist incubation, there was no consistent treatment effect on Fe oxides. Al oxide concentration was not affected by incubation water content, pH adjustment or P addition (Fig. 3c, d). There was no correlation between P pools and Fe/Al oxides.

In submerged incubation without P addition, RP was the largest percentage of P pools (around 70% of measured P), followed by NLP (about 20%) (Table 4). Only about 5% of measured P was in labile P (LP). With P addition on the other hand, LP was the largest pool (about 70%) whereas RP and NLP together were only about 30%. Moderately labile P was a small pool irrespective of P addition, less than 10% of measured P. In moist incubation without P addition, the proportion of RP, NLP and LP was similar as in submerged incubation (70%, 20% and 5% of measured P, respectively). With P added in moist incubation, about 40% of measured P was LP, 20–30% NLP and 30–40% RP. However, in submerged incubation, about 65% of measured P was LP, 15% NLP and less than 10% RP.

## 4 Discussion

This experiment showed that in sulfuric ASS, a large proportion of the added soluble P remained in available form, for at least over 2 weeks. Labile P, MLP and NLP were generally higher with P added than without P addition. P addition resulted in a 10-fold increase in the proportion of LP in total P detected, particularly in submerged incubation. Some of the added P was converted into NLP, likely through binding to Fe/Al oxides on soil particles. The lack of increase in RP with P addition suggests that the 2-week incubation was not long enough to convert P into very stable P forms. In general, P

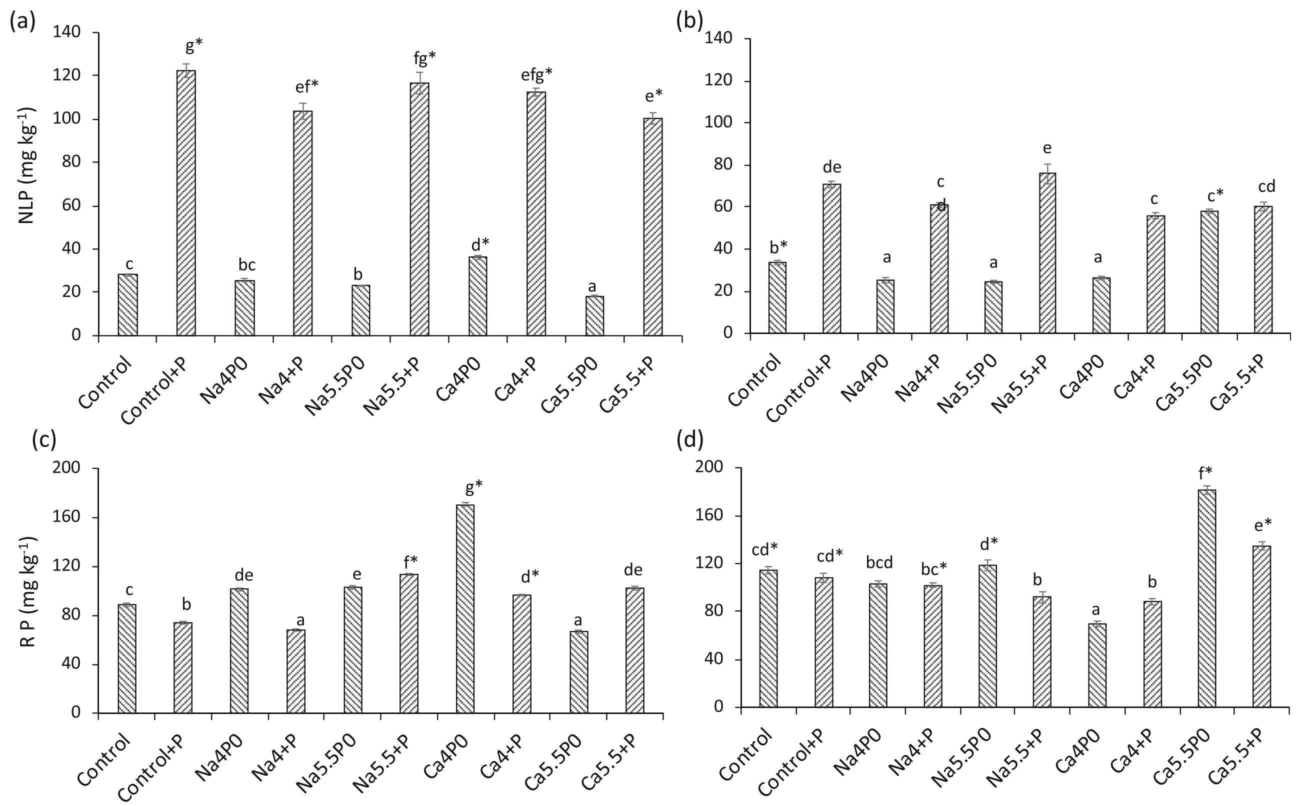

**Fig. 2** Non labile P (**a**, **b**) and residual P (**c**, **d**) pools in treatments with different amendments (for treatment names, see Table 1) after 2 weeks of submerged (**a**, **c**) or moist incubation (**b**, **c**). Columns with different letters are significantly different ($n = 4$, ± standard error, $P \leq 0.05$). Means with asterisk are significantly higher than the incubation with the other water content

pools were influenced by P addition and incubation water content (submerged or moist) whereas pH adjustment had little effect.

Without P addition, P pools differed little between submerged and moist incubations. Therefore, the second hypothesis (without P addition, available P will be lower in moist incubation compared to submerged incubation, due to oxidation and precipitation of $Fe^{3+}$) has to be declined. Labile P was slightly higher in submerged incubation than in moist incubation, and MLP was detectable only in submerged incubation. This suggests that submerged incubation enhanced mobilization of native soil P, possibly through reduction of Fe oxides to which P was bound (Zhang et al. 2003). However, Fe oxides were lower in submerged than in moist incubation only in ControlP0. Native soil P may have been mobilized by organic acid anions produced from decomposition of native OM at low oxygen concentrations which replaced P from binding sites (Ponnamperuma 1972). Redox potentials measured in the soil were not low, but low oxygen concentrations could occur in microsites, e.g. close to the bottom of the containers.

Labile P can be transformed into MLP or NLP by adsorption to soil minerals such as clays and Fe and Al oxides or by formation of salts with Ca, Fe or Al (Smeck 1985). With P added, LP and NLP in submerged incubation were up to twofold higher than those in moist incubation and MLP was detected only in submerged incubation. Thus, in submerged incubation, a greater proportion of the added P remained soluble than in moist incubation, although a greater proportion was also immobilized as NLP than in moist incubation. This apparent contradiction can be explained by the different sizes of the three pools in submerged incubation. Labile P was about fivefold greater than NLP and 10-fold greater than MLP. Thus, the increase in MLP and NLP had little impact on LP concentration. The higher MLP and NLP in submerged incubation than in moist incubation with added P may be due to greater diffusion of the added P throughout the soil which increased the likelihood of contact with soil particles and formation of stable P pools.

Increasing soil pH had no consistent effect on P pools without P addition. With P added in submerged incubation, LP was about 50% higher with Ca addition than the control which is in agreement with the first hypothesis (the effect of pH increase on soluble P and Fe will be greater in soil amended with inorganic P than without P addition). Although labile P was slightly lower in moist incubation than in submerged incubation, Fe oxides were higher in submerged than in moist incubation (except in ControlP0). Therefore, the lower labile P in moist incubation cannot be explained by enhanced formation of Fe oxides and thus greater surface area for P binding. Ca addition can result in formation of Ca

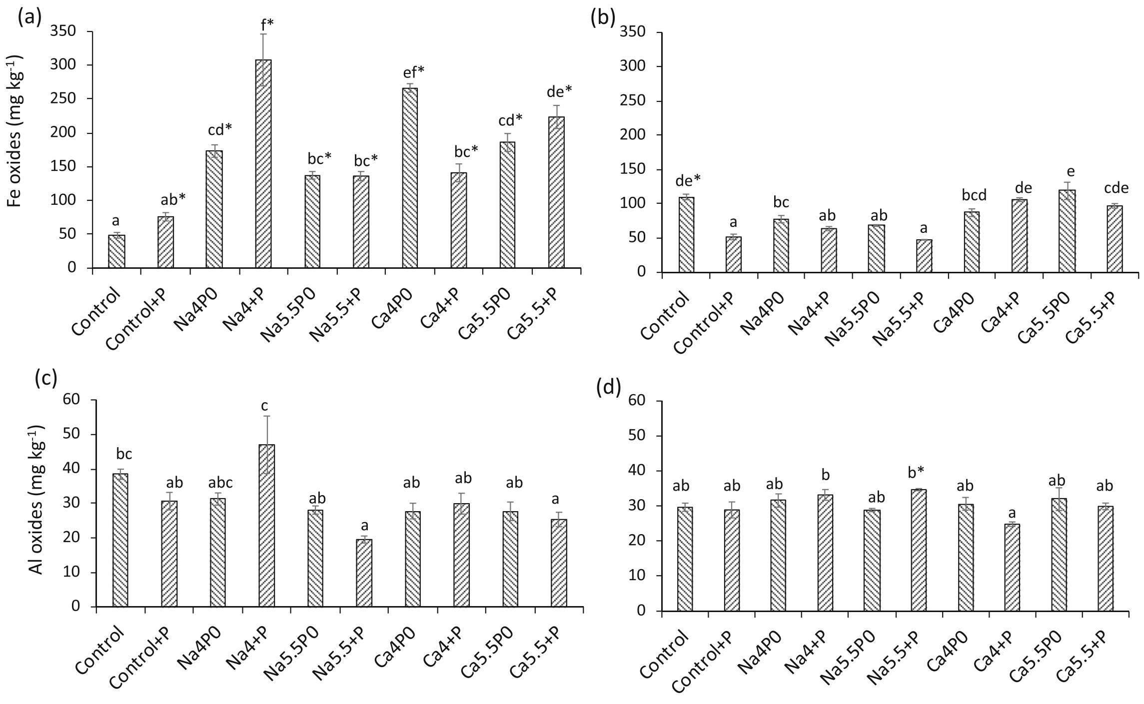

**Fig. 3** Fe oxides (**a**, **b**) and Al oxides (**c**, **d**) in treatments with different amendments (for treatment names, see Table 1) after 2 weeks of submerged (**a**, **c**) or moist incubation (**b**, **d**). Columns with different letters are significantly different ($n = 4$, ± standard error, $P \leq 0.05$). Means with asterisk are significantly higher than the incubation with the other water contents

phosphates (Curtin and Syers 2001), but this apparently did not happen in this soil because LP was higher with Ca addition than with Na addition or without pH adjustment. Thus, the third hypothesis (P availability will be lower in soils limed with $Ca(OH)_2$ compared to NaOH due to formation of calcium phosphate minerals) has to be declined. The higher LP with Ca addition may be due to replacement of sorbed P by $OH^-$ (McDowell et al. 2003). This did not occur with NaOH addition, likely because less $OH^-$ was added with the latter. In moist incubation, pH adjustment to 5.5 with $Ca(OH)_2$ increased RP compared to the control which indicates formation of very stable P forms. However, the higher RP did not affect NLP or LP, because the increase in RP was quite small (about 40 mg $kg^{-1}$).

In submerged incubation, Fe oxides were about threefold higher in pH-adjusted treatments compared to the controls. This can be explained by the lower solubility of Fe oxides at pH > 4 and the redox potential between 400 and 600 mV which reduces Fe solubility compared to lower redox potentials (Cook and Olive 2012). Iron oxides were higher in submerged incubation than in moist incubation which indicates that Fe reduction was not enhanced in submerged conditions, possibly because the redox potential was similar as in moist incubation. In moist incubation, sulfate ions could have been released by pyrite by oxidation (Johnson and Hallberg 2005). Sulfate ions may react with Na or Ca added with the neutralizing agents and enhance the formation of Ca/Na hydrous

**Table 4** Percentage of P pools in total extracted P with different amendment treatments incubated under submerged and moist conditions (for treatment names, see Table 1)

| Incubation | P pool | ControlP0 | Control+P | Na4P0 | Na4+P | Na5.5P0 | Na5.5+P | Ca4P0 | Ca4+P | Ca5.5P0 | Ca5.5+P |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Submerged | LP | 5.7 $^{abc}$ | 64.8 $^{e}$ | 6.4 $^{bc}$ | 62.9 $^{d}$ | 5.2 $^{ab}$ | 62.4 $^{d}$ | 4.2 $^{a}$ | 73.5 $^{f}$ | 6.8 $^{c}$ | 74.2 $^{f}$ |
| | MLP | 5.9 $^{c}$ | 7.2 $^{f}$ | 4 $^{a}$ | 5.9 $^{c}$ | 6.8 $^{ef}$ | 6 $^{cd}$ | 5 $^{b}$ | 5.1 $^{b}$ | 6.5 $^{de}$ | 6.3 $^{cd}$ |
| | NLP | 21.3 $^{d}$ | 17.5 $^{bc}$ | 18.2 $^{c}$ | 18.9 $^{c}$ | 16 $^{b}$ | 16 $^{b}$ | 15.9 $^{b}$ | 11.5 $^{a}$ | 18.7 $^{c}$ | 9.7 $^{a}$ |
| | RP | 67.1 $^{d}$ | 10.5 $^{a}$ | 71.4 $^{e}$ | 12.3 $^{b}$ | 72 $^{e}$ | 15.6 $^{c}$ | 74.9 $^{f}$ | 9.9 $^{a}$ | 68 $^{d}$ | 9.8 $^{a}$ |
| Moist | LP | 3.6 $^{a}$ | 43 $^{d}$ | 5.1 $^{ab}$ | 42.5 $^{d}$ | 4.3 $^{ab}$ | 35.2 $^{c}$ | 5.9 $^{b}$ | 34.1 $^{c}$ | 3.5 $^{a}$ | 35.1 $^{c}$ |
| | MLP | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | NLP | 22 $^{bcde}$ | 22.6 $^{bcde}$ | 18.7 $^{ab}$ | 21.5 $^{bcd}$ | 16.5 $^{a}$ | 29.3 $^{f}$ | 25.9 $^{ef}$ | 25.6 $^{def}$ | 23.4 $^{cde}$ | 20.0 $^{abc}$ |
| | RP | 74.4 $^{e}$ | 34.4 $^{a}$ | 76.2 $^{ef}$ | 36 $^{a}$ | 79.2 $^{f}$ | 35.5 $^{a}$ | 68.2 $^{d}$ | 40.3 $^{b}$ | 73.1 $^{e}$ | 44.9 $^{c}$ |

Different letters indicate significant differences between treatments ($n = 4$, ± standard error, $P \leq 0.05$)

sulfate coatings on soil particles. These coatings can reduce the rate of oxygen diffusion and thereby limit oxidation of Fe minerals (Blowes et al. 1991).

## 5 Conclusions

This study showed that added soluble P remained available 2 weeks after addition and little had entered the more stable P pools. This suggests that added P was not quickly bound on Fe minerals in the acid sulfate soil used in this study. Soil water content influenced P pools only with P addition where a greater proportion of the added P remained soluble in submerged compared to moist incubation. Increasing the pH had little effect on P pools. The results suggest P amendment to acid sulfate soils could be an effective strategy to increase available P and promote plant growth. However, high rates of P fertilizer addition may also increase P loss via runoff or seepage. To further assess the effect of P addition and water content on P pools in ASS, studies with a range of P concentrations over longer periods (several months) are needed.

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# Chapter 3

## Threshold of labile phosphate in a sandy acid sulfate soil

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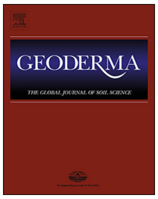

# Threshold for labile phosphate in a sandy acid sulfate soil

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## ABSTRACT

In acid soils, plant availability of fertiliser P is affected by phosphate binding to Fe or Al oxide minerals. However, little is known about the P pools in acid sulfate soils after phosphate fertilisation, or the capacity of the oxyhydroxy sulfate mineral jarosite ($KFe_3(OH)_6(SO_4)_2$) found in these soils, to bind or release added phosphate. Sulfuric acid sulfate soil (initial pH 3.5, adjusted to 5.5) was amended with phosphate at 0, 96, 385, 578 and 770 mg P $kg^{-1}$ and incubated under submerged conditions. Soil phosphate pools (labile phosphate, moderately labile phosphate, non-labile phosphate and residual phosphate) and amorphous Fe/Al were measured after two and four weeks. Phosphate sorption and its release were determined in incubated soils and jarosite, separately. In soil, maximum phosphate sorption was about 350 mg phosphate $kg^{-1}$. Labile phosphate was higher than the control (without phosphate addition) at phosphate rates above 350 mg P $kg^{-1}$ and was more than 50% of added phosphate. The length of submergence had little effect on phosphate pools. Below the threshold of 350 mg phosphate $kg^{-1}$, absorbed phosphate was strongly bound to the soil whereas 50% of sorbed phosphate was released above the threshold. The high phosphate binding capacity of jarosite (maximal 360 mg phosphate $kg^{-1}$) confirmed the importance of Fe oxyhydroxy sulfate minerals in P binding in acid soils. However, at maximal phosphate binding, up to 20% of bound phosphate could be released, suggesting that phosphate bound to jarosite may become plant available. The results of this study will help inform fertiliser requirements for crop growth or remediation of acid sulfate soils using plants.

## 1. Introduction

Acid sulfate soils (ASS) are soils with sulfidic (mostly pyrite/$FeS_2$) or sulfuric (pH < 4) materials, and are wide-spread in coastal areas and inland along lakes, rivers and wetlands (Andriesse and Van Mensvoort, 2006; Fitzpatrick et al., 2008). They are often used for crop production, e.g. as rice paddies (Dent and Pons, 1995). Plants require phosphorus (P) for growth; however, phosphate availability in soils often limits plant P uptake, even after addition of P fertiliser (Bovill et al., 2013). Low phosphate availability in acid soils is due to the reaction of P with acidic cations (Fe/Al) that occur as free cations in the soil solution, as exchangeable ions of soil colloids or as amorphous/crystalline mineral oxides (Sanyal and De Datta, 1991). Reactions of soluble P species with such acidic cations lead to the formation of poorly soluble P salts or adsorption of P to soil colloids (Pierzynski et al., 2005). Both amorphous Fe and Al are found in ASS because amorphous Fe oxides exist under changing redox potential conditions and amorphous Al oxides are formed in environments with changing acidity (Cornell and Schwertmann, 2003). Amorphous oxides have a higher reactive surface for phosphate sorption than crystalline oxides. Further, ASS contain pyrite under submerged conditions and jarosite, schwertmannite and goethite when they are oxidised (Fanning et al., 2017; Fitzpatrick et al., 2017). When phosphate is added to acid soils, the phosphate concentration remains low due to adsorption of P to Fe/Al oxides, particularly acid oxalate extractable oxides of Fe/Al (Ayenew et al., 2018; Freese et al., 1992). However, above a threshold that is dependent on the maximal adsorption capacity of the soil, soluble P strongly increases (Rubio et al., 2008). For example, Ayenew et al. (2018) reported a threshold of 150 mg phosphate $kg^{-1}$ in acid soils above which the concentration of available P increased sharply. In a slightly acidic soil (pH about 5.5), Bond et al. (2006) found a much higher threshold (5800 mg phosphate $kg^{-1}$). The large differences in phosphate threshold values indicates that soil properties are important for phosphate binding. To improve P fertiliser efficiency, it is necessary to understand the processes that influence phosphate availability after fertiliser addition.

The solubility of Fe minerals and capacity to bind phosphate depends on the redox potential (Kleeberg et al., 2013). For example, reductive dissolution of Fe oxides can lead to release of previously bound phosphate (Peretyazhko and Sposito, 2005; Zhang et al., 2003). Jarosite

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($KFe_3(OH)_6(SO_4)_2$), an oxyhydroxy sulfate mineral, precipitates under strong oxidising and acidic conditions and is commonly found in ASS (Bigham and Nordstrom, 2000; Dent, 1986). Jarosite may play an important role in phosphate binding through sulfate-phosphate anion exchange or ligand exchange mechanism which involves the replacement of one or more surface hydroxyl groups by phosphate (Wang et al., 2013; Zhong et al., 2007). However, little is known about the fate of P fertilisers in acid sulfate soils. Understanding nutrient availability in ASS is important because they are used for crop production and because plants are being increasingly used for remediation of ASS (Gardner et al., 2018). In our previous study (Mayakaduwage et al., 2019) where phosphate was added at 770 mg phosphate $kg^{-1}$ to an ASS adjusted to pH 5.5, more than 60% of the added phosphate was in the labile pool after two weeks submerged incubation. In this study, we addressed the following questions: which phosphate pools occur in acid sulfate soil after phosphate addition, do they change with time in submerged conditions, and how much of the bound P can be released? We also wanted to determine the capacity of jarosite to bind and subsequently release phosphate. The hypotheses were (i) labile phosphate will remain low in acid sulfate soils below a threshold representing the phosphate binding capacity of the soil, (ii) non-labile and residual phosphate will increase with phosphate addition rate, particularly above the threshold, (iii) only a small proportion of phosphate bound to the soil can be released, (iv) P pools change little over time because two weeks is sufficient time for the added phosphate to be bound to the soil, and (v) jarosite has a strong capacity to bind phosphate; little of the phosphate bound to jarosite can be released.

## 2. Materials and methods

Samples of an acid sulfate soil (ASS) with sulfuric materials containing jarosite ($KFe_3(OH)_6(SO_4)_2$) segregations were collected in a reclaimed tidal wetland from at 80–150 cm at Gillman in the Barker Inlet of South Australia (34°49′47.25″S; 138°32′40.24″E). This depth was chosen because it is extremely acidic (Thomas, 2011). The soil is classified as sulfuric soil (Acid sulfate soil classification), peaty, sulfuric, hypersalic Hydrosol (Australian Soil classification) or Salic Fluvisol (hyperthionic, drainic) (World Reference Database identification keys). For properties see Mayakaduwage et al. (2019). Briefly, it has pH 3.2 (1:1 water slurry), sand 890 g $kg^{-1}$, total P 0.30 g $kg^{-1}$, total Fe 22 g $kg^{-1}$, jarosite 258 g $kg^{-1}$. The soil was dried at 40 °C and sieved to $<$ 2 mm.

To extract jarosite, the soil was dried at 60 °C for 48 h. Large soil aggregates were broken apart by hand and sieved to obtain $<$ 4 cm fraction. Bright yellow segregations of jarosite were picked out manually, ground with mortar and pestle and sieved to $<$ 250 µm.

### 2.1. Experimental design

#### 2.1.1. Soil incubation

After mixing the soil with reverse osmosis (RO) water (water from which ions were removed, with similar properties as distilled water) at a 1:2 ratio, the soil pH was increased to 5.5 ± 0.5 by adding 0.5 N $Ca(OH)_2$ drop-wise and mixing it thoroughly. Soil pH was measured again after 24 h and adjusted with $Ca(OH)_2$ if necessary. Once the target pH was reached, the soil was dried in a fan-forced oven for 36 h at 30 °C to maximum water holding capacity followed by measuring the pH in a 1:1 soil/water ratio. The final pH values were 5.5 ± 0.1. Soil (20 g dry weight equivalent) was placed in 70 mL plastic containers. For soil experiment 1, phosphate ($KH_2PO_4$) was thoroughly mixed at 0, 96, 385, 578 and 770 mg phosphate $kg^{-1}$, referred to as P0, P96, P385, P578 and P770. These rates are very high when calculated on a per ha basis (770 mg phosphate $kg^{-1}$ is equivalent to about 2000 kg $ha^{-1}$), but were chosen because a preliminary experiment showed that the soil has a very high phosphate sorption capacity. In soil experiment 2, phosphate addition rates between 90 and 380 mg phosphate $kg^{-1}$ were investigated in greater detail to determine rate at which labile P strongly increased. Phosphate was mixed at 0, 90, 130, 190, 230, 290, 330 and 380 mg P $kg^{-1}$, referred to as P0, P90, P130, P190, P230, P290, P330 and P380. Next, soils were mixed with RO water and then covered by a 3.5 cm water layer which was maintained throughout the incubation. After closing the vials tightly, they were was incubated at 25 °C in the dark. In soil experiment 1, sampling was carried out after 2 and 4 weeks. Soil experiment 2 was incubated only for 2 weeks because in soil experiment 1 the measured parameters changed little from 2 to 4 weeks. P release was determined by shaking 0.5 g dry soil equivalent with 10 mL of RO water for 72 h. Then phosphate in the supernatant was determined.

#### 2.1.2. P binding and release by jarosite

In the jarosite experiment, a 100 mg phosphate $L^{-1}$ stock solution was prepared with $KH_2PO_4$ and diluted with RO water to achieve six different phosphate concentrations: 0, 5, 10, 20, 40, 50 and 60 mg $L^{-1}$. Jarosite (0.5 g) was added to 10 mL of each phosphate solution, giving phosphate concentrations of 0, 100, 200, 400, 800, 1000 and 1200 mg $kg^{-1}$. The suspensions were shaken in an end-over-end shaker overnight at room temperature, followed by centrifugation and filtration.

To measure phosphate release, phosphate-loaded jarosite was separated from the solution by filtration and dried at room temperature for 24 h. The dry weight of jarosite was measured and RO water was added to achieve the same solid/solution ratio as in the first part of the experiment. The suspensions were shaken overnight and phosphate in the supernatant was determined after centrifugation and filtration.

### 2.2. Analyses

Soil analyses were carried out as described in Mayaduwage et al. (2019). Briefly, soil pH was measured in a 1:1 soil: water slurry. Maximum water holding capacity was determined after Wilke (2005), soil texture after Gee and Or (2002), soil total P according to Hanson (1950). Concentrated nitric acid dissolution (Zarcinas et al., 1996) followed by inductively coupled plasma optical emission spectroscopy (ICP-OES, Agilent, Mulgrave, Australia) was used to measure total nitric acid soluble Fe in the soil. The sequential fraction method of Ivanoff et al. (1998) was used to determine soil P pools. Labile phosphate (LP), moderately labile phosphate (MLP) and non-labile phosphate (NLP) pools were extracted by sequentially shaking soil (equivalent to 1 g dry) with 0.5 M $NaHCO_3$ (for 16 h), 1 M HCl (for 4 h) and 0.5 M NaOH (for 16 h) respectively. The malachite green method (Ohno and Zibilske, 1991) was used to measure phosphate in the centrifuged and filtered extracts. Residual phosphate (RP) in the remaining soil pellet was measured as described for total soil P. Phosphate in overlying water was determined by the malachite green method. Soil redox potential was measured by inserting the probe (Model IJ64, Ionode, Australia) into the soil without removing overlying water. Absolute redox potential values were calculated after correction to the standard hydrogen electrode. In soil experiment 1, after 2 weeks and in the jarosite experiment after overnight shaking, the concentration of bound P (which may also include P in pore water) was calculated as the difference between initial P addition rate and P in the overlying solution.

Acid oxalate was used to extract amorphous Fe/Al and associated P (Schwertmann, 1964). Soil (0.4 g dry weight equivalent) was shaken with 40 mL of acid oxalate reagent (0.114 M ammonium oxalate and 0.086 M oxalic acid) in the dark for 4 h at room temperature. Then the samples were centrifuged to obtain a clear extract. The extracts were filtered, 10-fold diluted and acidified with 0.2% v/v nitric acid. Fe, Al and P in the extracts were determined by ICP-AES and referred to as acid oxalate extractable Fe, Al and P. The jarosite concentration in the soil was determined by measuring sulfur extracted by 4 M HCl and 1 M KCl and calculating the difference as described in Sullivan et al (2018).

The phosphate sorption data of the soil and jarosite were used to calculate Langmuir and Freundlich isotherms (Fig. S1). The Langmuir equation was used to calculate maximum P sorption of soil and jarosite which were 588 and 357 mg P kg$^{-1}$, respectively.

The contribution to phosphate sorption by jarosite to soil phosphate sorption was calculated as

$$\text{Phosphate sorption \% by jarosite} = \frac{\dfrac{\textit{Maximum adsorption}}{\textit{capacity of jarosite}} \times \text{Jarosite concentration}}{\text{Amount of phosphate adsorbed by soil}} \quad (1)$$

### 2.3. Data analysis

There were four replicates per treatment and sampling time. After confirming that the data were normally distributed (W test), they were subjected to one-way analysis of variance (ANOVA), separately for two and four weeks. Differences between means were compared by Tukey's multiple comparison tests at 95% confidence interval using GenStat 15th edition (VSN Int. Ltd, UK). Paired t-tests were carried out for each treatment to compare two and four week sampling times.

## 3. Results

Redox potential was 500–400 mV in P0, but 300 mV in P96, P385, P578 and P770 (Fig. S2). In the first soil experiment, bound P was similar after two and four weeks. It increased with phosphate addition rate (Fig. 1a). Compared to P96, bound P was three-fold higher in P385 and five to six-fold higher in P578 and P770. Of the phosphate added, nearly 100% was bound in P96, 75% in P385 and P578, but only 60% in P770 (Fig. 1a). Labile phosphate was higher in P amended treatments than the control (P0) (Fig. 1b). Compared to P0, labile phosphate was three to five-fold higher in P96, 25-fold higher in P385 and P578 and 30-fold higher in P770. There was little difference between two and four weeks sampling times.

When phosphate addition rates between 0 and 380 mg kg$^{-1}$ were studied in greater detail in soil experiment 2, labile phosphate after 2 weeks was similar in P90 and P130 where it was about three to five-fold higher than the control (Fig. 1c). Between P190 and P330, labile phosphate was about two-fold higher than in P90. Labile phosphate then increased more than two-fold from P330 to P380.

Moderately labile phosphate was not detectable (< 0.5 mg kg$^{-1}$). After two weeks, non-labile phosphate was similar in P0, P96 and P385 (Fig. 2a). It was 80% higher than the control in P578 and two-fold higher in P770. After four weeks compared to the control, non-labile phosphate was 20% higher in P96, 50% higher in P385 and P578 and two-fold higher in P770. Non-labile phosphate differed little between two and four weeks sampling times. Residual P was not influenced by P rate or incubation time (Fig. 2b).

Phosphate release was lowest in the control (Fig. 2c). After two weeks, phosphate release was two-fold higher than the control in P96 and about 30-fold higher at the higher phosphate addition rates. After four weeks compared to the control, phosphate release was two-fold higher in P96, 10-fold higher in P385 and nearly 20-fold higher in P578 and P770. In P578 and P770, phosphate release was about 50% higher after four than after two weeks.

After two weeks, acid oxalate extractable Fe was about two-fold higher in P0, P96 and P385 than in P578 and P770 (Fig. 3a). Acid oxalate extractable Fe differed little among phosphate rates after four weeks due to a two-fold increase in P578 and P770. After 2 weeks, acid oxalate extractable P compared to the control was two to three-fold higher in P96 and 10 to 20-fold higher at the higher phosphate rates (Fig. 3b). Acid oxalate extractable P increased up to two-fold from two to four weeks in P385, P578 and P770 but did not change over time in P0 and P96. After two weeks, acid oxalate extractable Al was up to two-fold higher in P0, P96 and P385 than P578 and P770 (Fig. 3c), but after four weeks, acid oxalate extractable Al differed little among treatments.

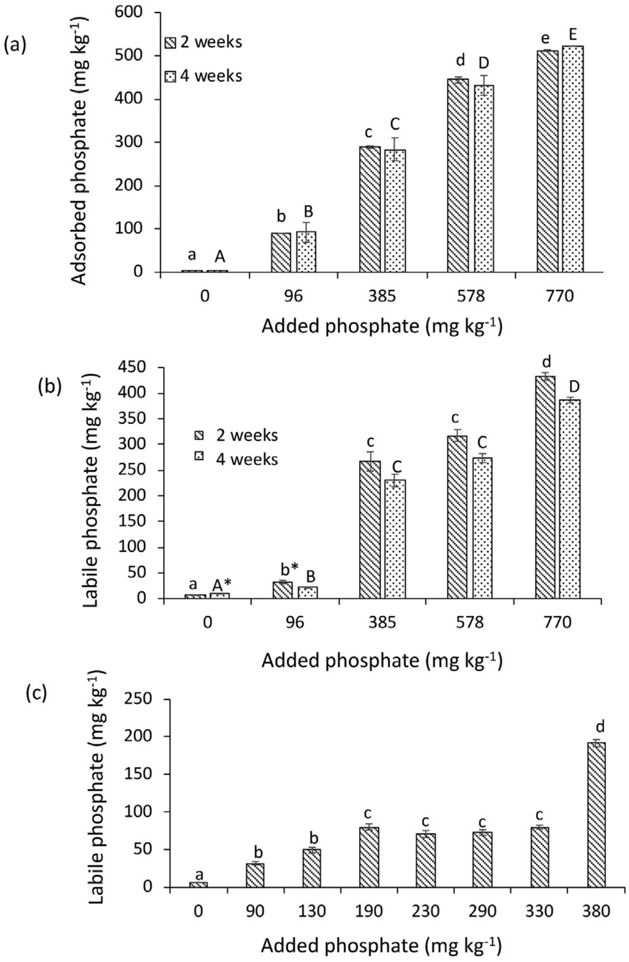

**Fig. 1.** Adsorbed phosphate after two and four weeks (a), labile phosphate after two and four weeks (b) in soil experiment 1 and labile phosphate in soil experiment 2 after two weeks (c) in soil amended with inorganic phosphate ($KH_2PO_4$) at 0 to 770 mg kg$^{-1}$. Columns with different letters are significantly different with lower case letters for two weeks, upper case letters for four weeks (n = 4, ± standard error, $P \leq 0.05$). Means with asterisks are significantly higher at one sampling time than the other.

After both two and four weeks, non-labile phosphate ranged between 8% and 16% of total extracted phosphate with little difference among treatments (Table 1). Of total extracted phosphate, labile phosphate was between 2% and 11% in P0 and P96 and about 50% in P385, P578 and P770. Residual P represented 75–88% of total extracted phosphate in P0 and P96 whereas it was only 30–43% in P385, P578 and P770.

Binding of phosphate to jarosite increased with phosphate rate between P100 and P1000, but then declined to the P1200 rate (Fig. 4a). Compared to P100, sorbed phosphate was two to three-fold higher in P200 and P400, five-fold higher in P800 and 10-fold higher in P1000 whereas it was only eight-fold higher in P1200. Release of phosphate from phosphate-loaded jarosite compared to P200 was about two-fold higher in P400 and 10-fold higher in P800 and P1200 (Fig. 4b). At maximum phosphate binding, 10–20% of bound phosphate was released. Based on maximum phosphate binding capacity of jarosite and concentration of jarosite in the soil, about 50% of phosphate binding in Gillman ASS can be attributed to jarosite.

## 4. Discussion

The study showed that there is a threshold for phosphate binding in

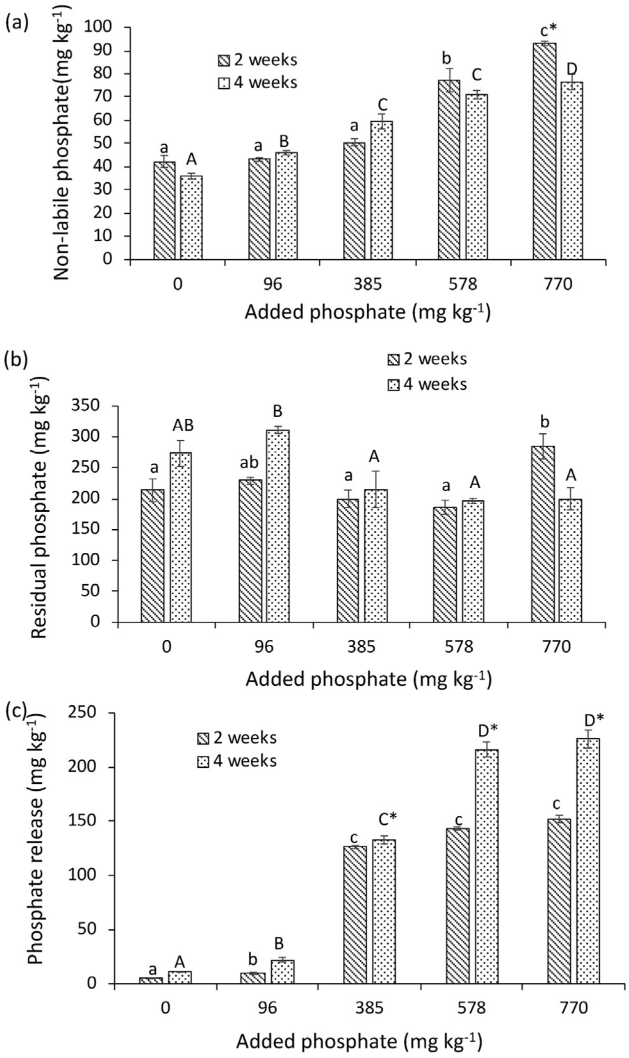

**Fig. 2.** Non-labile phosphate (a), residual phosphate (b) and phosphate release (c) after two and four weeks in soil amended with phosphate ($KH_2PO_4$) at 0, 96, 385, 578 and 770 mg kg$^{-1}$. Columns with different letters are significantly different with lower case letters for two weeks, upper case letters for four weeks (n = 4, ± standard error, $P \leq 0.05$). Means with asterisks are significantly higher at one sampling time than the other.

the jarosite containing ASS used in this study. The threshold is at a phosphate addition rate of about 350 mg phosphate kg$^{-1}$ because below about 350 mg phosphate kg$^{-1}$, labile phosphate remained low and represented about 20% of added phosphate, confirming the first hypothesis (labile phosphate will remain low below a threshold representing phosphate sorption capacity of the soil). Thus below the threshold about half of the added phosphate was sorbed to the soil.

It should be noted that we used the sequential fraction method of Ivanoff et al. (1998) to determine soil P pools because it was easy to perform and gave consistent results. The method was developed to fractionate organic P in highly organic aquatic soils. The soil used in this experiment had low organic matter content, which may explain why moderately labile P (extracted with 1 M HCl) which specifically extracts organic P was not detectable. However, the following extraction of non-labile P with 0.5 M NaOH should also be suitable for this soil because it extracts Fe and Al bound P (Ivanoff et al., 1998).

Of the sorbed phosphate below the threshold, only about 25% could be released by shaking with water which indicates that most sorbed phosphate was strongly bound to the soil. This is in agreement with previous studies that showed that P availability in soils remains low up to a threshold (Ayenew et al., 2018; Recena et al., 2016). The threshold is lower than the calculated maximum phosphate binding capacity of the soil which was 588 mg phosphate kg$^{-1}$. The lower threshold value is likely due to the fact that labile phosphate did not differ significantly between P380 and P578, but the Langmuir equation considered them as different.

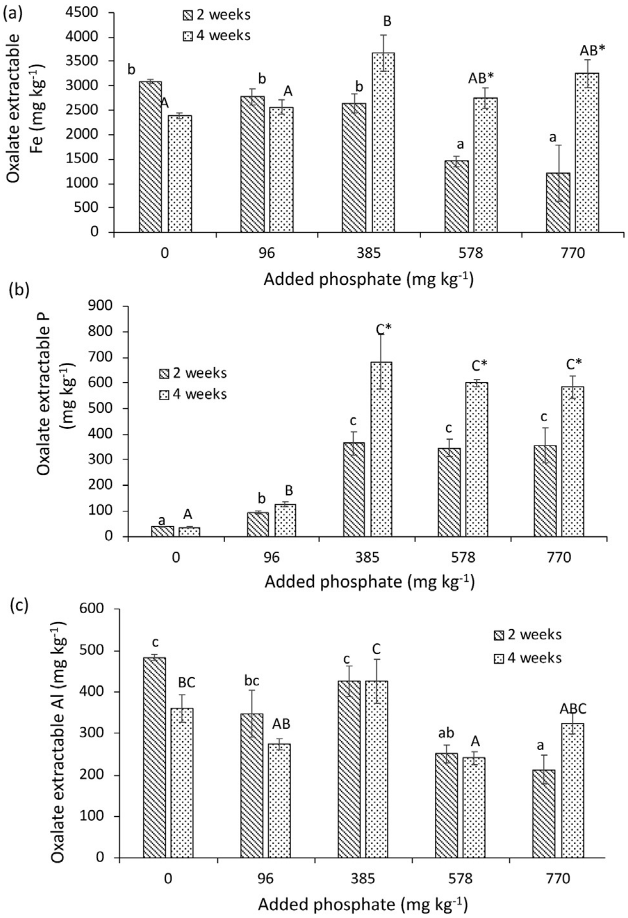

**Fig. 3.** Acid oxalate extractable Fe (a), P (b) and Al (c) after two and four weeks in soil amended with phosphate ($KH_2PO_4$) at 0, 96, 385, 578 and 770 mg kg$^{-1}$. Columns with different letters are significantly different, with lower case letters for two weeks, upper case letters for four weeks (n = 4, ± standard error, $P \leq 0.05$). Means with asterisks are significantly higher at one sampling time than the other.

**Table 1**
Percentage of phosphate pools (labile phosphate (LP), non-labile phosphate (NLP), residual phosphate (RP)] in total extracted phosphate after 2 and 4 weeks in soil amended with phosphate ($KH_2PO_4$) at 0, 96, 385, 578 and 770 mg kg$^{-1}$. Values of one sampling time with different letters are significantly different (n = 4, ± standard error, $P \leq 0.05$).

| | Phosphate addition rate (mg kg$^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| Length of incubation | Phosphate pool (mg kg$^{-1}$) | 0 | 96 | 385 | 578 | 770 |
| 2 weeks | LP | $2^{a}$ | $11^{b}$ | $52^{c*}$ | $55^{c}$ | $53^{c}$ |
| | NLP | $16^{c*}$ | $14^{bc}$ | $10^{a}$ | $13a^{bc}$ | $11^{ab}$ |
| | RP | $81^{b}$ | $75^{b}$ | $39^{a}$ | $32^{a}$ | $35^{a}$ |
| 4 weeks | LP | $3^{a}$ | $4^{a}$ | $46^{b}$ | $51^{c}$ | $58^{d}$ |
| | NLP | $11^{a}$ | $8^{a}$ | $12^{a}$ | $13^{a}$ | $11^{a}$ |
| | RP | $86^{c}$ | $88^{c}$ | $43^{b}$ | $36^{ab}$ | $30^{a}$ |

Iron minerals are known to play an important role in binding P, particularly in acidic soils (Boukemara et al., 2017; Liptzin and Silver, 2009). Amorphous Fe, determined as acid oxalate extractable Fe in this study, is typically present in high concentrations in ASS due to pyrite

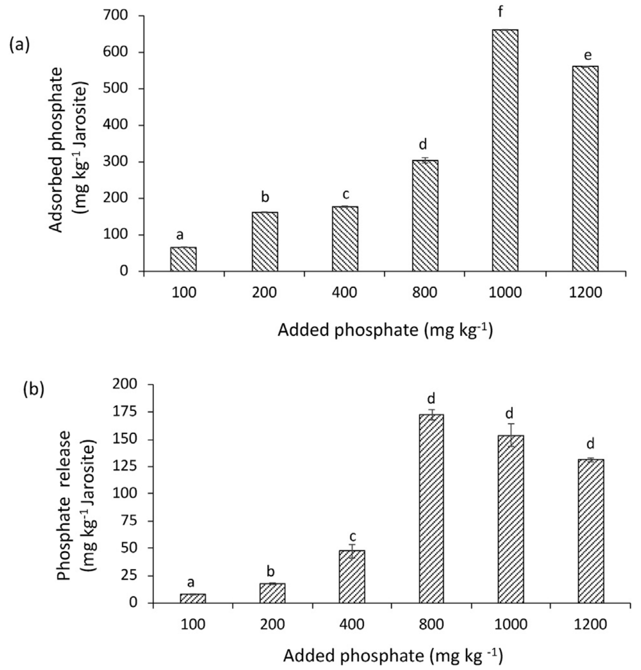

**Fig. 4.** Phosphate binding by jarosite shaken with solutions containing 100, 200, 400, 800, 1000 and 1200 mg phosphate $L^{-1}$ as $KH_2PO_4$ (a) and phosphate release from phosphate-loaded jarosite (b). Columns with different letters are significantly different (n = 4, ± standard error, $P \leq 0.05$).

oxidation (Mosley et al., 2014). The increase in acid oxalate extractable P from the control to 96 mg $kg^{-1}$ was of similar magnitude as the increase in bound P which suggests that P was mainly bound to amorphous Fe and possibly also amorphous Al. However, acid oxalate extractable Fe was four to eight-fold higher than acid oxalate extractable Al which indicates that in this soil, amorphous Fe is the main P binding mineral.

Above the threshold of about 350 mg added phosphate $kg^{-1}$, labile phosphate increased strongly and was more than 50% of P added although bound and non-labile phosphate also increased with phosphate addition rate. This indicates that although some of the added phosphate was bound the soil, sufficient phosphate was added to increase plant-available P. On the other hand, residual phosphate was variable and not affected by phosphate addition which suggests that it did not contribute to phosphate sorption. Nevertheless, residual phosphate was a large pool. Thus, small changes in its concentration, e.g., an increase by 50 mg phosphate $kg^{-1}$ may not change this pool significantly. Other studies also reported that large and stable P pools change little with phosphate addition (e.g., Jiménez et al., 2019; Lehmann et al., 2001). Therefore the second hypothesis (non-labile and residual phosphate will increase with phosphate addition rate, particularly above the threshold) can only be accepted for non-labile phosphate.

The about three-fold increase in acid oxalate extractable P at phosphate rates $\geq$ 350 mg $kg^{-1}$ compared to lower phosphate rates suggests that P bound in this soil was bound to Fe or co-precipitated as Fe-phosphate solid phases. Compared to below the threshold, a larger proportion of bound phosphate could be released by shaking in water (50% compared to 25%). Thus, the third hypothesis (only a small proportion of phosphate bound to the soil can be released) has to be declined. The greater phosphate release after 4 weeks compared to 2 weeks may be due to Fe reduction which would reduce the number of binding sites for phosphate and mobilise P from Fe-phosphate (Chacon et al., 2006). The decline in redox potential during the experiment from 500 to 300 mV in treatments with phosphate amendment supports this explanation.

Amorphous Fe can react with phosphate either by formation of Fe-phosphate or by binding phosphate to anion exchange sites (Sample et al., 1980). Acid oxalate extractable phosphate was about four to eight-fold higher above the threshold than below which supports the existence of a threshold. However, acid oxalate extractable P did not increase with phosphate rate above the threshold. This can be explained by the increase of overall surface negative charge as more phosphate is bound which reduces further binding of phosphate to the mineral surface (Rajan, 1976). The higher acid oxalate extractable P after four weeks than two weeks could be due to slow diffusion of phosphate into the interior of the mineral after initial surface adsorption (Froelich, 1988).

Labile P, non-labile P and residual P changed little over time which confirms the fourth hypothesis (phosphate pools change little over time because 2 weeks is sufficient time for the added phosphate to be bound to the soil). Even a shorter exposure time may have been sufficient as Boukemara et al. (2017) showed that in acid mine sediments with jarosite, maximum phosphate sorption was reached after 4 h. Thus in this soil, the different phosphate pools had equilibrated after two weeks and are likely to remain stable unless the equilibrium is disrupted, e.g. by removing labile phosphate via leaching or a further decrease in redox potential.

To determine phosphate sorption to jarosite, jarosite was shaken with phosphate solutions in an end-over-end shaker overnight at room temperature, followed by centrifugation and filtration. We assumed that overnight shaking of jarosite with phosphate solution is sufficient to reach maximum phosphate sorption because Boukemara et al. (2017) studied phosphate sorption in acid mine sediments where jarosite was a principal constituent. Maximum phosphate sorption was reached after 4 h. The fifth hypothesis (jarosite has a strong capacity to bind phosphate; little of the phosphate bound to jarosite can be released) can only be partially accepted because although jarosite had a strong capacity to bind phosphate, a proportion (50–70%) of the bound phosphate could be released. The high phosphate binding capacity of jarosite and the finding that about 50% of phosphate bound to this soil can be attributed to jarosite confirm the importance of Fe minerals to phosphate binding in ASS. This suggests that the remaining 50% of phosphate binding to the soil is possibly controlled by other minerals in Gillman ASS with anion exchange sites, such as goethite (Thomas, 2011). About 30–50% of phosphate sorbed to jarosite was released by shaking with water, which is similar as phosphate release of the soil. Thus, phosphate bound to jarosite could become available to plants upon rewetting.

This study showed that in Gillman ASS adjusted to pH 5.5, phosphate additions above about 350 mg $kg^{-1}$ are required to increase available phosphate and thereby potentially enhance plant growth, e.g. for remediation using plants (Gardner et al., 2018). This rate is equivalent to about 1000 kg P $ha^{-1}$ which is much higher than the rate that would be added to a crop annually (20–50 kg $ha^{-1}$). However, this amount of P could be added in several applications until phosphate binding sites are saturated. Adjustment of the pH to 5.5 in the field may be possible by liming or addition of organic matter and flooding to stimulate sulfate reduction which consumes protons. Microbial activity induced by organic matter addition may have the additional benefit of decreasing soil redox potential which would reduce phosphate binding sites. In our previous study with this soil, we showed that increasing the pH to 5.5 increased labile phosphate after phosphate addition (Mayakaduwage et al., 2019). This can be explained by the effects of $OH^-$ on Fe minerals including exchange of phosphate with $OH^-$ minerals and reducing the number of anion binding sites (Oburger et al., 2011). Thus, even greater amounts of phosphate may need to be added to increase labile P if the soil pH is not increased.

## 5. Conclusions

The results show that phosphate additions above about 350 mg $kg^{-1}$ are required to increase available phosphate in Gillman ASS when the pH is adjusted to 5.5. Below 350 mg $kg^{-1}$, a high proportion of added phosphate remained strongly bound to the soil, particularly to amorphous Fe. Jarosite had a high capacity to bind phosphate and contributed about 50% of phosphate binding in this soil. Further research could assess the role of other Fe minerals such as goethite on phosphate availability in ASS under submerged conditions. Laboratory and field trials of P dynamics in amended ASS would be beneficial to further inform development of remediation strategies.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2020.114359.

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# Chapter 4

# Phosphorus pools in acid sulfate soil are influenced by soil water content and form in which P is added

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| Contribution to the Paper | Performed the experiment, analysed the samples, data analysis and interpretation of data, writing the manuscript.<br>I hereby certify that the contribution is accurate | | |
| Overall percentage (%) | 70 | | |
| Certification: | This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper. | | |
| Signature | | Date | 26/08/2020 |

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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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# Phosphorus pools in acid sulfate soil are influenced by soil water content and form in which P is added

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## ABSTRACT

Phosphorus availability in acid soils is influenced by interactions of P and Fe-oxides which are modulated by soil water content. Decomposition of organic material influences these interactions. But little is known about the effect of P added as inorganic P or manure on P pools in an acid sulfate soil under varying water content. Sandy acid sulfate soil (pH adjusted to 5.5) was amended with 400 mg P $kg^{-1}$ as inorganic P, manure or combinations of inorganic P and manure. Amendment treatments included inorganic P alone (100F), manure alone (100M) and P added as 50% inorganic P + 50% manure (50M/50F) as well as an unamended control. The soil was submerged for 2 weeks, then the overlying water was removed and the soil dried to maximum water-holding capacity. The soil was incubated for 4 weeks at this water content followed by submergence for 2 weeks. In the unamended control, P pools were very low and not affected by soil water content. At the end of the first submergence, labile phosphate and oxalate extractable P were two-fold higher in 100F than in 100M and 50M/50F. Oxalate extractable Fe was higher in 100M than 100F and remained unchanged until the end of the experiment. In the following moist incubation and after the second submergence, labile phosphate remained unchanged in 100M, but halved in 100F. Labile phosphate strongly increased in 50M/50F and was about two-fold higher than in the other two amended treatments. Oxalate extractable P was two-fold higher in the moist period than at the end of the first submergence in manure treatments and 30% higher in 100F. It then decreased again after re-submergence in the manure treatments and became lower than in 100F. It can be concluded that with inorganic P addition, labile phosphate was initially very high, but then strongly decreased in the moist period due to removal of phosphate with the overlying water and to binding of P to amorphous Fe where it remained after re-submergence. With manure on the other hand, labile phosphate was lower than with 100F initially, but then remained stable. Phosphate in the overlying water was low with manure which indicates that, in contrast to inorganic P addition, the threat of P transfer into the surrounding environment is low.

## 1. Introduction

In acid soils, P availability strongly depends on interactions between P and Al/Fe-oxides as well as soil water content (Nguyen and Marschner, 2005; Torrent et al., 1992; Wisawapipat et al., 2017). Added phosphate can be sorbed to anion exchange sites on Fe/Al oxides (Ayenew et al., 2018) and particularly amorphous Fe oxides (Haynes, 1982; Li et al., 2012; Winkler et al., 2016). However, when the soils are flooded, the consequent lowering of the redox potential can solubilise Fe-oxides thereby releasing bound P (Shenker et al., 2005; Wisawapipat et al., 2017). In contrast, when previously flooded soils are drained and Fe-oxides are formed under aerobic conditions, the phosphate concentration in the soil solution can decrease due to sorption on newly formed Fe-oxides (Sah et al., 1989).

Availability of P is also influenced by addition of organic materials. Organic materials can release inorganic P directly and after mineralisation of organic P (Sharpley and Moyer, 2000). Further, organic amendments influence P availability indirectly. Firstly, organic acid anions produced during decomposition can exchange bound phosphate from anion exchange sites, releasing P into the soil solution (Hue, 1991). Secondly, bound organic acid anions can prevent re-adsorption of phosphate (Reddy et al., 1980). Thirdly, organic matter decomposition by microbes results in oxygen consumption which can lower the redox potential to where Fe(III) oxides are reduced and dissolved (Todorova et al., 2005; Yi et al., 2012).

Acid sulfate soils (ASS) are widespread in coastal areas and around rivers and lakes (Andriesse and Van Mensvoort, 2006; Fitzpatrick et al., 2008) where they are exposed to periods of flooding and drying. Acid

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sulfate soils are soils with sulfidic (mostly pyrite/$FeS_2$) materials formed under reducing conditions, but they can become extremely acidic (pH < 4, referred to as sulfuric) when exposed to oxygen. Sulfate reduction consumes protons and therefore raises the pH. But when sulfuric ASS are flooded, sulfate reduction may be low because sulfate reducers are inhibited by low pH (Berner, 1984). Acid sulfate soils are important for crop production because they often occur in rice paddies (Dent and Pons, 1995). For agricultural use, ASS and particularly sulfuric ASS, are often limed to minimise the risk of acidity stress for crops. In earlier studies, we showed that in a sulfuric ASS with pH adjusted to 5.5 under submerged conditions, at least 350 mg phosphate $kg^{-1}$ had to be added to increase labile P above the unamended control (Mayadukawage et al., 2019; 2020). We found that the Fe(III) oxysulfate mineral jarosite played an important role in P binding in this soil (Mayakaduwage et al., 2020).

As outlined above, the effect of P addition on soil P pools may depend on the form in which P is added as well as soil water content. But little is known about the effect of P added in inorganic form or as manure on P pools in ASS exposed to varying water content. Using the same soil as in our previous studies (Mayadukawage et al., 2019; 2020), the first aim of this study was to investigate the effect of P added as manure, inorganic P or combinations of manure and inorganic P on labile P (preliminary experiment). The second aim was to determine P pools in this soil amended with manure, inorganic P or a 1:1 mix of manure and inorganic P under changing water regime (submerged, moist, re-submerged – main experiment). Manure was chosen as organic amendment that is decomposed more slowly than most plant residues as manures are already strongly decomposed during passage through the guts of animals (Hungate, 2013).

The following hypotheses were tested:(1) Labile phosphate will be lower with manure than inorganic fertiliser alone but later differences among amended treatments will be smaller as manure P is mineralised, (2) P associated with Fe-oxides (determined as oxalate extractable P) will be lower with manure than inorganic P because soluble phosphate is quickly sorbed to anion exchange sites in the soil or forms P salts whereas phosphate release is slow from manure and organic acid anions generated during decomposition reduce P sorption, (3) when submerged conditions are followed by moist incubation, labile phosphate will decrease more strongly with inorganic P than manure because manure mineralisation provides a continuous source of labile phosphate.

## 2. Materials and methods

Sulfuric (pH < 4) acid sulfate soil was collected in the Barker Inlet, Gillman, South Australia (34°49′47.25″S; 138°32′40.24″E). The soil is classified as sulfuric soil (Acid sulfate soil classification), peaty, sulfuric, hypersalic Hydrosol (Australian Soil classification) or Salic Fluvisol (hyperthionic, drainic) (World reference base identification keys). Properties of the soil were pH 3.2 (1:1 water slurry), sand 89%, silt 7%, clay 4%, total P 300 mg $kg^{-1}$, total N 900 mg $kg^{-1}$, labile P 7.6 mg $kg^{-1}$, maximum water holding capacity (WHC) 140 g $kg^{-1}$, total organic C 19 g $kg^{-1}$, total Fe 22 g $kg^{-1}$. The soil was dried at 40 °C in a fan-forced oven and sieved to < 2 mm. Sheep manure was dried at 40 °C for 2 days, ground and then sieved to < 2 mm. It had following properties: pH 6.8, total organic C 186 g $kg^{-1}$, total N 7.3 g $kg^{-1}$, water extractable N 0.57 g $kg^{-1}$, total P 4.2 g $kg^{-1}$, water extractable P 0.48 g $kg^{-1}$, total Fe 5.3 g $kg^{-1}$ and amorphous Fe 0.26 g $kg^{-1}$.

The sieved soil was mixed with reverse osmosis (RO) water at a 1:2 soil:water ratio. Soil pH was increased by adding 0.5 N $Ca(OH)_2$ and mixing it with soil until the pH of the soil slurry was 5.5 ± 0.5 24 h after adjustment. Then the soil was dried in a fan-forced oven at 30 °C for 36 h until the water content was 100% WHC. The final pH at 100% of WHC in a 1:1 soil/water ratio was 5.5 ± 0.1. Soil equivalent to 30 g dry weight was placed in a 70 ml plastic container and 400 mg P $kg^{-1}$ was added as either manure alone, inorganic fertiliser (as $KH_2PO_4$) alone or combination of manure and inorganic fertilisers. This rate was selected to detect measurable P changes in the labile P fraction. Based on our previous study with this soil (Mayakaduwage et al., 2019; 2020), labile P only increased significantly compared to the unamended control at phosphate addition rates of ≥350 mg $kg^{-1}$.

**Table 1**
Names of amendment treatments with different proportions of P and N added with total P addition 400 mg $kg^{-1}$ and total N addition 733 mg $kg^{-1}$.

| Treatment | P with manure | N with manure | Inorganic P | Inorganic N |
|---|---|---|---|---|
| Amendment (mg $kg^{-1}$) | | | | |
| 100M | 400 | 733 | 0 | 0 |
| 75M/25F | 300 | 550 | 100 | 183 |
| 50M/50F | 200 | 367 | 200 | 367 |
| 25M/75F | 100 | 183 | 300 | 550 |
| 100F | 0 | 0 | 400 | 733 |

### 2.1. Preliminary experiment

The aim of this experiment was to select the P amendment treatments to be used in the main experiment. There were six treatments with four replicates in which 400 mg P $kg^{-1}$ was added as 100% sheep manure (100M), 75% sheep manure and 25% inorganic fertiliser (75M/25F), 50% sheep manure and 50% inorganic fertiliser (50M/50F), 100% inorganic fertiliser (100F) (Table 1) and an unamended control. To ensure that microbes were adequately supplied with N, the same amount of N was added in the amended treatments, equivalent to the total N added with 100% manure (733 mg N $kg^{-1}$). In the other treatments, the difference between this amount and that added with manure was supplied as $NH_4Cl$. Manure, inorganic P and inorganic N were thoroughly mixed with the soil. Then, RO water was added so that the soil surface was fully covered by water (about 30 ml) and the lids of the containers were screwed on tightly to minimise entry of air. The soil was incubated at 25 °C in the dark under submerged conditions and destructively sampled in weeks 2 and 4 to measure labile P.

### 2.2. Main experiment

The unamended control and three amendment treatments were used (100M, 50M/50F, 100F). The amendment treatments were selected because in preliminary experiment, they differed in labile P as well as in the changes over time.

As described for the preliminary experiment, soil pH was adjusted to 5.5 using 0.5 N $Ca(OH)_2$ and the same amount of N and P (equivalent to 100M) was added to all amended treatments. Next, RO water was added so that the soil surface was fully covered by water and then incubated submerged for 2 weeks (from week 0 to week 2). After 2 weeks, the overlying water was removed and analysed for inorganic P. Then the soils were dried to 100% WHC (referred to as moist) and maintained at this water content for 4 weeks (from week 2 to week 6). After 6 weeks, the soil was re-submerged and incubated for another two 2 weeks under submerged conditions (from week 6 to week 8). During submergence, container lids were screwed on tightly whereas during incubation at 100% WHC, lids were placed on the containers, but not screwed on to allow for gas exchange. The soil was incubated at 25 °C in the dark. Destructive sampling was carried out at the start of the experiment (0 weeks), and at the end of weeks 2, 4, 6 and 8.

### 2.3. Analyses

Soil Eh was determined by inserting a redox electrode (Model IJ64, Ionode, 159 Australia) about 1 cm into the soil. Absolute redox potential values were calculated by correction to the standard hydrogen electrode. Maximum WHC was determined using a sintered glass funnel connected to a 1 m water column (Wilke, 2005). Soil texture was determined by the hydrometer method (Gee et al., 2002). Total Fe in the soil and manure was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES, Agilent, Mulgrave, Australia) after dissolution in aqua regia ($HNO_3$:HCl 1:3) (Zarcinas et al., 1996). Total organic carbon in soil and sheep manure was determined by $K_2Cr_2O_7$ and $H_2SO_4$ oxidation by titration with acidified $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ (Walkley and Black, 1934). The phosphovanado molybdate method (Hanson, 1950) was used to determine total P after acid digestion with nitric acid-hydrochloric acid at a 1:3 ratio. Total N in manure and soil was measured by a modified Kjeldahl method (Bremner and Breitenbeck, 1983). Water soluble phosphate and inorganic N in sheep manure was determined after extraction with hot water as described in Konieczyński and Wesołowski (2007): 30 ml of hot (85 °C) RO water were added to 1 g manure, shaken for 2 h and filtered. Extracts were analysed for phosphate with the malachite-green method (Ohno and Zibilske 1991).

Labile phosphate was determined by shaking soil (equivalent to 1 g dry) with 0.5 M $NaHCO_3$ for 16 h. The phosphate concentration in the extracts was determined by the malachite green method after centrifugation and filtration (Ohno and Zibilske, 1991). At the end of week 2 and week 8, the overlying water was decanted and analysed for phosphate by the malachite green method (Ohno and Zibilske, 1991). The phosphate concentration in the overlying water was expressed in mg $kg^{-1}$ by calculating the amount of phosphate in the overlying water (concentration × volume, 30 ml) and dividing it by the soil dry weight per container (30 g). Amorphous Fe/Al were determined according to Schwertmann (1964). Soil (0.4 g dry weight equivalent) was shaken with 40 ml of acid oxalate reagent (0.114 M ammonium oxalate and 0.086 M 165 oxalic acid) for 4 h in the dark at room temperature. Then, the samples were centrifuged to obtain a clear extract followed by dilution and acidification with 0.2% v/v nitric acid. Fe, Al and P in the extracts were determined by ICP-AES. P in the extracts was measured to determine P associated with oxalate extractable F and Al.

### 2.4. Data analysis

There were four replicates per treatment and sampling time. After confirmation of normality (W test), data were analysed by repeated measures ANOVA with treatments as factors and time as repeated measure in SPSS statistics version 25. Tukey's multiple comparisons test was used to determine significant differences among treatments and sampling times.

## 3. Results

### 3.1. Preliminary experiment

In week 2 compared to the control, labile phosphate was five-fold higher in 100M, 10 and 12-fold higher in 75M/25F and 50M/50F and about 20-fold higher in 25M/75F and 100F (Fig. 1). From week 2 to week 4, labile phosphate increased by 40% in 100M and by 10% in 100F whereas it decreased by about 20% in 75M/25F, 50M/50F and 75M/25F. It didn't change over time in the control. In week 4, labile phosphate compared to the control was about six-fold higher in 100M, 75M/25F and 50M/50F whereas it was 15-fold higher in 25F/75F and 100F.

### 3.2. Main experiment

Labile phosphate was always lowest in the control and four to five-fold higher in 100M (Fig. 2a). It remained stable over time in these two treatments. At the start of the experiment (week 0), labile phosphate was about 10-fold higher in 50M/50F and 100F than in the control. After two weeks submergence (week 2) compared to the start, labile phosphate was about 30% lower in 50M/50F, but didn't change in 100F. Of the added P, about 40% was labile in the treatments with manure and 75% in 100F. Two weeks after removal of the overlying water and drying to 100% WHC (week 4), labile phosphate in 50M/50F was two-fold higher than at the end of submergence (week 2), but then decreased by about 30% from week 4 to week 6 (moist period). In 100F, labile phosphate was about 70% lower in the moist period (weeks 4 and 6) than at the end of the submerged period (week 2). Compared to the control, labile phosphate in the moist period was about 10-fold higher in 50M/50F, but only five-fold higher in 100F. Re-submergence after week 6 didn't affect labile phosphate in 50M/50F and 100F. In 50M/50F labile phosphate was about two-fold higher than in 100M at most sampling times except in week 2, where the two treatments didn't differ in labile phosphate. From week 4 onwards, labile phosphate was about 40% of added P in 100M and 100F and 75% in 50M/50F.

Phosphate in the overlying water, which was removed after the first and second submergence period (weeks 2 and 8), was lowest in the control (Fig. 2b). In week 2, phosphate in the overlying water compared to the control was 20, 100 and 200-fold higher in 100M, 50M/50F and 100F, respectively. In the amended treatments, phosphate in the overlying water at the end of the second submergence period (week 8) was 30% lower in 100M, but 60% lower in 50M/50F and 80% lower in 100F than in week 2. In week 8 compared to the control, phosphate in the overlying water compared to the control was 10, 30 and 40-fold higher in 100M, 50M/50F and 100F, respectively.

The redox potential was similar in all treatments at the start of the experiment (week 0) and in week 4 (after two weeks at 100% WHC) (Fig. 3). However, at the end of the first submergence period (week 2), the moist period (week 6) and the second submergence period (week 8), the redox potential was about 15% lower in 100M and 50M/50F than in 100F and the control.

At the start of the experiment (week 0), oxalate extractable Fe was

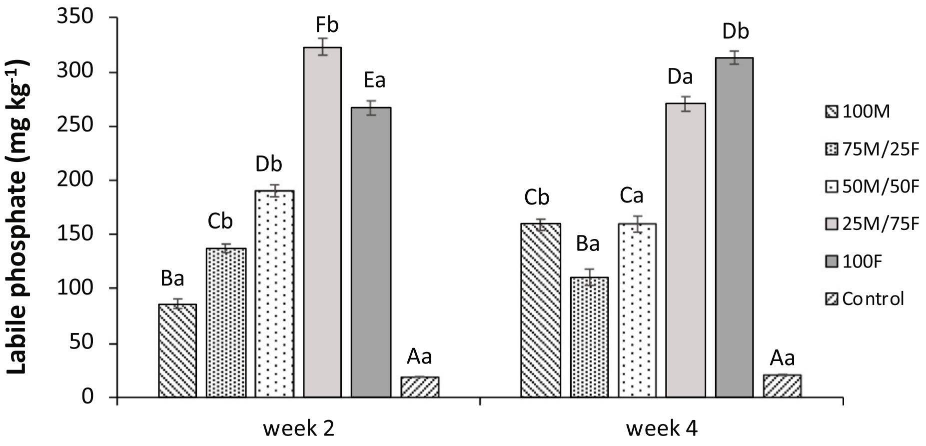

**Fig. 1.** Labile phosphate in treatments with different amendments (for treatment names see Table 1) after 2 and 4 weeks of submergence in the preliminary experiment. Different upper-case letters (A, B, C) indicate significant differences among treatments at each sampling date. Different lower-case letters (a, b, c) show significant differences in a treatment between sampling times (n = 4, means ± SE, $P \leq 0.05$).

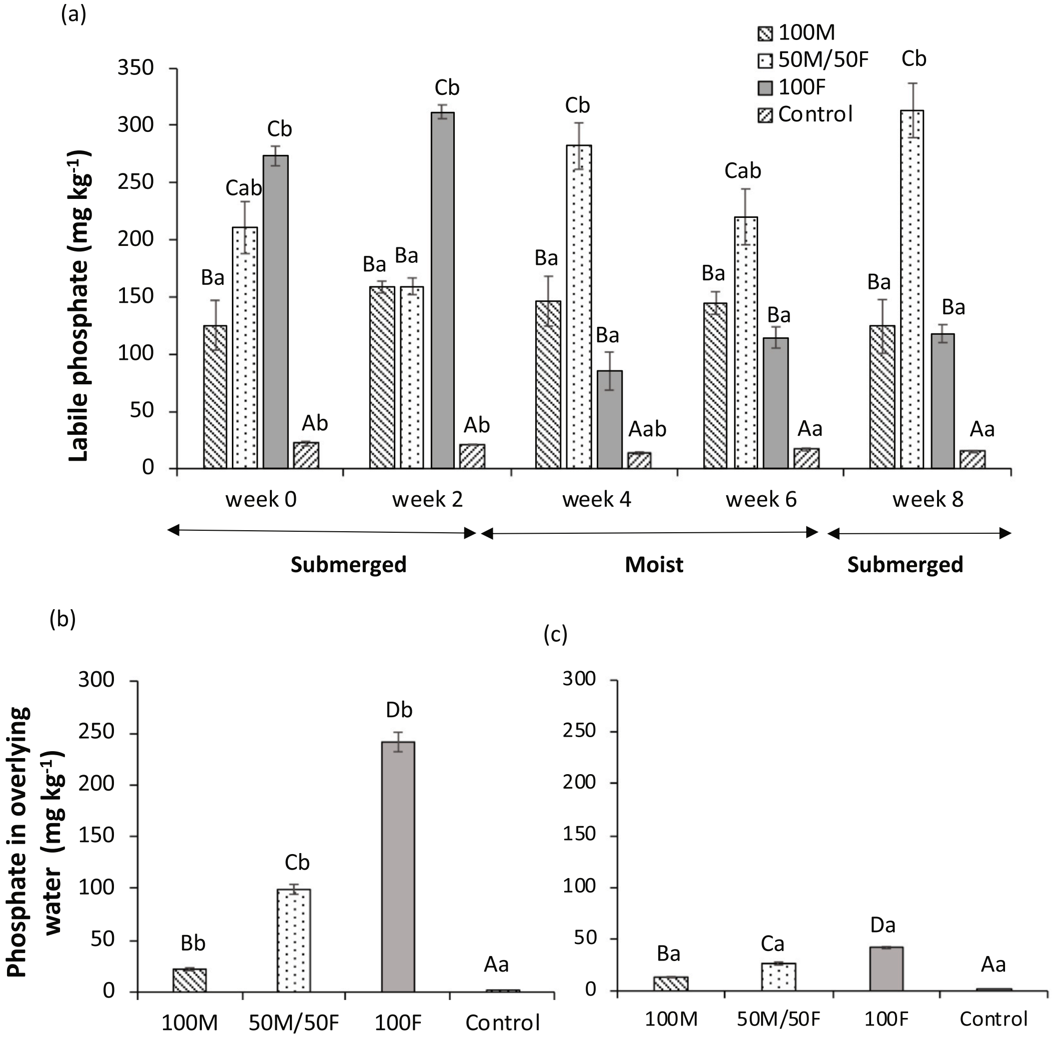

**Fig. 2.** Labile phosphate in in treatments with different amendments (for treatment names see Table 1) in weeks 0, 2 (submerged), 4, 6 (moist) and 8 (submerged) in the main experiment (a) and phosphate in the overlying water in weeks 2 (b) and 8 (c). Different upper-case letters (A, B, C) indicate significant differences among treatments at each sampling date. Different lower-case letters (a, b, c) show significant differences in a treatment over time (n = 4, means ± SE, $P \leq 0.05$).

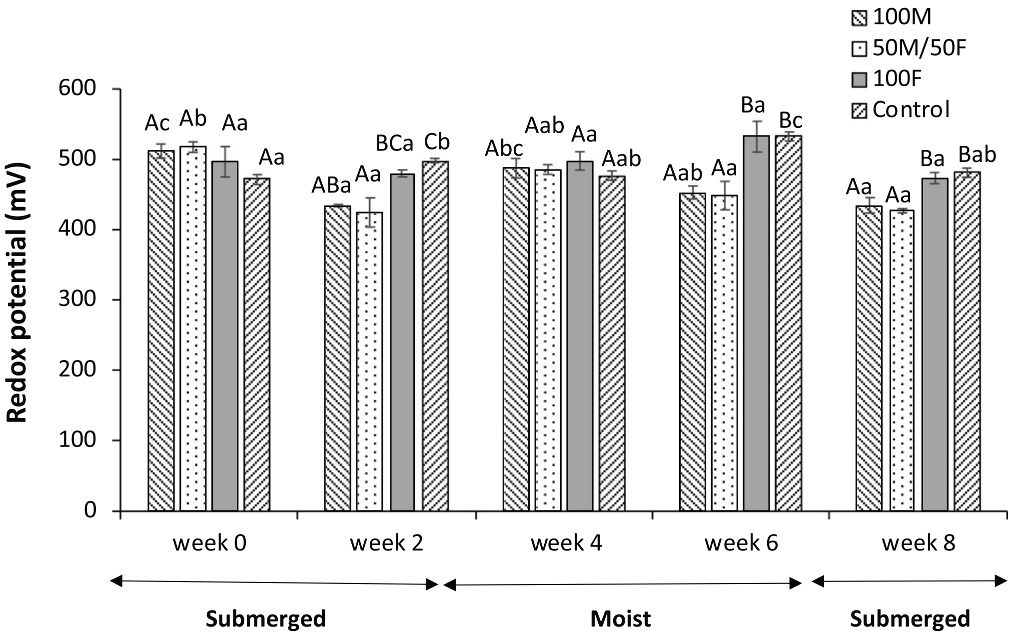

**Fig. 3.** Redox potential in treatments with different amendments (for treatment names see Table 1) in weeks 0, 2 (submerged), 4, 6 (moist) and 8 (submerged) in the main experiment. Different upper-case letters (A, B, C) indicate significant differences among treatments at each sampling date. Different lower-case letters (a, b, c) show significant differences in a treatment over time (n = 4, means ± SE, $P \leq 0.05$).

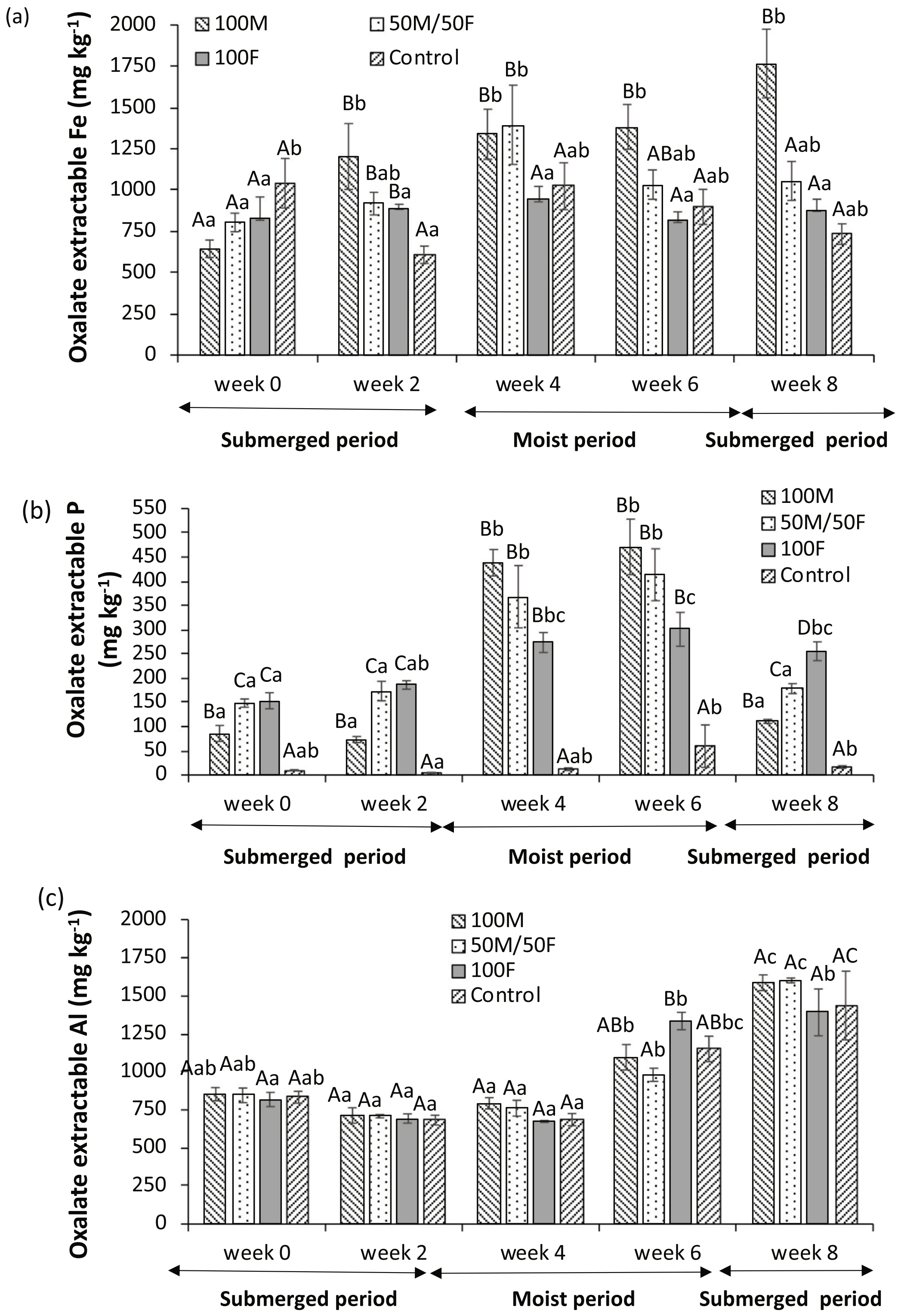

**Fig. 4.** Oxalate extractable Fe (a), P (b) and Al (c) in treatments with different amendments (for treatment names see Table 1) in weeks 0, 2 (submerged), 4, 6 (moist) and 8 (submerged) in the main experiment. Different upper-case letters (A, B, C) indicate significant differences among treatments at each sampling date. Different lower-case letters (a, b, c) show significant differences in a treatment over time (n = 4, means ± SE, $P \leq 0.05$).

similar in all treatments (Fig. 4a). At all other sampling times, oxalate extractable Fe was up to two-fold higher in 100M than the control. Oxalate extractable Fe increased two-fold in the first submergence period (from week 0 to week 2) in 100M, remained unchanged in 50M/50F and 100F and halved in the control. Thus in week 2, oxalate extractable Fe compared to the control was two-fold higher in 100M and 50% higher in 50M/50F and 100F. From then on, oxalate extractable Fe did not change significantly until the end of the experiment.

Oxalate extractable P was lowest in the control where it changed little during the experiment (Fig. 4b). At the start of the experiment (week 0) and in week 2, oxalate extractable P compared to the control was five-fold higher in 100M and 10-fold higher in 50M/50F and 100F.

In the moist period (weeks 4 and 6), oxalate extractable P was higher than during the previous submergence period in the amended treatments. It differed little between week 4 and week 6. Compared to week 0, oxalate extractable P at the end of the moist period (week 6) was five-fold higher in 100M, two-fold higher in 50M/50M and 30% higher in 100F. In both weeks 4 and 6 compared to the control, oxalate extractable P was about 20-fold higher in the amended treatments. From the end of the moist period (week 6) to the end of the second submergence period (week 8), oxalate extractable P decreased by 70% in 100M and by 50% in 50M/50F but remained unchanged in 100F and the control. Compared to the control, oxalate extractable P in week 8 was five-fold higher in 100M, 10-fold higher in 50M/50F and 15-fold higher in 100F.

Oxalate extractable Al differed little among treatments and was higher at the end of the moist period (week 6) and the end of the second submergence period (week 8) than at the previous sampling times (Fig. 4c).

## 4. Discussion

The results of the experiments showed that P pools in ASS are influenced by both soil water content and the form in which P is added.

The first hypothesis (labile phosphate will be lower with manure than inorganic fertiliser alone but later differences among amended treatments will be smaller as manure P is mineralised) can be confirmed. With the inorganic fertiliser (soluble P) labile P was initially high, of which a large proportion was removed with the overlying water in week 2. In contrast, mineralisation of manure P provided a continuous source of phosphate. It should be noted that we showed earlier in this soil that the pH increase to 5.5 reduced P binding compared to the original pH 3.2, likely due to competition of OH– with phosphate (Mayakaduwage et al., 2019). Thus labile phosphate may be overestimated compared to acidic ASS in the field. But to enable crop growth, landholders would add lime to increase the pH. Therefore the results of this experiment would be applicable to ASS used for cropping.

In the following, the results of the main experiment will be discussed separately for the three periods (first submergence, moist incubation and second submergence), focussing on P pools and oxalate extractable Fe.

### 4.1. First submergence (weeks 0–2)

Phosphate in the overlying water was higher with only inorganic fertiliser than treatments with manure because of the higher proportion of water-soluble P added with inorganic fertiliser. This high phosphate concentration could lead to undesirable algal growth in surrounding water bodies (McDowell et al., 2020). The increase in oxalate extractable Fe in 100M from week 0 to week 2 could be due to chelation of Fe by organic anions from the manure (Hue, 1991) which increased its extractability with oxalate. Despite the increase in oxalate extractable Fe, oxalate extractable P didn't increase from week 0 to week 2 in 100M indicating low P release from manure which is consistent with the unchanged labile phosphate.

In agreement with the second hypothesis [P associated with Fe-oxides (determined as oxalate extractable P) will be lower with manure than inorganic P], oxalate extractable P was two-fold higher in 100F than 100M. This can be explained by rapid sorption of the soluble phosphate from the inorganic fertiliser to anion exchange sites in the soil (Sample et al., 1980) whereas phosphate release from manure is slow. Further, organic acid anions released during manure decomposition may reduce P sorption (Hue, 1991; Wisawapipat et al., 2017). However, the second hypothesis has to be declined for the following moist period (see below). In both submerged periods, the redox potential was lower in treatments with manure than the control and 100F which can be explained by oxygen consumption by heterotrophic microbes decomposing the manure. However, the decrease in redox potential was relatively small (about 50 mV). This suggests that the vials used for the experiment were not completely sealed and allowed some oxygen in. In a submerged soil profile, particularly at greater depth, the redox potential is likely to be much lower if sufficient decomposable organic C is present.

### 4.2. Moist period (weeks 4–6)

In treatments with manure, the redox potential was higher after 2 weeks moist incubation (week 4) than at the end of the submergence (week 2) indicating diffusion of oxygen into the soil. As a result of the increase in redox potential in the manure treatments, the redox potential was similar in all treatments in week 4. However after four weeks moist incubation (week 6), the redox potential in the manure treatments was lower than in 100F and the control. This suggests that oxygen diffusion into the moist soil did not completely compensate for oxygen consumption by microbes stimulated by organic C in the manure.

The third hypothesis (when submerged conditions are followed by moist incubation, labile phosphate will decrease more strongly with inorganic P than manure) can be confirmed. Whereas labile phosphate in 100F decreased by about 70% from week 2 to week 4, it remained unchanged in 100M. The strong decrease in 100F can be explained by removal of soluble phosphate with the decanting of the overlying water. With the overlying water, about 250 mg $kg^{-1}$ phosphate were removed in 100F which is very close to the reduction in labile phosphate by about 200 mg $kg^{-1}$ from the end of the submergence to week 4. Another possible reason for the decrease in labile phosphate is sorption of phosphate to Fe oxides (Yuan and Lavkulich, 1994) as indicated by the higher oxalate extractable P in week 6.

In 100M on the other hand, labile phosphate was similar in the moist period as in week 2 (end of first submergence). The lack of change in labile phosphate from week 4 to the moist period is likely because of continuous mineralisation of manure P and because little phosphate was removed with the overlying water (only about 25 mg $kg^{-1}$). However, oxalate extractable P in 100M was about five-fold higher in the moist period than in week 2. More oxidising conditions in the moist period could have increased formation of Fe oxides (Fiedler and Sommer, 2004). But this does not seem to be the case in this experiment as oxalate extractable Fe did not change from submerged to moist conditions, likely because the redox potential also changed little. Apparently, P mineralised from manure was preferentially bound to amorphous Fe as labile phosphate was similar in the moist period as in week 2. Additionally, uptake of phosphate by microbes may have prevented an increase in labile phosphate. It is also possible that organic P from manure directly bound to Fe oxides in the soil without mineralisation (Martin et al., 2004). Ognalaga et al. (1994) proposed that organic phosphates can be bound to Fe oxides via ligand exchange between the phosphate group and surface-reactive hydroxyls.

In 50M/50F, both labile phosphate and oxalate-extractable P were higher in the moist period than at the end of the submerged period although about 100 mg $kg^{-1}$ phosphate were removed with the overlying water. The released P may have come mainly from mineralisation of manure P as indicated by the strong increase in oxalate extractable P in 100M. Organic acid anions produced during manure decomposition may have prevented a stronger increase in oxalate extractable P in 50M/50F and allowed more of the mineralised P to remain labile (Johnson and Loeppert, 2006).

The higher oxalate extractable Fe in 100M than in treatments without manure (100F and the control) from week 2 onwards is likely due to stabilisation of amorphous Fe by the manure through prevention of the formation of crystalline Fe oxides (Jones et al., 2009). It should be noted that in dry conditions in the field, soils may dry to below 100% WHC which is likely to induce greater formation of Fe oxides.

### 4.3. Second submergence (week 8)

Two weeks after submerging the soils a second time (week 8), oxalate extractable P was lower in the treatments with manure. The decrease in oxalate extractable P in the manure treatments occurred although oxalate extractable Fe did not change from week 6 to week 8. This suggests that previously bound P was exchanged from amorphous Fe by other anions, likely organic acid anions produced during manure decomposition (Hunt et al., 2007; Wisawapipat et al., 2017). In 50M/50F, the decrease in oxalate extractable P was associated with a corresponding increase in labile phosphate. In 100M on the other hand, labile phosphate was stable from week 6 to week 8. This suggests that the released oxalate extractable P was either immobilised by microbes or remained in organic form in solution and therefore not detectable as phosphate.

In all amended treatments, phosphate in the overlying water was lower at the end of the second submergence (week 8) than the end of the first submergence (week 2). The decrease was greatest in 100F and smallest in 100M. In 100F, the low phosphate in the overlying water in week 8 can be explained by the removal of P in the overlying water after the first submergence and the low labile P which indicates that very little of the added P had remained water-soluble. In the treatments with manure on the other hand, phosphate in the overlying water was lower in week 8 although labile P in week 8 had not changed compared to week 2 in 100M and was higher than in week 2 in 50M/50F. Bicarbonate used for extraction of labile phosphate does not only assess the phosphate in the soil solution, but also that weakly bound to anion exchange sites on soil particles (Soinne, 2009). Thus in 100M and 50M/50F, apparently a large proportion of labile P was bound to soil particles.

## 5. Conclusion

This study showed that P pools in the sandy acid sulfate soil at pH 5.5 are strongly influenced by soil water content, but this effect was modulated by the form in which P was added. Addition of inorganic P resulted in a high concentration of labile phosphate within the first two weeks of submergence, however when the overlying water which contained large amounts of phosphate was removed and the soil then remained at 100% maximum water-holding capacity for 2–4 weeks, labile P was lower than in the first two weeks. Even subsequent submersion of the soil did not increase labile P again suggesting that the remaining P was strongly bound. This indicates that the risk of P transfer in the surrounding environment is high shortly after inorganic P addition, but is low once the first flush of released P is removed.

Manure addition increased labile phosphate in the first submergence period to a smaller extent than inorganic P, but in the following moist period and after re-submergence, labile P was similar as with inorganic P. The lower oxalate extractable P with manure under submerged conditions suggests that compounds released during manure decomposition reduced P binding to Fe oxides. It can be concluded that manure may provide a sustained P source for plants whereas inorganic P addition can enhance the risk of P transfer into the environment. Experiments with other acid sulfate soils and field studies should be conducted to assess the effect of inorganic P and organic amendments on soil P pools.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2020.114692.

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# Chapter 5

## Phosphorus pools in acidic acid sulfate soil are influenced by pH, water content and addition of organic matter

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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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# Phosphorus pools in acidic acid sulfate soil are influenced by pH, water content and addition of organic matter

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## Abstract

Purpose

Determine the effect of pH, soil water content and organic matter availability on soil P pools and Fe minerals in acidic acid sulfate soils which are common in paddy rice fields.

Methods

An acidic acid sulfate soil (original pH 3.2 or adjusted to pH 5.5) was amended with mangrove root pieces to achieve an organic carbon additions of 50% or 150% of native soil organic carbon. Then the soil incubated for 12 weeks with with four weeks each in submerged, then moist and then resubmerged conditions. At the end of each four-week period, soil P pools (labile P, moderately labile P, non-labile P and residual P), oxalate extractable Fe/Al and potential phosphate P sorption were measured.

Results

In the submerged periods, addition of mangrove roots decreased the redox potential and increased oxalate-extractable Fe, but only at pH 5.5 indicating that reducing microbes were constrained by the low pH of the original soil. Labile phosphate was increased up to two-fold with mangrove roots compared to the unamended control, with greatest increase at 150% OC and pH 5.5. The increase in labile phosphate was likely due to release of bound P by Fe reduction and of P from mangrove roots. Mangrove root addition enhanced phosphate sorption during the first 8 weeks, suggesting that mangrove roots provided more P binding sites in cell walls.

Conclusion

It can be concluded that addition of organic matter can enhance P availability in acid sulfate soils by providing P and enhancing soil P release.

Key words: Acid sulfate soils, Organic carbon addition, Phosphorus pools, Phosphate sorption, pH increase

## Introduction

In acidic soils, P availability is governed by changes in Fe forms because P is mainly bound to Fe minerals (Torrent et al. 1992). In oxidising conditions, $Fe^{3+}$ precipitates to form Fe-oxides which can then bind P on positively charged sites (Sanyal and De Datta 1991). Oxygen consumption by heterotrophic microbes can lead to reducing conditions when soils are submerged which results in $Fe^{3+}$ oxide reduction, releasing $Fe^{2+}$ as well as P bound to the Fe oxides (Shenker et al. 2005; Todorova et al. 2005). The development of reducing conditions is strongly dependent on availability of organic carbon (OC) because OC is the energy source for heterotrophic reducing microbes (Yi et al. 2012). Another factor influencing redox reactions in soils is pH as most reducing bacteria are inactive at pH < 5 (Berner 1984; Neculita et al. 2007). These processes have been studied in a range of acidic soils, but the role of these processes in acid sulfate soils (ASS) is unclear.

Acid sulfate soils are widespread in coastal areas and inland wetlands as well as in paddy rice fields (Fitzpatrick et al. 2008). Under submerged conditions, sulfate is reduced by heterotrophic bacteria and reacts with Fe to form pyrite. Compared to other soils, microbial reduction and release of P in ASS under submerged conditions can be constrained by low pH and the often low amount of native available OC (Dent, 1986). This was shown in previous studies with acidic ASS where, after submergence, strong reduction only occurred in OC-amended soils and when the pH was increased to >5 (Kölbl et al. 2019; Yuan et al. 2015). Drainage of previously submerged ASS can result in strong pH decrease (often to pH < 4) due to the oxidation of pyrite (Mosley et al. 2014).

An important limitation for plant growth in ASS is P deficiency, likely due to binding of P to Fe oxides. However, the effect of oxidizing and reducing conditions on P pools and Fe minerals in ASS may differ from those in other acidic soils because of the very low pH and the presence of pyrite. Therefore it is critical to understand the factors influencing P availability in ASS in submerged and oxidizing conditions. In our previous studies (Kölbl et al. 2019; Yuan et al. 2015), ASS were amended with mature wheat straw which has a low N and P concentrations. Thus, the main effect of straw amendment was addition of available OC. But the influence of other nutrients such as P that can be added though organic amendments to ASS is not clear.

We aimed to determine the effect of OC addition rate, pH increase, and changes in water content on P pools and phosphate sorption in an acidic ASS. OC was added in the form of mangrove root pieces, as this OC source occurs globally in many coastal ASS areas. We hypothesized that (1) OC addition reduces redox potential and increases soluble P, the effect is greater at high OC addition rate, (2) The OC effect is greater when the pH is increased because microbial activity is constrained by low pH, and (3) OC addition increases potential P sorption because it provides P binding sites.

## Materials and Methods

Sandy acid sulfate soil with sulfuric material was collected in the Barker Inlet, Gillman, South Australia (34°49'47.25"S; 138°32'40.24"E). The area where the soil was collected was covered with mangrove forest before it was drained and then acidified. The soil profile is classified as sulfuric soil according to Australian acid sulfate soil classification (Fitzpatrick 2013) and Hypothionic Gleysol (Drainic, Hypersulfidic) according to the World Reference Base for Soil

Resources. Sulfuric material was taken at a depth of 80-150 cm. This depth was chosen because it is strongly acidic and with little fresh organic matter (Kölbl et al. 2019; Thomas 2011). After drying at 40 °C in a fan-forced oven, the sulfuric material was sieved to < 2 mm. The properties were pH 3.2, sand 89%, silt 7%, clay 4%, total P 0.30 g $kg^{-1}$, maximum water holding capacity (WHC) 140 g $kg^{-1}$, total organic C 19 g $kg^{-1}$ and total Fe 22 g $kg^{-1}$.

Mangrove roots (*Avicennia marina* L.) were collected at a depth of 10-20 cm from a mangrove woodland at the Gillman site (34°48'32.91"S; 138°31'37.24"E). The roots were dried at 40 °C in a fan-forced oven and cut into pieces of 0.5 cm. The roots had following properties: total organic C 414 g $kg^{-1}$, total P 0.62 g $kg^{-1}$, water extractable P 0.30 g $kg^{-1}$, total Fe 1.8 g $kg^{-1}$, oxalate extractable Fe 0.10 g $kg^{-1}$ and oxalate extractable Al 0.12 g $kg^{-1}$.

Experimental design

Soil incubation

Thirty g air-dried soil was placed into 70 mL plastic containers. The mangrove root pieces were mixed into the soil to achieve an organic carbon (OC) addition of 50% or 150% of native SOC. The OC addition rates with mangrove roots were based on Kölbl et al. (2019) who showed that OC additions of $\geq$ 50% of native soil OC (as wheat straw) and adjustment of pH values $\geq$ 5 enhanced microbial reduction reactions in acidic ASS. Such high OC additions may occur in soils as dense root mats. Total OC, P, Fe and oxalate extractable Fe added with mangrove roots (mg $kg^{-1}$) were 284, 14.1, 4.1 and 2.3 with 50% OC and 851, 42.3, 12.3 and 6.8 with 150% OC. Soil pH treatments were original pH or adjusted to 5.5 using 0.5 N $Ca(OH)_2$. There were six treatments with four replicates; 50% OC addition with and without pH adjustment (50+pH and 50-pH), 150% OC addition with and without pH adjustment (150+pH and 150-pH) and unamended controls with and without pH adjustment (UA+pH and UA-pH). The treatments were incubated for 12 weeks consisting of three four-week periods: submerged, then moist, followed by another submerged period to assess the effect of changing redox potentials on soil biochemistry. To initiate the first submerged period, reverse osmosis (RO) water was added so that the soil surface was fully covered by a 1 cm layer of water and the lids of the containers were screwed on tightly to minimise entry of air. The containers were incubated in the dark at 25 °C for 4 weeks. Then the overlying water was removed and the vials were placed in a fan-forced oven at 30 °C. After the soil had dried to its maximum water holding capacity (100% WHC), the vials were removed from the oven and incubated at 25 °C for 4 weeks. During this moist period, 100% WHC was maintained by weighing the vials regularly and adding water if required. In previous studies, we found that 100% WHC provides sufficient moisture and oxygen for pyrite oxidation, whereas oxidation is inhibited at 40% WHC (Jayalath et al. 2016a).

After 4 weeks of moist incubation, RO water was added so that the soil surface was covered with a 1 cm layer of water to start the re-submerged period which was continued for another 4 weeks. Soil pH and redox potential were measured every two weeks by inserting the electrodes about 1 cm into the soil. Destructive sampling was carried out at the end of each period (after 4, 8 and 12 weeks from the start). There were four replicates per treatment and sampling time.

### Phosphate sorption potential of incubated soil

To determine phosphate sorption potential, a 100 mg phosphate $L^{-1}$ stock solution was prepared with $KH_2PO_4$ and diluted with RO water to achieve five phosphate-P concentrations: 0, 5, 10, 20, 40 and 50 mg $L^{-1}$. Soil (0.5 g) was added to 10 mL of each phosphate solution, giving phosphate concentrations of 0, 100, 200, 400, 800 and 1000 mg $kg^{-1}$ (referred to as P100, P200, P400, P800 and P1000). The suspensions were shaken in an end-over-end shaker overnight at room temperature, followed by centrifugation and filtration.

### Analyses

Soil pH was measured in a 1:1 (w/w) soil to water ratio using a calibrated glass electrode. Redox potential was determined by inserting a combination redox probe (Model IJ64, Ionode, Australia) into the soil. Absolute redox potential values were calculated after correction to the standard hydrogen electrode. Maximum WHC (matric potential -10 kPa) was measured according to Wilke (2005). Soil texture was determined by the hydrometer method (Gee and Or 2002). For total Fe in the soil and mangrove roots were digested with aqua regia ($HNO_3$:HCl 1:3). Iron was measured by inductively coupled plasma optical emission spectroscopy (ICP-OES, Agilent, Mulgrave, Australia) (Zarcinas et al. 1996). Total organic carbon in soil and mangrove roots was determined according to Walkley and Black (1934). To determine total P, soil and mangrove roots were digested with nitric acid and hydrochloric acid at a 1:3 ratio. Phosphate-P in the extracts were measured by the phosphovanado molybdate method (Hanson 1950).

Water soluble phosphate P in mangrove roots was extracted with hot water as described in Konieczyński and Wesołowski (2007). One g mangrove roots was shaken for 2 h with 30 ml of hot (85 °C) RO water. After filtration, the extracts were analysed for phosphate according to the malachite-green method (Ohno and Zibilske 1991). Soil phosphate-P pools were determined by the Ivanoff et al. (1998) sequential fraction method. Labile, moderately labile and non-labile P pools were separated by sequential extraction of 1 g soil with 0.5 M $NaHCO_3$ for 16 h for labile P, followed by extraction with 1 M HCl for 4 h for moderately labile P. The final extraction for non-labile P was with 0.5 M NaOH for 16 h. After shaking, centrifugation and filtration, phosphate in the extracts was determined by the malachite green method. Residual P in remaining soil pellet was determined as described for total P. At the end of submerged and re-submerged periods, the overlying water was decanted and analysed for phosphate by the malachite-green method. To allow comparison to P pools in the soil, phosphate in the overlying water is expressed in mg $kg^{-1}$ (phosphate concentration x volume of overlying water/0.03 kg soil). Oxalate extractable Fe/Al and P in mangrove roots and soil were determined according to Schwertmann (1964). Soil or roots (0.4 g dry weight equivalent) was shaken with 40 ml of acid oxalate reagent (0.114 M ammonium oxalate and 0.086 M 165 oxalic acid) in the dark for 4 h at room temperature. Then the samples were centrifuged to obtain a clear extract followed by dilution and acidification with 0.2% v/v nitric acid. Fe, Al and P in the extracts were determined by ICP-AES.

For phosphate sorption, the filtered supernatant was analysed for phosphate-P by the malachite green method. The amount of sorbed phosphate was calculated as the difference in phosphate concentration between the initial solution added and the supernatant.

Data analysis

There were four replicates for each treatment and sampling time. After confirmation of normality by the Shapiro-Wilk test, data were analysed by repeated measures ANOVA with treatments as factors and time as repeated measure in SPSS statistics version 25. For P sorption, data was analysed by two-way ANOVA with P rate amendment and treatment as factors, for each sampling time separately. Differences between means were compared by Tukey's multiple comparison tests at 95% confidence interval using GenStat 15th edition (VSN Int. Ltd, UK). The multiple comparison test was chosen because we wanted to compare all treatments with each other.

## Results

At the end of the submerged period compared to UA-pH, labile P was 40% higher in the 150+pH treatment and about 10-25% higher in the other treatments, except for 50-pH (Fig. 1a). From the end of the submerged period to the end of the moist period, labile P decreased by 90% in soils without OC amendment whereas it decreased about 60% in amended soils. From the end of the moist period to the end of the submerged period, labile P increased two-fold in amended treatments and four-fold in unamended soils, labile P was highest in 150-pH and lowest in the unamended treatments. Only in the 150% OC treatments, labile P was affected by pH, it was about 20% higher in 150+pH than 150-pH.

At the end of the first submergence period compared to UA-pH, non-labile P was about 70% higher in OC amended soils, but 25% lower in UA+pH (Fig. 1b). From the end of the submerged period to the end of the moist period, non-labile P increased by 40% in unamended soils and decreased by 20% in 150+pH, but changed little in the other treatments. At the end of the moist period, non-labile did not differ among treatments. From the end of the moist period to the end of the re-submerged period, non-labile P increased by 25% in 50% OC treatments, but did not change in the other treatments. At the end of the re-submerged period, non-labile P was 20% lower in 150% OC treatments than 50% OC and unamended treatments. Residual P was similar in all treatments and did not change during the experiment (Fig. 1c).

In the submerged periods, redox potential was lower in pH adjusted than unadjusted treatments (Fig. 2a). In week 0 (24 h after OC addition), redox potential was about 30% lower in treatments with pH adjustment than without (Fig.2a). By the end of the submerged period (week 4), redox potential was lowest in OC amended soils with pH adjustment. It was two- to three-fold higher in the other treatments, with highest values in 50-pH and UA-pH. In OC amended soils with pH adjustment, redox potential doubled from week 4 to 6 whereas it changed little in the other treatments. During the moist period (weeks 6 and 8), the redox potential was about 20% higher in UA-pH than in the other treatments. From week 8 to 10, the redox potential decreased by 40% in 150+pH and by 15% and 150-pH, whereas it changed little in the other treatments. After re-submergence (weeks 10 and 12) compared to UA-pH, the redox potential was about 50% lower in 150% OC treatments and 25% lower in 50% OC treatments.

At the end of the submerged and re-submerged periods, phosphate in the overlying water was lower in the unamended controls and 50% OC treatments than in 150% OC treatments (Fig. 2b). After the first submergence, compared to the unamended soils, phosphate in the overlying water was six-fold higher in 150+pH and 50+pH, but 10-fold higher in

150-pH. At the end of re-submergence, in all the treatments except 50+pH, phosphate in overlying water was three to six-fold higher than in the first submergence, with the greatest difference in 150% OC treatments. At the end of the re-submerged period, phosphate in overlying water did not differ among unamended soils and 50% OC treatments. It was six and 12-fold higher in 150+pH and 150-pH than in the unamended soils.

Throughout the experiment, oxalate extractable Fe was similar and low in unamended soils and OC amended soils without pH adjustment (Fig. 3a). At the end of the submerged periods, it was two and five-fold higher in pH adjusted soils with 50% and 150% OC additions. But in these treatments, oxalate extractable Fe was 50% lower in at the end of the moist period than in the submerged periods. Thus, at the end of the moist period, oxalate extractable Fe was higher than the unamended soils only in 150+pH.

Oxalate extractable P differed little among treatments or incubation periods (Fig. 3b). At the end of submerged period, oxalate extractable Al (Fig. 3c) was about 30% higher in treatments with pH adjustment than without. At the end of the moist period, it differed little among treatments. From the end of the submerged period to the end of the moist period, oxalate extractable Al decreased by 20% in 50+pH and 150+pH but increased by 30% in UA-pH, it changed little in the other treatments. From the end of the moist period to the end of the re-submerged period, oxalate extractable Al increased by 50% in 50+pH and did not change in other treatments. At the end of the re-submergence, oxalate extractable Al was about 30% higher in 50+pH than in the unamended soils.

Sorbed phosphate was similar in all treatments at P100 and increased with rate of phosphate addition (Fig. 4). At the end of the submerged period (Fig. 4a), sorbed phosphate increased with phosphate addition rate more strongly in OC amended soils with pH adjustment than without. However in the unamended treatments, sorption increased with phosphate addition rate more strongly in UA-pH than in UA+pH. Compared to P100, sorbed phosphate increased five-fold in 50+pH and UA-pH and seven-fold in 150+pH. Phosphate sorption increased least in UA+pH where it was only two-fold higher at P1000 than at P100.

At the end of the moist period at P200 and P400, sorbed phosphate of OC amended soils was up to two-fold greater in pH adjusted than unadjusted treatments (Fig. 4b). However at higher rates, phosphate sorption of OC amended soils differed little between pH treatments. In the unamended soils on the other hand, phosphate sorption was up to two-fold greater in UA-pH than UA+pH at P400 and above. Compared to P100, phosphate sorption at P1000 was about seven-fold greater in OC amended soils, five-fold greater in UA-pH but only two-fold higher in UA+pH.

Sorbed phosphate at the end of the re-submerged period differed among treatments only at P400 and P1000 (Fig. 4c). At P400, phosphate sorption was lowest in 50+pH and about two-fold higher in the other treatments, phosphate sorption was two-fold higher in 50-pH than 50+pH. At P1000, phosphate sorption was lowest in 150-pH, three-fold higher in UA-pH and up to two-fold higher in the other treatments. Sorbed phosphate was about two-fold higher in 150+pH and UA+pH than in the respective treatments without pH adjustment. At P1000 compared to P100, sorbed phosphate was three-fold higher in 150-pH, about ten-fold higher in UA-pH and about five-fold higher in the other treatments.

## Discussion

This study showed that addition of organic matter in the form of mangrove roots increased labile P and phosphate sorption compared to the unamended control. Root addition also reduced the redox potential in submerged conditions and oxalate-extractable Fe, but only when the pH had been increased to 5.5.

Hypothesis 1 (OC addition reduces redox potential and increases soluble P) can be confirmed, but only for the 150% OC addition rate which led to higher labile P and overlying water P than the unamended soils. This can be explained by two processes, release of P associated with Fe due to reduction of Fe (Kirk 2004; Ponnamperuma 1984) and of from the mangrove roots. When the pH was increased to 5.5, both OC rates reduced the redox potential to a similar extent in the first submerged period. The redox potential in these treatments was about 250 mV which is considered to be too high for Fe reduction at pH 5.5. However, the increased oxalate extractable Fe in 50+pH and 150+pH indicates that Fe reduction which induces conversion of crystalline Fe to amorphous (oxalate extractable) Fe (Chacon et al. 2006). The reduction despite the high measured redox potential can be explained by formation of micro-scale sites with low redox potential around organic matter particles (Stockdale et al. 2009). Iron in roots can not explain the higher oxalate extractable Fe in amended treatments at pH 5.5 because the maximum total Fe added with roots was only 12 mg kg$^{-1}$ which is three orders of magnitude lower than the difference in oxalate extractable Fe between amended and unamended treatments. In the re-submerged period, the redox potential was lower with 150% OC than 50% OC which indicates that the higher OC rate provided a longer lasting OC source for microbes. Only 150% OC increased overlying water P which could be due to the greater P addition with the higher amount of OC and, in the second submerged period, the lower redox potential than with 50% OC.

The lower redox potential in treatments where the pH was increased to 5.5 suggests that microbes in the original soil (pH 3.2) are constrained by the low pH. This is consistent with previous evidence that low pH limits reduction reactions in soils as most reducing bacteria are inactive at pH < 5 (Berner 1984; Neculita et al. 2007). Thus Hypothesis 2 (OC effect is greater when the pH is increased) can be confirmed.

Hypothesis 3 (OC addition effect is greater in submerged than moist conditions) can be confirmed for redox potential and oxalate extractable Fe where differences among treatments were greater in the two submerged periods than the moist period. Labile P differed little among treatments in the first submerged period, and the increase in labile P in amended compared to unamended treatments represented less than 5% of added P. Thus, P release from the added mangrove roots was low. The increase in non-labile P in amended compared to unamended treatments at the end of the first submergence period was greater than the amount of P added with 50% OC and similar to the amount of P added with 150% OC. With 50% OC, the higher non-labile P may be due to conversion of residual P to non-labile P. Conversion of residual P may also have contributed to the higher labile P with 150% OC. Despite the similar labile P in all treatments in the first submerged period, P in the overlying water was low in unamended soils and the 50% OC treatments, however the P concentration in the overlying water was 100-fold lower than that of labile P in the soil. The higher phosphate concentration in the overlying water with 150% OC is likely due to inorganic P released during decomposition of the mangrove roots, but the increase represented less than 0.5% of P added with the roots. The high concentration of labile P and phosphate in the overlying water with mangrove roots could increase plant growth. On the other hand, a high phosphate concentration in the overlying water may be undesirable because it could lead to eutrophication in surrounding water bodies (McDowell et al. 2020).

Labile P was higher in the first submergence period than later. This indicates that the higher redox potential after drying to 100% WHC lead to stronger binding of P likely because Fe-oxides are formed under aerobic conditions and subsequent sorption of phosphate on newly formed Fe-oxides (Sah et al., 1989). This which was not completely overcome in the following re-submergence period despite the low redox potential in the OC amended treatments at pH 5.5. At the end of the moist period, the unamended soil contained very little labile P, thus the main source of labile P in OC amended treatments was likely the added mangrove roots that were mineralised.

Mangrove root addition may have also increased labile P indirectly by two mechanisms related to organic acids released during decomposition of the roots which can exchange bound P by anion exchange (Hue 1991) and by binding to potential P binding sites (Johnson and Loeppert, 2006). The latter mechanism would reduce potential P binding which occurred after the second submergence period, but not after the first.

Phosphate sorption differed among amendment treatments only at the higher P addition rates. This indicates that at the lower P addition rates, soil particles in the unamended soil provided sufficient sorption sites. Only at higher P rates, the additional P sorption sites provided by the mangrove roots became apparent. After the first submergence, maximum P sorption was greater in OC amended than unamended soils which can be explained by binding to root cell walls and to Fe oxides on the roots (Reef et al. 2010). The lack of effect of OC addition in the re-submerged period could be due to decomposition of roots in the previous periods which reduced the number of potential P binding sites in cell walls (Richardson et al. 2011). The higher P sorption in OC amended soils at pH 5.5 compared to the original pH is likely due to the higher oxalate extractable Fe as a result of the lower redox potential during the submerged periods. Oxalate extractable Fe indicates amorphous Fe which has a high P binding capacity (Sample et al. 1980). In the unamended treatments on the other hand, phosphate sorption was greater at pH 3.2 than pH 5.5 at the end of the first submergence and the moist period. This may be due to the enhanced electrostatic repulsion caused by increased negative surface charge or competition of OH- with added phosphate ions for binding sites (Haynes 1982; Sato and Comerford 2005).

## Conclusion

This study showed that mangrove root amendment supplied not only organic C, but also P to the ASS and thus improve P availability to plants. Compared to unamended soil, decomposition of mangrove roots in submerged conditions resulted in a lower redox potential which enhanced release of P bound to Fe-oxides in the soil as well as of P from mangrove roots. On the other hand, mangrove root addition increased P sorption in the first 8 weeks which may limit P loss into the surrounding ecosystem. Particularly with 150% of native OC addition, the effect on redox potential was greater with pH increase to 5.5, suggesting that microbial activity is limited by the low pH of the original soil (pH 3.2). The results suggest that decomposing roots play an important role for redox conditions and P availability in mangrove areas subjected to changes in water content.

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Fig. 1. Labile phosphate P (a), non-labile phosphate P (b) and residual phosphate P (c) at the end of the submerged, moist and re-submerged periods in unamended soil (UA) or soil with 50 or 150% OC at pH adjusted to 5.5 (+pH) or original pH 3.2 (-pH). Different upper case letters indicate significant differences between treatments at each sampling date. Different lower case letters show significant differences in a treatment over time (n = 4, means ± SE, $P \leq 0.05$).

Fig. 2. Redox potential (a) at the end of the submerged, moist and re-submerged periods and phosphate P in the overlying water (b) at the end of the submerged and re-submerged periods in unamended soil (UA) or in soil with 50 or 150% OC at pH adjusted to 5.5 (+pH) or original pH 3.2 (-pH). Different upper case letters indicate significant differences between treatments at each sampling date. Different lower case letters show significant differences in a treatment over time (n = 4, means ± SE, $P \leq 0.05$).

Fig. 3. Oxalate extractable Fe (a), P (b), and Al (c) at the end of the submerged, moist and re-submerged periods in unamended soil (UA) or in soil with 50 or 150% OC at pH adjusted to 5.5 (+pH) or original pH 3.2 (-pH) Different upper case letters indicate significant differences between treatments at each sampling date. Different lower case letters show significant differences in a treatment over time (n = 4, means ± SE, $P \leq 0.05$).

Fig 4. Phosphate sorption at the end of the submerged, moist and re-submerged periods in unamended soil (UA) or in soil with 50 or 150% OC at pH adjusted to 5.5 (+pH) or original pH 3.2 (-pH). Different upper case letters indicate significant differences between treatments (n = 4, means ± SE, $P \leq 0.05$).

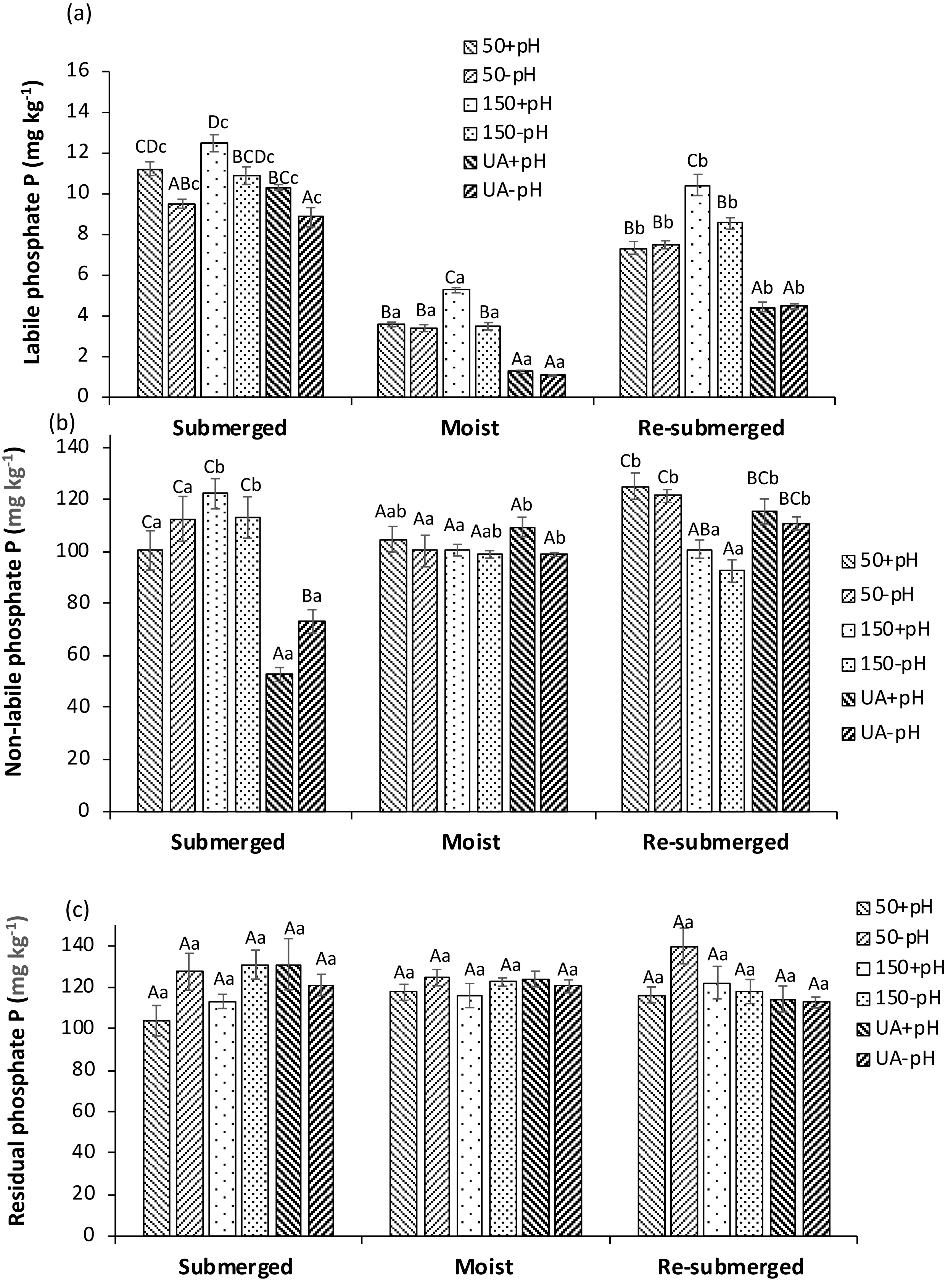
(a)
Labile phosphate P (mg kg$^{-1}$)
0
2
4
6
8
10
12
14
16
▧ 50+pH
▨ 50-pH
☐ 150+pH
⬚ 150-pH
▧ UA+pH
▨ UA-pH
CDc
ABc
Dc
BCDc
BCc
Ac
Ba
Ba
Ca
Ba
Aa
Aa
Bb
Bb
Cb
Bb
Ab
Ab
Submerged
Moist
Re-submerged
(b)
Non-labile phosphate P (mg kg$^{-1}$)
0
20
40
60
80
100
120
140
Ca
Ca
Cb
Cb
Aa
Ba
Aab
Aa
Aa
Aab
Ab
Ab
Cb
Cb
ABa
Aa
BCb
BCb
▧ 50+pH
▨ 50-pH
☐ 150+pH
⬚ 150-pH
▧ UA+pH
▨ UA-pH
Submerged
Moist
Re-submerged
(c)
Residual phosphate P (mg kg$^{-1}$)
0
20
40
60
80
100
120
140
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
Aa
▧ 50+pH
▨ 50-pH
☐ 150+pH
⬚ 150-pH
▧ UA+pH
▨ UA-pH
Submerged
Moist
Re-submerged

Fig. 1.

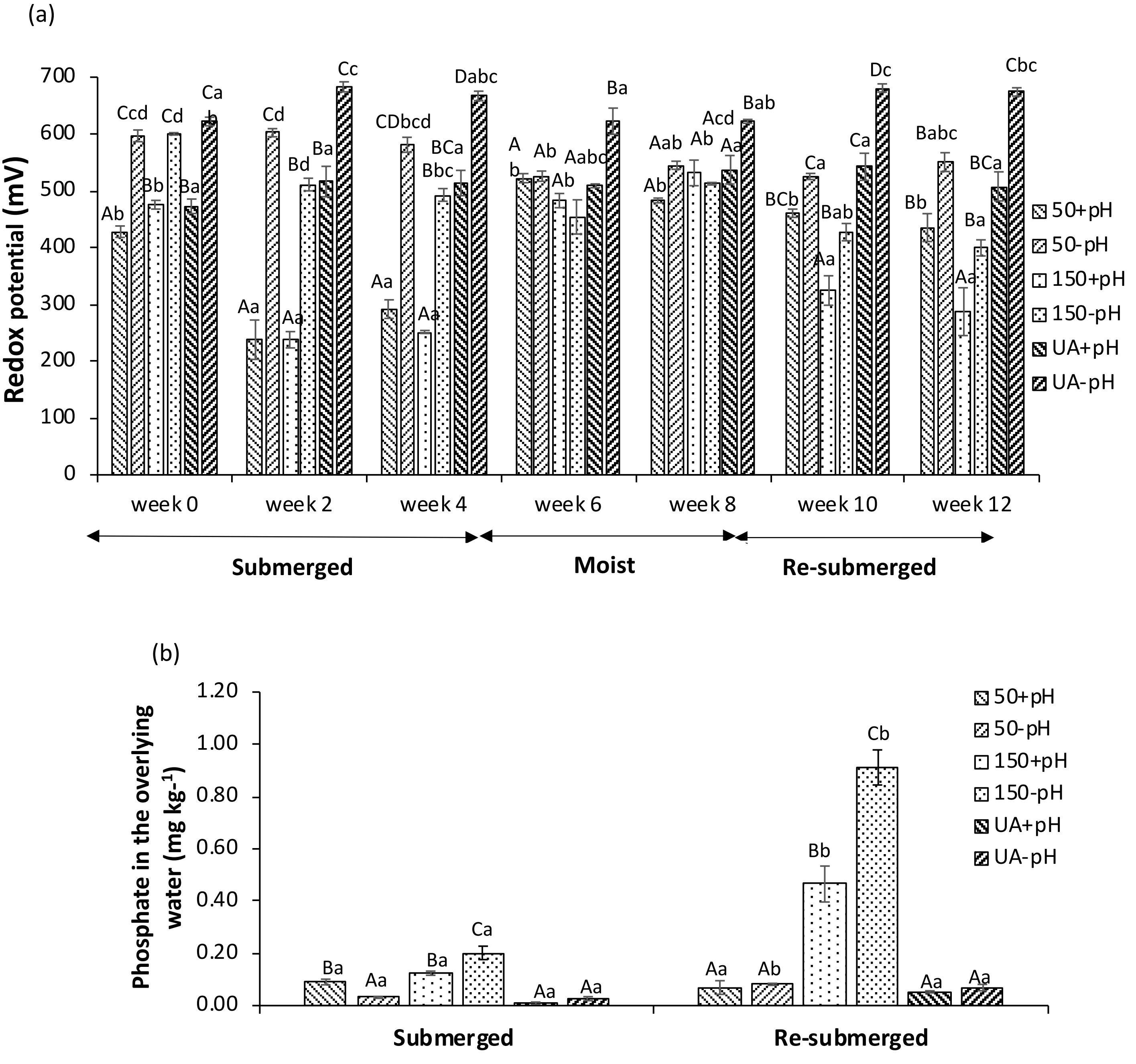
(a)
Redox potential (mV)
0
100
200
300
400
500
600
700
Ab
Ccd
Bb
Cd
Ba
Ca
b
Aa
Cd
Aa
Bd
Ba
Cc
Aa
CDbcd
Aa
Bbc
BCa
Dabc
A
b
Ab
Ab
Aabc
Ba
Ab
Aab
Ab
Acd
Aa
Bab
BCb
Ca
Aa
Bab
Ca
Dc
Bb
Babc
Aa
Ba
BCa
Cbc
week 0
week 2
week 4
week 6
week 8
week 10
week 12
Submerged
Moist
Re-submerged
▧ 50+pH
▨ 50-pH
☐ 150+pH
▩ 150-pH
▧ UA+pH
▨ UA-pH
(b)
Phosphate in the overlying water (mg kg-1)
0.00
0.20
0.40
0.60
0.80
1.00
1.20
Ba
Aa
Ba
Ca
Aa
Aa
Aa
Ab
Bb
Cb
Aa
Aa
Submerged
Re-submerged
▧ 50+pH
▨ 50-pH
☐ 150+pH
▩ 150-pH
▧ UA+pH
▨ UA-pH

Fig. 2.

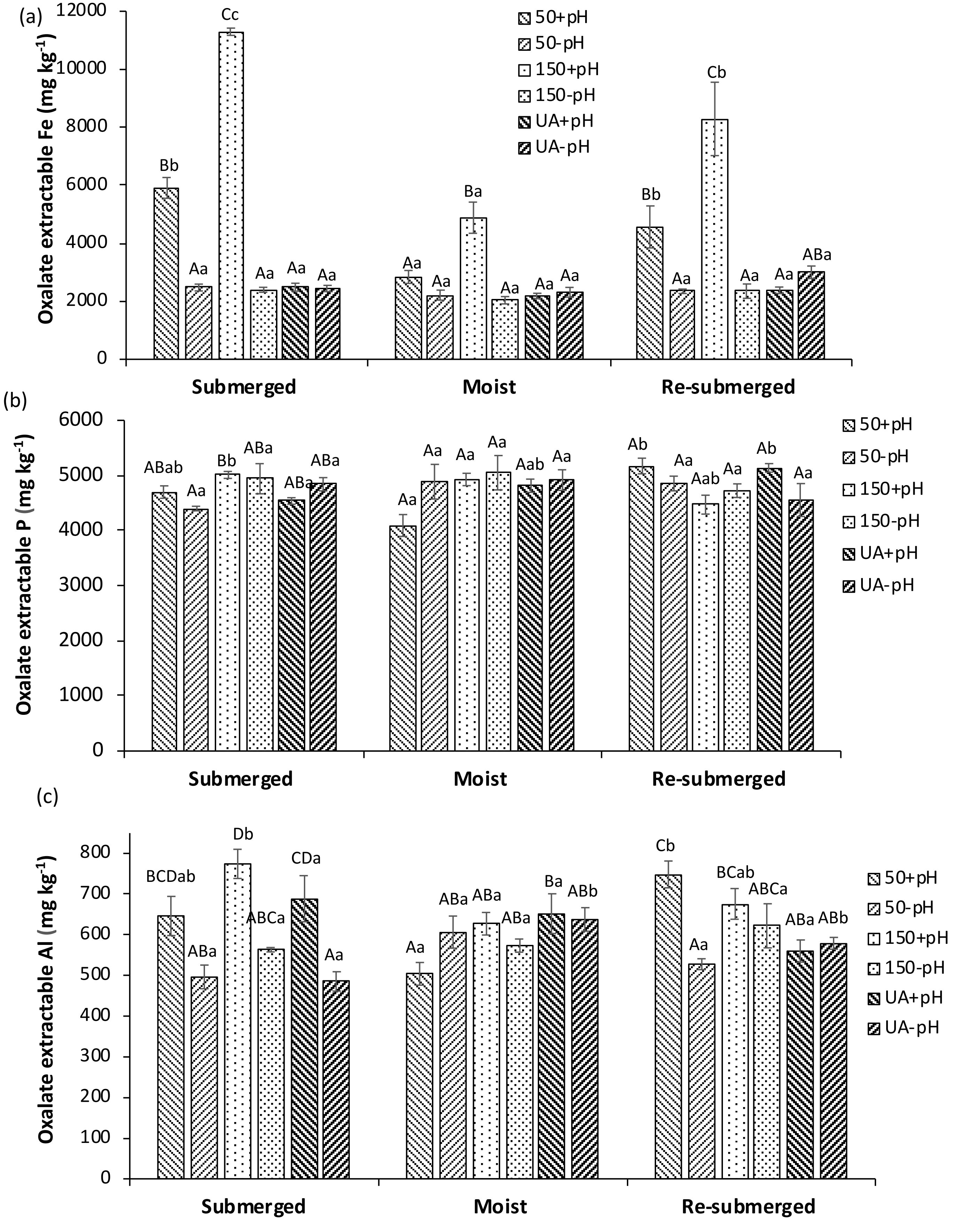
(a)
Oxalate extractable Fe (mg kg$^{-1}$)
12000
10000
8000
6000
4000
2000
0
Bb
Aa
Cc
Aa
Aa
Aa
Aa
Aa
Ba
Aa
Aa
Aa
Bb
Aa
Cb
Aa
Aa
ABa
Submerged
Moist
Re-submerged
▧ 50+pH
▨ 50-pH
▦ 150+pH
▦ 150-pH
▧ UA+pH
▨ UA-pH
(b)
Oxalate extractable P (mg kg$^{-1}$)
6000
5000
4000
3000
2000
1000
0
ABab
Aa
Bb
ABa
ABa
ABa
Aa
Aa
Aa
Aa
Aab
Aa
Ab
Aa
Aab
Aa
Ab
Aa
Submerged
Moist
Re-submerged
▧ 50+pH
▨ 50-pH
▦ 150+pH
▦ 150-pH
▧ UA+pH
▨ UA-pH
(c)
Oxalate extractable Al (mg kg$^{-1}$)
800
700
600
500
400
300
200
100
0
BCDab
ABa
Db
ABCa
CDa
Aa
Aa
ABa
ABa
ABa
Ba
ABb
Cb
Aa
BCab
ABCa
ABa
ABb
Submerged
Moist
Re-submerged
▧ 50+pH
▨ 50-pH
▦ 150+pH
▦ 150-pH
▧ UA+pH
▨ UA-pH

Fig. 3.

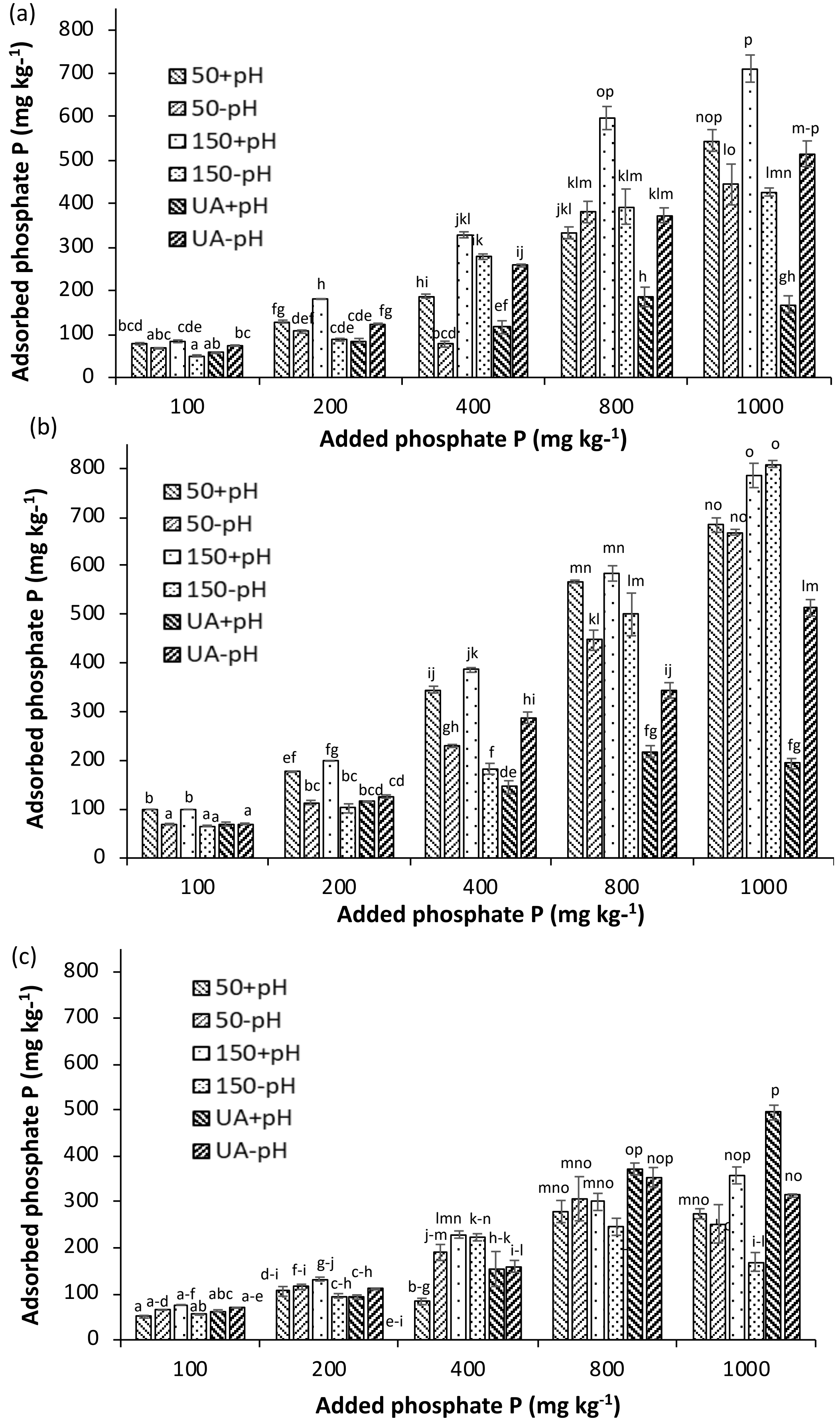
(a)
Adsorbed phosphate P (mg kg$^{-1}$)
800
700
600
500
400
300
200
100
0
▧ 50+pH
▨ 50-pH
☐ 150+pH
▦ 150-pH
▧ UA+pH
▨ UA-pH
bcd
abc
cde
a
ab
bc
fg
def
h
cde
cde
fg
hi
bcd
jkl
k
ef
ij
jkl
klm
op
klm
h
klm
nop
lo
p
lmn
gh
m-p
100
200
400
800
1000
Added phosphate P (mg kg$^{-1}$)
(b)
Adsorbed phosphate P (mg kg$^{-1}$)
800
700
600
500
400
300
200
100
0
▧ 50+pH
▨ 50-pH
☐ 150+pH
▦ 150-pH
▧ UA+pH
▨ UA-pH
b
a
b
aa
a
ef
bc
fg
bc
bcd
cd
ij
gh
jk
f
de
hi
mn
kl
mn
lm
fg
ij
no
no
o
o
fg
lm
100
200
400
800
1000
Added phosphate P (mg kg$^{-1}$)
(c)
Adsorbed phosphate P (mg kg$^{-1}$)
800
700
600
500
400
300
200
100
0
▧ 50+pH
▨ 50-pH
☐ 150+pH
▦ 150-pH
▧ UA+pH
▨ UA-pH
a
a-d
a-f
ab
abc
a-e
d-i
f-i
g-j
c-h
c-h
e-i
b-g
j-m
lmn
k-n
h-k
i-l
mno
mno
mno
op
nop
mno
nop
i-l
p
no
100
200
400
800
1000
Added phosphate P (mg kg$^{-1}$)

Fig 4:

# Chapter 6

## Conclusions and future research

## 6.1 Conclusion

Large areas of ASS are used for cropping, e.g. paddy rice cultivation where soils are exposed to changes in soil water content. Further, remediation of ASS by utilisation of plants has been investigated (Gardner et al., 2018). To enhance crop growth, ASS may be limed or amended with fertilisers. However, little is known about the effect of these factors on P availability in ASS. Studies in acidic non-ASS showed that P availability is influenced by soil pH, aeration and P addition (Chien et al., 1996; Scholz, 2015; Willard, 1979). In this project, the effects of these factors on P availability and P pools were assessed in a sandy acidic ASS (pH<4). The results of this study can help land managers to improve crop P uptake in ASS.

In the experiment described in Chapter 2 (Mayakaduwage et al., 2019), the soil was amended with two neutralizing agents (NaOH or $Ca(OH)_2$) to achieve soil pH 4 or 5.5, without or with addition of inorganic P equivalent to 20 kg $ha^{-1}$. The soil was incubated for two weeks at 25 °C in either submerged or moist conditions (100% of maximum water holding capacity). Adjustment of pH had little effect on the measured parameters, but addition of P increased all measured P pools. With P addition, labile and non-labile P pools were up to two-fold higher in submerged incubation than moist incubation, likely due to release of P through solubilisation of Fe oxides at low redox potential (Sallade and Sims, 1997). Labile P represented 70% and 40% of measured P under submerged and moist incubation, respectively. It can be concluded that a high proportion of added P remained available, particularly under submerged conditions.

However, the fate of added P is likely to be affected by the rate at which P is added which was investigated in the experiment described in Chapter 3 (Mayakaduwage et al., 2020a). The soil pH was adjusted to 5.5. Then the soil was amended with phosphate at 0-770 mg P $kg^{-1}$ and incubated under submerged conditions. Soil phosphate pools were measured after two and four weeks. Phosphate sorption and its release were determined in incubated soils and isolated jarosite. Labile phosphate was higher than the control (without phosphate addition) at phosphate rates above 350 mg P $kg^{-1}$ and was more than 50% of added phosphate. Hence, maximum phosphate sorption of the soil was about 350 mg phosphate $kg^{-1}$ which was similar to the maximum phosphate sorption of isolated jarosite. Below the threshold of 350 mg phosphate $kg^{-1}$, absorbed phosphate was strongly bound to the soil whereas above the threshold 50% of sorbed phosphate could be released by shaking with water. The high phosphate binding capacity of jarosite confirmed the importance of Fe oxyhydroxy sulfate minerals in P binding in acid soils. The lack of change in labile P from two to four weeks indicated that most P was bound to Fe oxides within 2 weeks. The threshold of labile P is high compared to usual P fertiliser addition rates, but repeated P additions could gradually reach this threshold by saturating P binding sites.

In the field, ASS may be exposed to repeated wetting and drying cycles which may affect P availability and binding. Further, in the previous experiments, P was added as inorganic soluble P, but farmers or environmental managers may also add P in form of organic amendments which could affect the fate of P. These knowledge gaps were addressed in Chapter 4 which aimed at determining the effect of changes in soil water content and form of P added on P availability and P pools. The soil (pH adjusted to 5.5) was unamended or amended with 400 mg P kg$^{-1}$ as inorganic P (100F), manure (100M) or as 50% inorganic P + 50% manure (50M/50F). The soil was submerged for two weeks, then the overlying water was removed and the soil dried to maximum water-holding capacity. Then soil was incubated for four weeks at this water content followed by another submergence for two weeks. In the unamended control, P pools were very low and not affected by soil water content. At the end of the first submergence, labile phosphate and oxalate extractable P were two-fold higher in 100F than in 100M and 50M/50F, likely because soluble P was added in 100F. In the following moist incubation and after the second submergence, labile phosphate remained unchanged in 100M, but halved in 100F. This suggests that previously released P was adsorbed to Fe minerals formed under oxidizing condition which was counteracted by sustained P release by manure. This P release may be due to direct release of inorganic P from the manure, mineralisation of organic P, the lower redox potential which increased solubility of Fe minerals releasing P, or by organic acid anions produced during decomposition replacing bound phosphate (Bril and Salomons, 1990; Hue, 1991; Johnson and Loeppert, 2006). The latter two mechanisms are indicated by the lower oxalate extractable P in manure treatments after re-submergence compared to 100F. It can be concluded that the initially high labile P with inorganic P addition could pose an environmental threat by causing eutrophication.

The low phosphate in the overlying water with manure suggests that the threat of P transfer into the surrounding environment is low.

Manure or inorganic P may be added to ASS in some situations, such as in agricultural areas. However, many ASS are formed in mangrove wetlands and therefore contain mangrove roots which could P influence availability and P pools. Soil pH can also influence soil P pools and P binding capacity (Devau et al., 2009). In most previous experiments in this thesis, the pH of the acidic ASS was increased to pH 5.5 which did not allow to study the effect of pH on P pools. The experiment described in Chapter 5 addressed these points by amending the soil, left at original pH 3.2 or adjusted to pH 5.5, with mangrove root pieces to achieve organic carbon additions of 50% or 150% of native soil organic carbon. Then the soil was incubated for four-week periods each in submerged, then moist and then resubmerged conditions. At the end of each four-week period, soil P pools, oxalate extractable Fe/Al and potential phosphate sorption were measured. Reducing microbes were apparently constrained by the low pH of the original soil because addition of mangrove roots decreased the redox potential and increased oxalate-extractable Fe in the submerged periods only in treatments adjusted to pH 5.5. The higher labile phosphate with mangrove roots was likely due to release of bound P after Fe reduction and of P from mangrove roots. Mangrove root addition enhanced phosphate sorption during the first 8 weeks, suggesting that mangrove roots provided more P binding sites in cell walls and associated Fe oxides. However after re-submergence, phosphate sorption was not affected by organic carbon addition indicating the loss of binding sites due to decomposition.

## 6.2 Future research

The experiments in this thesis provided new information about the effect of different factors such as soil pH, addition of P and soil water content on P availability in ASS. This information could be used to improve management of P availability for crops grown in ASS, e.g. rice, or for revegetation. However, this study has also revealed research gaps which could be addressed by future studies.

In this thesis, the fate of added P could only be assessed by comparing P amended with unamended soil. However, P addition may have also induced changes in native soil P which could not be detected in the experiments described here. The fate of added P could be determined by using $^{32}P$ labelled inorganic P and measuring $^{32}P$ in the different P pools (Di et al., 1997).

In this PhD project, P pools were measured by extraction with different solutions. But the extractions may not have detected the P pools completely and some P pools, e.g. organic P were not assessed. In-depth information about inorganic and organic P pools could be obtained by synchrotron K-edge X-ray absorption near-edge structure (XANES) (Andersson et al., 2019) for inorganic P forms and $^{31}P$ NMR for organic P pools (Doolette et al., 2017).

The role of microbes in the experiments could not be determined. To assess this, community composition and activity of microbes involved in P mobilisation by organic P decomposition, P solubilisation and Fe reduction could be determined by using metagenomic sequencing and real-time PCR of phosphatase, Fe reductase genes and genes involved in organic acid release (Chhabra et al., 2013; Huang et al., 2016; Saleh-Lakha et al., 2005).

The experiments were conducted using an acidic sandy ASS. However, the texture of ASS can vary widely, ranging from sandy to clayey soils. Texture is likely to affect P binding as clay has a higher P binding capacity than sand (Ge et al., 2019). Not all ASS become highly acidic when exposed to oxygen because they have a high pH buffer capacity or low pyrite content (Fitzpatrick et al., 2011; Fitzpatrick et al., 2009). This may also affect the fate of added P. Therefore, future experiments should be conducted using ASS that vary in texture and pH to ensure that the results are applicable to a range of different ASS.

Incubation times in submerged or moist conditions in this project ranged from two to four weeks. This length was chosen because P pools and redox potential seem to stabilize within about two weeks. However, the redox potential in submerged conditions remained quite high, possibly due to relatively thin water film which allowed some $O_2$ diffusion into the soil. A further reduction of the redox potential is likely to increase solubility of Fe minerals and therefore release of bound P. In the field, ASS may remain submerged for several months and the redox potential can reach levels sufficient for sulfate reduction, particularly in presence of available organic C (Berner, 1984; Johnston et al., 2009). To better understand P pools under such conditions, future experiments should include longer incubation periods. In submerged conditions, a low final redox potential could be reached by addition of decomposable organic matter and/or by incubation in $O_2$ depleted atmosphere (Kölbl et al., 2019).

Laboratory incubation experiments can help understand mechanisms and processes. The field situation differs in a number of points from the laboratory studies in this thesis. In the field, pH adjustment is difficult and may be confined to the top soil. Further, added P or organic matter may not be uniformly distributed in the soil. These factors are likely to reduce the effect of the amendments on soil P pools and P availability to crops. Therefore field experiments are needed to provide landholders with the information required to manage their ASS.

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