Accumulation and depletion of soil Phosphorus pools

A Thesis submitted to the University of Adelaide in fulfilment

of the requirement for the degree of

Doctor of Philosophy

Khuyen Thi Kim Hoang

School of Agriculture, Food and Wine

The University of Adelaide

February 2020

Dedicated to my family

Table of contents

Acknowledgements

I would like to express my sincere gratitude to my principal supervisor, Professor Petra Marschner who has guided, supported and encouraged me with valuable feedback throughout my PhD studies. I am greatly indebted to her for her endless efforts in introducing me to a marvellous world of soil science and helping me by reading and correcting the manuscripts as well as providing feedback. I also would like to thank my co-supervisor, Dr Ashlea Doolette for her valuable comments and support.

Special thanks to Colin Rivers for his technical assistance in the lab, Dr Sean Mason for his help in collection Birchip Soil and Garry Smith for sludge sampling. I extend my thanks to the members of Petra's group, including Dr. Trung Ta Nguyen, Dr Tan Dang, Kehinde Olajide Erinle, Thi Hoang Ha Truong, Yanchen Yang, Thi Huong Xuan Le, Sonia Mayakaduwage, Thi Thanh Hue Ngo and Mihiri Seneviratne for their advice and support during my experiments.

My deep gratitude to my parents and brothers and sisters for their encouragement, support and constant encouragement. Finally my deepest gratitude to my daughters (Mia and Amy) for their endless love and inspiration during my PhD study.

Above all, I am extremely grateful to Vietnamese Government for my PhD scholarship to study at the University of Adelaide.

Abstract

In this thesis, three experiments were carried out to assess the effect of straw or sewage sludge on soil P pools and plant P uptake.

To assess the impact of straw and inorganic P amendment on P pools and wheat growth, barley straw (C/P 255) was added to a loamy sand alone or with inorganic P to reduce the C/P ratio to 127 or 25 (straw treatments). Other treatments included inorganic P alone at the same rates as in the straw treatments. P pools increased with P addition rate and were higher with inorganic P alone than straw treatments except MBP which was greater in the straw treatments. Wheat growth for 5 weeks reduced HCl P, phosphatase P, citrate P and $CaCl_2$ P. Barley straw was added into another set and after three weeks incubation. Straw addition also reduced HCl P and citrate P but increased phosphatase P, $CaCl_2$ P and MBP. It can be concluded that P pools are affected mainly by P addition rate whereas the form in which P is added is less important.

To assess the effect of longer term P fertilisation on P pools and the influence of straw addition and plant growth, two experiments were carried out with silt loam which had been amended with 0, 10 and 20 kg P ha^{-1} a^{-1} for 7 years (referred to 0P, 10P and 20P). In the first experiment, soil was incubated for 4 months without or with 5 g kg^{-1} barley straw (C/P 255). After 4 weeks of wheat growth in 10P and 20P, citrate P was 25% higher and HCl P 25% lower than before planting. Plant P uptake was higher with straw which indicates that microbial biomass P is an important P source for plants. In the second experiment, wheat was grown for 5, 10 and 15 weeks in unamended soils from the field trial. After 10 and 15 weeks, HCl P and citrate P in 10P and 20P were reduced by 20% compared to the start.

In the third experiment, a loamy soil was amended with the same amount of N and P (43 mg P kg^{-1} soil and 60 mg N kg^{-1} soil) as sludge and inorganic nutrients. Sludge or inorganic N and P were added either alone (100S and 100F), or 75% S+25% F, 50% S+50% F and 25% S+75% F. Before planting, HCl and citrate P was about four-fold higher in amended treatments than the control. Leachate N increased with proportion of F and leachate P with >50% sludge was higher than in the control. After 5 weeks of growth compared to the control, shoot P uptake was about 50% higher in 100S and 75S25F and three-fold higher in treatments with ≥50% F. HCl P in amended treatments was lowest in 100F. Citrate P was higher in treatments with

≥50% sludge than in 100F. Leachate inorganic P was lowest in 100S and increased with proportion of F. Available N and leachate inorganic N were very low after harvest.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I acknowledge that copyright of published works contained within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Khuyen Thi Kim Hoang

24 February 2020

Structure of this thesis

The thesis is organised into 5 chapters and is presented as a combination of papers that have been published and unpublished work written in a manuscript style. This chapter also includes the proposed objectives of the research presented in this thesis

Chapter 1 provides an overview of the literature review on the characteristic of P and transformation of soil P pools

Chapter 2 comprises a paper published in the *Journal of Soil Science and Plant Nutrition.* It describes the effect of soil amendments on P pools and the depletion by plants and microbes.

Chapter 3 describes a paper published in the *Journal of Soil Science and Plant Nutrition*. It consists of the effect of long-term P fertilisation and the influence of straw addition and plant growth on soil P pools.

Chapter 4 comprises an unpublished work using sewage sludge combined with inorganic P and N fertiliser to access their effect on soil P pools, P leaching and plant P uptake.

Chapter 5 contains general conclusions from all chapters and recommendation for future research.

Chapter 1

Introduction and literature review

1. Introduction and literature review

1.1 Introduction

Phosphorus (P) is an essential nutrient for plant growth and influences the productivity and health of terrestrial and aquatic ecosystems (Condron & Schjonning, 2004). Phosphorus (P) is a structural component of the nucleic acids, coenzymes, phosphoproteins, ATP and phospholipids. In general, P deficiency stimulates root growth, particularly the development of lateral or fibrous roots (Gahoonia & Nielsen, 2004). At low P supply, plants can be stunted, thin stemmed and spindly with dark or bluish-green leaves (Sharpley & Tunney, 2000). Since a large proportion of P in soil is poorly available to plants (Holford, 1997), P deficiency is one of the main constraints to crop growth and production (Vance et al., 2003). Therefore, it is often necessary to apply soluble sources of P, for example fertilizers, to soils in order to increase the P available to plants. However, following its application P often rapidly undergoes fixation, sorption and complexation within the soil and transformation into the organic pool (Richardson, 2001, Schachtman et al., 1998) rendering it unavailable for plant uptake. Therefore, P fertilizer addition often has to be higher than crop demand (George et al., 2016) and consequently results in high fertilizer costs for the grower/farmer because large amounts of fertilizer need to be applied. Furthermore, this can also become problematic when the applied P is not taken up by the plant. Phosphorus may be leached or removed in runoff and can result in eutrophication of waterways (Frossard et al., 2000).

Economic and environmental risks associated with the overuse of P fertilisers have led to use of alternative P sources such as manures, crop residues and compost. Organic soil amendments can directly increase P available for plant uptake in soil through P contained in the material. Soil organic amendments can also indirectly increase in soil P availability by

increasing P mobilisation and decreasing P fixation (Ayaga et al., 2006, Khan et al., 2009, Khan & Joergensen, 2009) through the production and release of organic acids during decomposition, stimulation of microbial activity or blocking of P fixing sites on soil particles. Organic acids released from crop residues or organic matter can increase P concentration by competing for P sorption sites (Iyamuremye et al., 1996) and chelation of Al and Fe in the soil solution which in turn reduces the precipitation of P (Fox & Comerford, 1990). Organic materials such as plant residues with a C/P ratio less than 300 are likely to induce net P mineralisation, while a C/P ratio >300 may induce P immobilisation (Brady & Weil, 2002, Alamgir et al., 2012). It has been shown that crop residues are an important source of P for crops, but little is known about how addition of residues and inorganic P affects P pools and P uptake in wheat.

This literature review will discuss P cycling in relation to the addition of inorganic P and organic amendments and plant P uptake.

1.2. Role of P in plants and P transport in plants

Phosphorus plays an essential role in plant cells and P-deficiency results in a spindly habit, acute leaf angles, suppression of tillering, prolonged dormancy, early senescence and decreased size or number of flowers and buds (Marschner & Rengel, 2012, Bergman, 1992). The appearance of dark green or blue-green older leaves is one of the first symptoms of P deficiency. Under P deficiency, sucrose and starch accumulate in leaves which reduces the expression of many genes involved in photosynthesis and decreases photosynthesis (Martin et al., 2002). Anthocyanin is produced due to the high concentration of leaf sucrose and results in development of red, purple or brown pigments in leaves and along veins (Amtmann et al., 2005, Müller et al., 2005). The accumulation of anthocyanin is thought to protect

nucleic acids from UV damage and prevent chloroplasts from photo-inhibitory damage (Hoch et al., 2001).

Phosphorus availability often limits plant growth in both natural and agricultural systems (Vance et al., 2003, Güsewell, 2004). Plant roots take up inorganic phosphate (Pi) as $H_2PO_4^-$ and HPO_4^{2-} to a minimum P concentration of 10 µM (Schachtman et al., 1998) and plant available Pi in the rhizosphere soil solution is rapidly depleted due to the slow replenishment of Pi in the rhizosphere soil solution (Barber, 1995). Soil solution concentrations are low because of sorption of P to binding sites on soil particles, formation of poorly soluble P salts and complex organic P forms have to be solubilized or mineralized to release Pi available for plant uptake. The main pathways by which nutrients are delivered to the plant root are mass flow and diffusion. Mass flow, the convective flow of water to plant roots, accounts for less than 5% of the P plant demand (Lambers & Chapin III, 1998) with the remainder of P delivery through diffusion (Jungk, 1994; Olsen et al., 1962; Barber, 1962). Diffusion depends on the Pi concentration gradient between the bulk soil and root surface, which is created by Pi uptake by plant roots. When plant roots remove Pi from the soil solution, a zone of P depletion zone is formed around the plant root, and a concentration gradient develops between the zone of depletion and the bulk soil (Syers et al., 2008) resulting in the net movement of P towards the plant root. However, the delivery of Pi by diffusion is too slow to meet plant demand which results in depletion of P in the rhizosphere. The rate of diffusion is related to the effective diffusion coefficient which is controlled by soil water content and depends on soil buffer capacity (Bhadoria et al., 1991). The extent of the depletion zone also depends on soil water content. Gahoonia et al. (1994) showed that the extension of the depletion zone was smaller, 0.1cm with 14 vol % compared to 0.2cm with 20 vol %.

Many plant species in P-limited environments have a number of adaptive features which enhance the acquisition of P from soil (Vance et al., 2003) for example changes in cellular metabolism and root morphology. Root morphology can be modified to explore the soil volume effectively and exploit localized patches of high Pi availability (Vance et al., 2003, Raghothama & Karthikeyan, 2005). Further, roots can increase P availability by acidification of the rhizosphere, exudation of organic anions and phosphatases or mycorrhizal associations (Ryan et al., 2001).

1.3. Forms of P in soil

Total P concentration in soils is high, from 200 to 3000 mg P kg^{-1} with older soils containing lower P and younger soils containing higher amounts of P (Tiessen, 2008, Harrison, 1987). However, only a small proportion of P is in soil solution which is immediately available to plants. Soil solution P concentration is commonly 1 µM (Richardson et al., 1994) but can be up to 5 µM (Conyers & Moody, 2009). Soil P can be divided into two groups, organic P (Po, from 20% to 80% total P) and inorganic P (Pi, 35% to 70% total P) (Brady & Weil, 2002, Harrison, 1987). The predominant forms of Pi in acid soils are Fe or Al phosphates, in alkaline soils Ca phosphates account for the main proportion. The main forms of P_o are mono and di-ester orthophosphates (e.g. phytate, DNA, RNA) and soil microbial biomass (Turner et al., 2002a). A large proportion of the P in soils has been found to exist in an organic form-the similar form to that found in living organisms (Potter & Benton, 1916) although fluxes of P through soil organic matter remain unclear since only a small amount of soil organic P has been identified as biomolecules of low molecular weight (e.g inositol hexakisphosphates) (McLaren et al., 2015b, Condron et al., 2005). Phospholipid and nucleic acids which are the most abundant forms of organic P in living cells are quickly degraded by microbes into more

persistent forms- inositol phosphates- the stable pool of soil organic P (Turner et al., 2002b). Phytate (*myo* stereoisomer of inositol hexakisphosphate), which is mainly found in the seeds of plants, accumulate in soil as the ability to bind to soil particles (Turner et al., 2002b). The component of soil organic P is large and complicated which requires a more novel technique such as ^{31}P NMR spectra of soil extracts (Vincent et al., 2012, Doolette et al., 2010, Doolette et al., 2011b).

The mobility and behaviour of Pi and Po vary and depend mainly on soil properties (soil pH, redox potential, soil moisture, temperature, physical chemical and surface properties (Shen et al., 2011)) and environmental conditions such as rainfall. For example P can be lost from soil by runoff, leaching or erosion (Sharpley et al., 2000). Seasonal changes in P fractions may provide some information on their bioavalability (Chen et al., 2003). Some studies of Perrott et al. (1990), Carelli et al. (2000) have found that the level of $NaHCO_3$-P_o in grassland and forest soils were greater but that of inorganic P fractions (bicarbonate extractable Pi and total Pi) were lower during autumn-winter than spring-summer which indicates that mineralization of $NaHCO_3$-P_o has been enhanced to meet the plant P demand in spring and summer while the accumulation of labile Po was increased by organic inputs and slow growth of plants and low microbial activity in late autumn and winter (Yeates, 1997).

Pi and Po in soils are present in different pools which can be classified according to their availability. These pools are available P, labile P and non-labile P.

Available P is present in the soil solution as orthophosphate anions which can be immediately utilized by plants. Depending on the pH of the soil solution, Pi is present as $H_2PO_4^-$ (acidic conditions) or HPO_4^{2-} (alkaline conditions) where the former is the favourable form for plants (Marschner & Rengel, 2012). Available P in the soil solution is quickly depleted by roots which

requires replenishing from labile P pools. Available Pi from fertilizers is soluble for a short time only before it is fixed or absorbed on clay particles or Fe/Al oxides/hydroxides. Organic amendments can provide available P by releasing Pi in the residue before decomposition and by mineralization of organic P.

Labile P is P that is less readily extractable and bound to soil particles or adsorbed to internal surfaces but can become plant-available. Labile Pi is released into the soil solution by solubilization and mobilization of adsorbed Pi, mineralization of Po, and turnover of the microbial biomass. As the available P concentration is low, labile P is the main pool of plant available P. Therefore, the ability to replenish soil solution P from labile P pools plays a vital role in providing P to plants and soil microbes. Labile absorbed Pi is in equilibrium with Pi in the soil solution (Holford, 1997). Labile Po is mostly in fresh residues and other Po forms such as adenosine phosphates (ATP, ADP, AMP), sugar phosphates and DNA, RNA. Labile Po can be released by enzymes such as phosphatases and phytase which hydrolyse P. Soil microbial biomass P (0.5-2.6 % of total soil P), a potentially important source of available P pool, is released through cell death and mineralization.

Stable P is occluded or strongly adsorbed to Fe/Al oxides/hydroxides or organic matter and therefore unavailable to plants. Inorganic P is strongly bound to soil particles or part of the soil mineral complex where it is either slowly exchangeable or non-exchangeable (Syers et al., 2008). Findings of Crews et al. (1995) demonstrated that predominant primary Ca-P in the parent soil was rapidly transformed by weathering into secondary adsorbed and mineral forms of inorganic P associated with Al and Fe and with further weathering, the secondary P was transformed to more recalcitrant occluded P forms. At low pH, variscite and strengite are the main P minerals whereas in soils with high pH, calcium P minerals such as apatite are

dominant (Savant & Racz, 1973). Stable organic P_o is resistant to mineralization by microorganisms (recalcitrant compounds) (Griffin et al., 2003). The chemical nature of P_o is elusive but it has been suggested to be polymeric molecules of high molecular weight containing P in monoester linkages (Doolette et al., 2011b, Doolette et al., 2010, Doolette et al., 2009, Smernik & Dougherty, 2007) such as inositol phosphates, especially myo-inositol hexakisphosphate (phytate) and to a lesser extent scyllo-inositol hexakisphosphate which are synthesized by plants and microbes (Dalal, 1977, Turner et al., 2002b). Phytate is negatively charged and is therefore bound tightly to soil particles which makes it poorly available to soil microbes (McKercher & Anderson, 1989). Phytate (or myo-Inositol hexa*kis*phosphate and lower phosphomonoesters of inositol) was thought to account for up to 80% of total P_o soil (Koopmans et al., 2003, Dao, 2004). Previous studies reported that phytate can be mineralized by micro-organisms (Hill et al., 2007), become stabilized with time binding to soil surfaces (Celi et al., 1999) or form insoluble metal complexes (He et al., 2006).

However, other studies have found that that inositol phosphates constitutes a relatively small fraction of total P_o, less than 15% in Australian soils (Cosgrove, 1963) which was similar to recent study of Doolette et al. (2011a) on 18 Australian soils using NMR.

The concentration of soil organic phosphorus varies, depending on elevation and climate (Chatterjee & Jenerette, 2015) because these factors influence not only plant P input but also soil microbial breakdown and transformation of organic P. For example, low temperatures and dry conditions, very wet conditions or acidic soils reduce plant growth as well as microbial activity (Van Meeteren et al., 2007, Hollings et al., 1969). Sumann et al. (1998) showed that the concentration of organic P decreased with increasing temperatures but increased with increasing precipitation (Sumann et al., 1998). The composition of organic P also depends on

climate. In soils with limited microbial activity due to cold and wet conditions, phosphomonoesters comprised the largest Po (83%) and trace amount of neo- and D-chiro-isomer of phytate (Zech et al., 1987, Gil-Sotres et al., 1990) which were absent in organic P of warm and dry Australian soils (Doolette et al., 2017).

1.4. P amendments and the fate of added P in soil

Phosphorus can be added to soil as inorganic fertiliser or organic amendments such as manures and crop residues. To improve crop production, farmers mainly rely on the application of inorganic P fertiliser which is easily absorbed to clay minerals, sesquioxides and organic matter or minerals (Holford, 1997). In Australia, superphosphate fertilisers have been used for more than 100 years as a main source of Pi in agricultural system (Glendinning, 2000). Phosphate rock (PR), a natural source of P which is mined commercially, is used as the raw material in fertiliser manufacture or can be applied directly to soil in broad acre agriculture. PR is usually carbonate-fluoro-apatite, Ca_{10} (PO_4, $CO_3)_6$ $F_{2\text{-}3}$ or carbonate-hydroxyl-flour-apatite, $Ca_{10}(PO_4, CO_3)_6$ $(OH,F)_{2\text{-}3}$. There are four major types of P resources mined in the world, P deposits include marine, igneous , metamorphic and biogenic (Van Straaten, 2002). The solubility of sedimentary PR is higher than rocks of igneous (from lava or magma) and metamorphic origin (Rajan et al., 1996) and carbonate substituted PR is more soluble than pure flouro-apatite containing little or no carbonate (Chien & Hammond, 1978). The ability of PR to supply P to plants depends on the rate of dissolution (Rajan et al., 1992) which, in turn, depends on soil parameters such as pH (Kanabo & Gilkes, 1987), their chemical composition, the particle size of the PR (Smyth & Sanchez, 1982, Khasawneh & Doll, 1979), soil calcium status (Wilson & Ellis, 1984) and temperature (Barrow & Shaw, 1975). The composition of PR

varies, for example pure flour-apatite contains 42% P_2O_5 and francolite (the carbonate-substituted form of apatite) contains 34% P_2O_5.

Organic amendments such as manures and plant residues can also be used to increase crop P availability. Therefore, returning crop residues to soil is important in P cycling. Application of crop residues and rock phosphate in field trip resulted in higher cereal yields and cereal P uptake than that of rock phosphate alone (Sharma & Prasad, 2003, Waigwa et al., 2003). After applying organic amendments, P availability depends on soil properties, cropping systems, climate conditions, organic amendment properties which influence the rate of residue decomposition, P release and microbial immobilization. Organic amendments not only add P, but also improve soil aggregation, increase microbial biomass and improve soil structure and water holding capacity (Haynes 1984).

It has been shown that 15% of shoot P uptake can be from plant residues which demonstrates that P derived from crop residues plays a vital role in P cycling in the soil-plant system (Noack et al., 2014). Plant residues contain both Pi and organic P (Po) which have a variety of pathways for transfer into soil P pools (Noack et al., 2012). Residue P can be released directly to soil as soluble orthophosphate which plants can use immediately, transform into labile or non-labile soil P pools or taken up by micro-organisms (Noack et al., 2014). For example, Martin and Cunningham (1973) reported rapid release of orthophosphate from plant residues (wheat roots) which was about 85% of total P- detected as. This P_o pool is available to plants, micro-organisms and can be adsorbed to soil particles (Noack et al., 2012).

Sewage sludge is another organic amendments which also improves soil structure, physical properties such as aggregation, crusting, bulk density, pore size distribution (Kladivko & Unger, 1994) and water-holding capacity (Lobo et al., 2013). To optimize the use of sewage

sludge as P fertiliser, it is important to understand P forms in the sewage sludge and their effect on soil P pools (Huang et al., 2008). Sewage sludge contains high proportions of labile P forms as inorganic phosphorus and non-apatite inorganic phosphorus which are associated with oxides and hydroxides of Al, Fe, Mn (González Medeiros et al., 2005). The organic P forms in sewage sludge include inositol phosphate, phospholipids, nucleic acids, phosphoproteins, various sugar phosphates (Maguire et al., 2001, Frossard et al., 1994). Huang et al. (2008) used Hedley fractionation to estimate Po and Pi in biosolids and found that the main P fraction was HCl-P (35%) followed by $NaHCO_3$-P (20%), water soluble P (12%), residual P (11%) and that Pi was the main P form in the $NaHCO_3$-P and water soluble P fractions. He et al. (2010) reported that organic P accounted for 24% of total P or 44.6% in HCl fraction. These findings suggest that sewage sludge could be a short and long-term source of P for plants. However, there are also limitations to sewage sludge application such as a high concentrations of heavy metals and toxic organic compounds, excess of labile organic matter and leachable P or pathogenic micro-organisms (McLaren et al., 2004, McBride, 2003).

1.5. The fate of P amendments in soil and their consequences for P availability to crops

The fate of Pi fertilizers depends on soil properties, including pH, water content, temperature, clay content and other environmental conditions like size and activity of microbes, organic matter content, P adsorption capacity and time (Nziguheba et al., 2000, Nziguheba et al., 1998). Rock phosphate provides only 10-20% soluble P directly to plants in the year of application and less than 50% in the following year (Bolland & Baker, 1998). Inorganic P in the soil solution can be quickly adsorbed to soil particles or react with Al and Fe in acidic soil or with Ca in alkaline soil to form poorly soluble compounds (Wang et al., 2007a). Continuous cultivation without fertiliser addition reduced total P content as well as organic P (by 3 mg P

kg^{-1} yr^{-1}) and Pi pools (Dalal, 1997) but the decrease of P_o was smaller than that of Pi pools (Wang et al., 2007b, McLaren et al., 2014). Therefore, managing P fertilizers in agricultural systems is important to maximise plant P uptake and reduce environmental issues and economic burden (Tunney et al., 1997).

Plant residues and organic amendments in general, increase P availability by two processes. Firstly decomposition/ mineralization of tissues to release available P and secondly, by the release of P sorbed to soil particles by organic anions which are produced during decomposition. Depending on the P concentration of the organic amendment, it can induce either net mineralization or net immobilization by microbes. According to Singh and Jones (1976) organic materials with C/P ratio <200 can cause net mineralization and increase available P in soil solution whereas organic materials with C/P ratio > 200 will result in net immobilization. However, P which is immobilized in the microbial biomass can become available to plants through the turnover of the microbial biomass (Singh et al., 1989). Decomposition of organic materials produces dissolved organic anions which can displace P from sorption sites, their affinity to P binding sites decreases in the following order oxalate>citrate>malate>>acetate (Shen et al., 1996). Other processes by which organic acid anions increase P availability include metal complexation, dissolution of Fe and Al oxides and by increasing the negative charge of soil particles which reduces P binding capacity (Easterwood & Sartain, 1990). Humic and fulvic acids produced during humification of organic matter can also reduce P adsorption of P and release P to soil (Sibanda & Young, 1986, Parfitt et al., 1977).

Most experiments on the effect of P amendments are short, lasting only one or two crop rotations or seasons (Maier et al., 2002, McLaughlin et al., 1988, Damon et al., 2014). However

farmers usually repeatedly apply fertilisers over many years in order to meet plant P requirements (Zhang & MacKenzie, 1997, Eichler-Löbermann et al., 2007, Ye et al., 2019, Jing et al., 2019). Therefore, long-term fertilization trials are useful to evaluate the effect of farmer practice on P pools and soil properties (Wang et al., 2007a, Laboski & Lamb, 2003). Compared to short P amendment experiments, long-term trials are likely to create more stable inorganic and organic P pools and better reflect P availability to crops. With a rate that is higher than that removed from the system, more than 50% of P added can be recovered in the upper layers of soil profile (McLaren et al., 2015a, Simpson et al., 2015) but the levels of P_o may not be affected (Cade-Menun et al., 2017, Tian et al., 2017). The studies of Schefe et al. (2015) indicated that there is no consistent relationship between soil P fertility and organic P sequestration in the Australian pasture system soils. However, a 37-year grazing experiment to identify the chemical nature of P_o in soils indicated that accumulation of P in the organic fraction eventually reaches a new equilibrium above which further P addition does not increase Po (McLaren et al., 2019). Another long-term trial in New Zealand found that Po pool increased over time but was accompanied by a decrease in Pi indicating that overtime inorganic P forms were converted to Po (Dodd et al., 2013). Thus availability of Po to plants may be important for sustainability of pastures (McLaren et al., 2019).

1.6. Plant strategies to increase P uptake

There are several strategies plants can implement to increase P uptake.

1.6.1. Root morphology

The diffusion rate of P is very slow, a millimeter per day (Barber, 1962, Barber, 1995, Hinsinger et al., 2005, Lewis & Quirk, 1967) which makes P the most immobile or least bioavailable macronutrient. Root architecture, root hair density and length are important for exploiting

the soil volume (Bates & Lynch, 1996). Root hairs are subcellular extensions from the root epidermis that increase the absorptive surface area of the root, allowing roots to explore a greater soil volume. By applying P^{33} to rape seedlings, Bhat and Nye (1973) showed that root hair length and density were higher under P deficient conditions. Similarly, root hair density increased five-fold compared to the control at low P availability (Ma et al., 2001). In a subsequent experiment Bhat and Nye (1974) also showed the role of root hairs in increasing the extent of the Pi depletion zone around roots. When transplanted from soil with high available P to soil with low P availability, root hair length in *Arabidopsis thailiana* doubled. On the other hand, when transplanted from a low to high-P soil, root hair length halved (Ma et al., 2001). Root hairs also improve dispersion of exudates such as citrate, malate, carboxylates which are important for mobilization of P in the rhizosphere (Ryan et al., 2001).

1.6.2. Root exudation

Organic anion exudation from plant roots is related to nutrient deficiency, particularly P or exposure to toxic cations, for example Al^{3+} (Jones & Darrah, 1994). The large electrical potential difference across the plasma membrane of plant cells ensures the electrochemical gradient for organic anion release without the direct expenditure of energy (Ryan et al., 2001). Organic acid anion release is likely regulated by both synthesis and transport across the plasma membrane (Ryan et al., 2001). Organic anions compete with phosphate for binding sites (e.g. Fe or Al oxides) in the soil (Jones & Darrah, 1994), particularly tricarboxylates because they form more stable ligand-metal complexes than di- and mono-carboxylates (Bolan et al., 1994, Hue et al., 1986). Bound organic anions can also block P sorption sites. Cluster roots, e.g. of white lupin, have very high organic anion exudation rates under P deficiency (Marschner et al., 1987) whereas other plant species such as lucerne (*Medicago*

sativa) and rice (*Oryza sativa)* have low organic anion exudation rates even when growing in low P soil (Richardson et al., 2011, Richardson et al., 2009).

1.6.3. Acidification

Acidification of the rhizosphere can enhance the solubility and mobility of soil P, particularly that of calcium phosphate (Gahoonia et al., 1992). The ratio of ammonium to nitrate uptake can control rhizosphere pH (Gahoonia & Nielsen, 1992), thus affect the degree of P depletion in the rhizosphere (Bhat et al., 1976). When the cation ammonium is taken up, roots release protons (H^+) into the soil solution which decreases the pH around the roots. Accordingly, when roots take up the anion nitrate it releases bicarbonate (HCO_3) and hydroxide (OH-) which increases the pH around the roots. Depending on soil type, root-induced pH changes in the rhizosphere may have a negative or positive effect on nutrient uptake and plant growth. In neutral or alkaline soils, increased pH in the rhizosphere will reduce the availability of P while in acidic soils, pH increase (between pH 4 and 7) can increase P availability and reduce Al toxicity (Marschner et al., 1987, Lindsay & Moreno, 1960).

1.6.4. Mycorrhiza

Mycorrhizal fungi play an important role in P uptake of most plants primarily by increasing the soil volume from which P can be taken up (Read & Perez-Moreno, 2003). Arbuscular mycorrhizal (AM) symbiosis are associations of plant roots with fungi that are generally beneficial for the plant. Mycorrhizae have been identified in thousands of plant species but there are only a few hundred fungal species (Redecker & Raab, 2006) and different plant biomes contain different types of mycorrhizal symbioses (Read & Perez-Moreno, 2003) although most plants establish AM symbiosis (Baxter & Dighton, 2001). Hyphae of AM can grow several centimeters into the soil surrounding the roots (Jansa et al., 2005) which helps

plants acquire resources from zones beyond the direct reach of roots and root hairs. Mycorrhizae translocate P through the extra-radical mycelium and release P from the hyphae in the roots which can then be taken up by root cells. The acquisition of P by mycorrhiza is also enhanced because hyphae have a diameter of 5-30 µm and therefore penetrate smaller soil pores than roots that have diameters of 50-100 µm (Li et al., 1991). The density of mycorrhizal hyphae can be very high, 50 m hyphae/g soil, compared to root densities of 0.1m root/g (Schnepf et al., 2008). Phosphorus taken up by hyphae can be stored in the hyphae itself or transported into the plant as short-chain polyphosphates (Bücking & Heyser, 2003, Takanishi et al., 2009). Storage of P in hyphae decreases the role of mycorrhiza in providing P to plants (Solaiman et al., 1999).

There is no conclusive evidence that the presence of mycorrhizae influences the weathering rates of apatite (Hutchens, 2009). This may be because many soil microorganism are able to release Pi from apatite (Jones & Smith, 2004). Enzymes which are produced by ectomycorrhizal fungi can hydrolyze organic P (Read & Perez-Moreno, 2003). Compared with non-mycorrhizal plants, the transfer of P to plants by *Hebeloma syrjense* accounted for 8% of the P added as organic material in 35 days, higher than in the of absence mycorrhizae where it was only 1% of P added (Tibbett & Sanders, 2002).

1.6.5. Rhizosphere microorganisms

Many studies have examined the role of rhizosphere microbes on root growth as well as nutrient uptake by plants (Yang & Crowley, 2000, Khan et al., 2007). Microbial density in the rhizosphere is high due to the presence of root exudates, for example sugars, organic anions and amino acids which are easily decomposable and thereby increase microbial density and activity in the rhizosphere (Marschner, 2008). Further, plant and soil physical/ chemical

characteristics also affect rhizosphere communities (Carelli et al., 2000). A given plant species may have a similar rhizosphere community composition in different soils (Miethling et al., 2000), but different plant species in the same soil often have distinct rhizosphere communities (Marschner et al., 2001). Rhizosphere microorganisms can either reduce plant available P by taking up P (immobilization), decomposing root exudates, inhibiting root growth or increase P by solubilizing poorly soluble inorganic P forms and mineralizing organic P

1.7. The role of microbes in P cycling

The available P pool is replenished by desorption of P which is adsorbed on the surface of clay minerals and through the decomposition or mineralization of labile organic P. Inorganic orthophosphate can be taken up by microbes (immobilization) thus reducing Pi in the soil solution. Mineralisation of Po by phosphatase or phytase released by microbes or plant roots is influenced by soil pH, water content, temperature and surface area of the soil particles (Shen et al., 2011, Oberson et al., 2005). At soil temperatures between 3 to 15^{o}C, net mineralization increases with temperature (Schmidt et al., 1999). Soil water content controls microbial activity and is a major factor that determines the rates of mineralization (Paul et al., 2003). At low water content, soil microbes accumulate organic and inorganic compounds which increases the osmotic potential inside their cells. When soils dry, substrate supply becomes increasingly limited as the pores drain and water films around aggregates become thinner and disconnected (Yan et al., 2015).

Microorganisms are a pool of P in soil and carry out numerous important processes in the biogeochemical P cycle (Oberson et al., 2005). Acidification by microbes can release P when P is bound to Ca (Schilling et al., 1998). Mineralization and immobilization by microorganisms

occurs at the same time in soil. Phosphorus immobilised in the microbial biomass is released into the soil solution when the microbes die (Dalal, 1977).

Microbes release low molecular weight organic anions like gluconate and oxalate which compete with Pi for sorption sites, consequently increasing Pi solubility (Illmer et al., 1995). But microbes can also decompose organic anions (van Hees et al., 2002) and therefore decrease P mobilization in the rhizosphere. In addition to their effect on P cycling, microorganism affect the physical properties of soil by producing extracellular polysaccharides and other cellular debris which help maintain soil structure and stabilize soil aggregates, this in turn can increase root growth and therefore P uptake (Rice et al., 2004)..

1.8. Methods to measure plant available P and P pools

P dynamics and P bioavailability in natural or managed ecosystems are determined by the complex interactions among inorganic, organic and microbial forms of P. Therefore, it is important to develop methods that can determine different forms of P in soil. Different extracting solutions can be used to determine plant available P which includes P in the soil solution and weakly adsorbed P. For example, dilute acids or acids buffered to certain pH values (e.g. H_2SO_4 acid (Truog, 1930), HCl acid (Bray & Kurtz, 1945) or mixture of two (Nelson et al., 1953)). In the Olsen or Colwell method, soil is shaken with 0.5 M $NaHCO_3$. Bicarbonate releases P by two main mechanisms (i) anion exchange with bound P and (ii) formation of $CaCO_3$ which reduces the concentration of Ca in solution and thereby promotes solubilization of Ca-P (Olsen et al., 1954). The Olsen and Colwell methods are best suited for alkaline soils. However, these method may not be suitable for strongly P-sorbing soils (Potter et al., 1991). Another method to determine plant available P is based on anion exchange membranes (Kouno et al. 1995). Soil is shaken with water and an anion exchange membrane (AEM).

Phosphate anions in the solution are bound to the AEM which decreases solution P concentration and therefore triggers further P release from labile P forms. The AEM acts as sink for P in a similar manner as a plant root. After shaking, the AEM is rinsed and shaken with a dilute acid to release bound P which can then be measured. In a range of soils, the AEM method has been shown to have a high correlation with plant P uptake and predicting wheat responses to fertilizers P application with 70% correct predictions compared to 55% in Colwell P method (Mason et al., 2010). Diffusive Gradients in Thin Films (DGT) has been used to predict crop response to P fertilizer application (Menzies et al., 2005) or wheat response to different forms of fertilizers (McBeath et al., 2007). Similar to the AEM principle, DGT technique uses a ferrihydrite gel as a sink for free P which continuously removes P from the solution which, in turn, induces replenishment of P from labile P forms into soil solution. The benefits of DGT compared with AEM are (1) less vulnerable to anionic interferences, pH, contact time between soil suspension and absorbent and changes in the soil:liquid ratio (Mason et al., 2008), (2) the diffusive layer in DGT limits the maximum flux of P from soil to the binding layer, (3) DGT can be put directly onto a saturated soil paste while AEM is deployed in soil suspension. However, the DGT method is time consuming and more studies are needed, for example on the impact of low soil water content and shorter incubation time to optimize its use (Mason et al., 2010, Mason et al., 2013).

Microbial biomass accounts for 2-3% of total organic carbon in soil and microbial biomass P can be 3-5 times higher than plant P uptake in semi-arid grassland (Cole et al., 1977). There are different methods to measure microbial biomass P (Brookes et al., 1982, Hedley & Stewart, 1982, McLaughlin et al., 1986). In the method of Brookes et al. (1982), soils are fumigated with $CHCl_3$ for 24 h. Then the soil is shaken with the Olsen extractant (0.5 M $NaHCO_3$ at pH 8.5 by NaOH) at a 1:20 (w/v) ratio of soil:solution. Microbial biomass P is the

calculated by difference between Pi extracted by the Olsen solvent from $CHCl_3$-fumigated soil and Pi extracted from unfumigated soil, divided by the conversion factor (k_p) 0.4. The k_p is used assuming that 40% of P in the biomass is extractable as Pi. In a study of McLaughlin et al. (1986) different biocides were compared. They found that fumigation with vapour is more effective than liquid. Hexanol and $CHCl_3$ gave the largest flushes of ^{32}P; advantages of hexanol are lower toxicity and easier handling compared to $CHCl_3$. The AEM method of Kouno et al. (1995) can also be used to determine microbial biomass P. Instead of using $CHCl_3$ vapour, Kouno et al. (1995) used liquid $CHCl_3$ to release P from microbial cells. Soil is shaken with water and an AEM is used to extract available P and in parallel, soil is also shaken with water, AEM and $CHCl_3$. The difference in P sorbed to the AEM between fumigated and unfumigated samples is microbial biomass P.

Single extractions with $NaHCO_3$ or NH_4F can estimate plant available P (Bray & Kurtz, 1945, Olsen et al., 1954), but provide no information about other soil P forms. P fractionation schemes involve sequential extraction or multiple extraction in parallel with a series of reagents to dissolve different forms of P based on the nature and strength of interactions between P moieties and other mineral and organic components (Condron & Newman, 2011). In P fractionation schemes, a sequence of extractants to selectively dissolve various forms of P based mainly on the nature and strength of interactions between P moieties and other mineral and organic components. P fractionation schemes involve the extraction of “loosely bound P” with a salt solution (1M NH4Cl), followed by extraction of iron- and aluminium-bound P with a combination of alkali reagents (0.5M $NaHCO_3$, 0.1 NaOH) which is then followed by extraction with acid (0.5M HCl, 0.5 M H2SO4) to recover calcium-bound P (Pierzynski et al., 2005). The most commonly used method is the Hedley procedure using 10 fraction steps (Hedley & Bernstein, 1982). In this method, a soil sample is sequentially

extracted with different extractants. In the first step, soil is shaken in water with an anion exchange resin to determine available P. Then the soil is extracted with 0.5 M $NaHCO_3$ for readily available P. This is followed by extraction with 0.1 M NaOH to extract P associated with iron and aluminium. Then, the sample is extracted with 1 M HCl to release P bound to Ca. Lastly, extraction with H_2SO_4 and H_2O_2 is used to determine residual P. The drawback of this method is that it is time consuming. More importantly, rhizosphere processes which solubilise P are not reflected in the method because it uses extractants that are unlikely to occur in soil (Johnson et al., 2003, Yang & Post, 2011). DeLuca et al. (2015) introduced a method to determine bioavailable P using four extractions in parallel: (1) 10 mM $CaCl_2$ (soluble P); (2) phosphatase (enzyme extractable P); (3) 10 mM citrate (citrate extractable P) (4) 1 M HCl (mineral occluded P). These extractants are thought to represent P can be solubilized by plants or microorganism to assess P which is (1) soluble/intercepted by the root, (2) enzyme hydrolysable (3), chelate extractable or forms complexes with organic acids, and (4) released by proton excretion (acidification). This method is thought to be applicable over a wide range of soil pH values. However, it does not assess microbial biomass P.

1.9. Conclusion and research gaps

Many previous studies investigated the effect of organic amendments like plant residues and sewage sludge on P availability (Zebarth et al., 1999, Franzluebbers, 2002). Celik et al. (2004) determined the effect of combinations of inorganic fertiliser with organic amendments on stable inorganic P forms. However, little is known about the effect of combined inorganic P and organic amendments on potentially plant available P pools. Further, there are few studies that assessed changes in P pools over time after straw amendment or during plant growth. These types of studies will help to gain an understanding about changes in P pools and how

they are influenced by management. Therefore, the objective of this study was to understand the effect of the addition of inorganic P and organic amendments on soil P pools and plant growth.

The underlying processes are shown in Figures 1 and 2. Figure 1 illustrates the effect of inorganic P and organic amendments on P pools and fluxes. Adding inorganic P can increase resin P in the soil solution which enhances microbial and plant P uptake but most of applied P is immobilized by adsorption into HCl labile P and citrate labile P pools. Resin P is directly accessible to plants and microbes. To assess HCl P and citrate P, plants and microbes release organic acids anions or protons. A proportion of organic P can be mineralized by phosphatases released by roots or microbes.

Figure 2 illustrates the influence of microbes on P pools and fluxes. Microbes take up P from the soil solution, mineralize organic P through release of phosphatase and mobilize HCl P and citrate P by releasing organic acid anions and protons. Compared to unamended soil, addition of high C/P residue enhances microbial activity and P uptake leading to net P immobilization in the microbial biomass.

Size of these P pools and how they change over time after straw addition and during plant growth were investigated in this thesis.

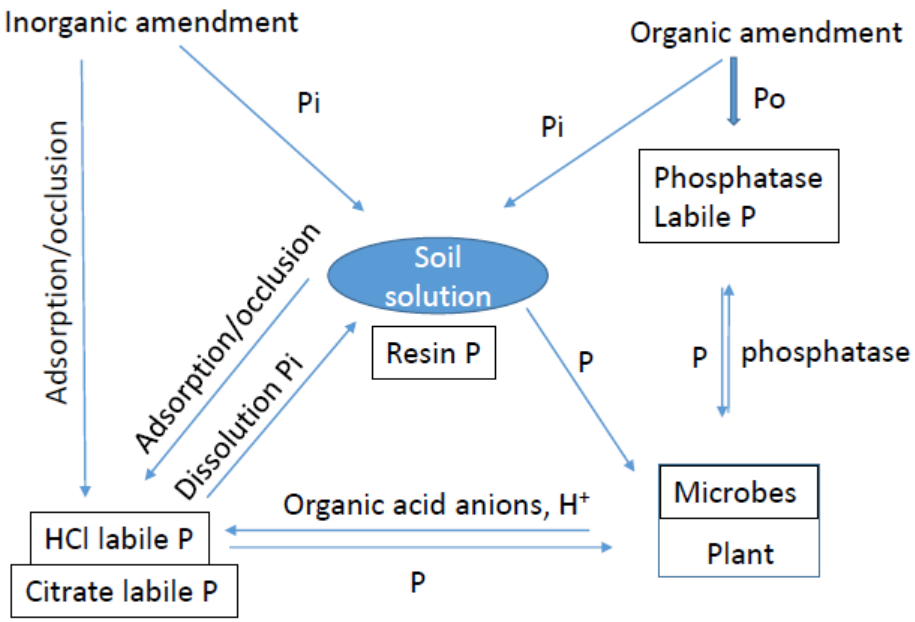

Figure 1: P pools in soil amended with inorganic P and organic amendments and mechanisms by which plants and microbes access these pools.

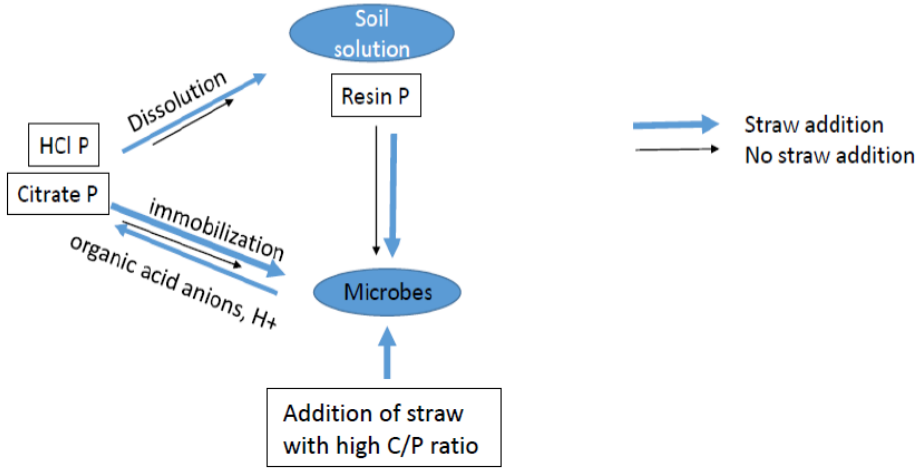

Figure 2: Influence of microbes on P pools in unamended soil (thin black arrows) and soil amended with high C/P straw (thick blue arrows).

To fulfil the objectives of this thesis, the study aims to:

1. Determine changes in P pools in soil amended with straw and inorganic P and the effect on wheat growth (Chapter 2). In this experiment, P was added as inorganic

Palone or in combination with wheat straw. The effects of plants and microbes stimulated by straw addition on P pools were investigated.

2. Using soil which had been subject to seven years of P fertiliser application, assess soil P pools and plant growth following incubation for four months with straw (Chapter 3). Soil that had received different P rates over seven years was used to assess the impact of four months incubation with wheat straw and subsequent plant growth on P pools.
3. Determine the effect of combined application of sewage sludge and inorganic P on soil P pools and wheat growth (Chapter 4). Soil was amended with the same amount of N and P as sludge and inorganic nutrients alone or in combination and then planted with wheat to evaluate the effect on soil P pools, on P leaching and plant P uptake.

Also included in this thesis are two appendices with additional experiments carried out during the PhD study. Appendix 1 describes the results of an experiment with different straw rates which provided useful information for the studies described in Chapters 2 and 3. Appendix 2 was a study with vermicompost carried out in collaboration with other students.

1.10. References

Alamgir M, McNeill A, Tang C & Marschner P 2012: Changes in soil P pools during legume residue decomposition. *Soil Biology and Biochemistry* **49,** 70-77.

Amtmann A, Hammond JP, Armengaud P & White PJ 2005: Nutrient sensing and signalling in plants: potassium and phosphorus. *Advances in Botanical Research* **43,** 209-257.

Ayaga G, Todd A & Brookes P 2006: Enhanced biological cycling of phosphorus increases its availability to crops in low-input sub-Saharan farming systems. *Soil Biology and Biochemistry* **38,** 81-90.

Barber SA 1962: A diffusion and mass-flow concept of soil nutrient availability. *Soil Science* **93,** 39-49.

Barber SA 1995: *Soil nutrient bioavailability: A Mechanistic Approach,* John Wiley & Sons.

Barrow N & Shaw T 1975: The slow reactions between soil and anions: 2. Effect of time and temperature on the decrease in phosphate concentration in the soil solution. *Soil Science* **119,** 167-177.

Bates T & Lynch J 1996: Stimulation of root hair elongation in Arabidopsis thaliana by low phosphorus availability. *Plant, Cell & Environment* **19,** 529-538.

Baxter JW & Dighton J 2001: Ectomycorrhizal diversity alters growth and nutrient acquisition of grey birch (Betula populifolia) seedlings in host–symbiont culture conditions. *New Phytologist* **152,** 139-149.

Bergman W 1992: Nutritional disorders of plants. *Development visual and analytical diagnosis. Jena, Germany: Gustav Fischer*.

Bhat K & Nye P 1973: Diffusion of phosphate to plant roots in soil. *Plant and Soil* **38,** 161-175.

Bhat K & Nye P 1974: Diffusion of phosphate to plant roots in soil: III. Depletion around onion roots without root hairs. *Plant and Soil***,** 383-394.

Bhat K, Nye P & Baldwin J 1976: Diffusion of phosphate to plant roots in soil. IV. The concentration distance profile in the rhizosphere of roots with root hairs in a low-P soil. *Plant and Soil* **44,** 63-72.

Bolan NS, Naidu R, Mahimairaja S & Baskaran S 1994: Influence of low-molecular-weight organic acids on the solubilization of phosphates. *Biology and Fertility of Soils* **18,** 311-319.

Bolland M & Baker M 1998: Phosphate applied to soil increases the effectiveness of subsequent applications of phosphate for growing wheat shoots. *Animal Production Science* **38,** 865-869.

Brady N & Weil R 2002: The nature and properties of soils . 2002. Upper Saddle River, Prentice Hall, NJ.

Bray RH & Kurtz L 1945: Determination of total, organic, and available forms of phosphorus in soils. *Soil Science* **59,** 39-46.

Brookes P, Powlson D & Jenkinson D 1982: Measurement of microbial biomass phosphorus in soil. *Soil Biology and Biochemistry* **14,** 319-329.

Bücking H & Heyser W 2003: Uptake and transfer of nutrients in ectomycorrhizal associations: interactions between photosynthesis and phosphate nutrition. *Mycorrhiza* **13,** 59-68.

Cade-Menun BJ, Doody DG, Liu CW & Watson CJ 2017: Long-term changes in grassland soil phosphorus with fertilizer application and withdrawal. *Journal of Environmental Quality* **46,** 537-545.

Carelli M, Gnocchi S, Fancelli S *et al.* 2000: Genetic diversity and dynamics of Sinorhizobium meliloti populations nodulating different alfalfa cultivars in Italian soils. *Applied and Environmental Microbiology* **66,** 4785-4789.

Celi L, Lamacchia S, Marsan FA & Barberis E 1999: Interaction of inositol hexaphosphate on clays: adsorption and charging phenomena. *Soil Science* **164,** 574-585.

Celik I, Ortas I & Kilic S 2004: Effects of compost, mycorrhiza, manure and fertilizer on some physical properties of a Chromoxerert soil. *Soil and Tillage Research* **78,** 59-67.

Chatterjee A & Jenerette GD 2015: Variation in soil organic matter accumulation and metabolic activity along an elevation gradient in the Santa Rosa Mountains of Southern California, USA. *Journal of Arid Land* **7,** 814-819.

Chen C, Condron L, Davis M & Sherlock R 2003: Seasonal changes in soil phosphorus and associated microbial properties under adjacent grassland and forest in New Zealand. *Forest Ecology and Management* **177,** 539-557.

Chien SH & Hammond LL 1978: A comparison of various laboratory methods for predicting the agronomic potential of phosphate rocks for direct application. *Soil Science Society of America Journal* **42,** 935-939.

Cole CV, Innis GS & Stewart J 1977: Simulation of phosphorus cycling in semiarid grasslands. *Ecology* **58,** 1-15.

Condron L & Schjonning P 2004: Phosphorus—surplus and deficiency. *Managing soil quality—Challenges In Modern Agriculture***,** 69-84.

Condron LM & Newman S 2011: Revisiting the fundamentals of phosphorus fractionation of sediments and soils. *Journal of Soils and Sediments* **11,** 830-840.

Condron LM, Turner BL & Cade-Menun BJ 2005: Chemistry and dynamics of soil organic phosphorus. *Phosphorus: Agriculture and The Environment* **46,** 87-121.

Conyers M & Moody P 2009: A conceptual framework for improving the P efficiency of organic farming without inputs of soluble P fertiliser. *Crop and Pasture Science* **60,** 100-104.

Cosgrove D 1963: The chemical nature of soil organic phosphorus. I. Inositol phosphates. *Soil Research* **1,** 203-214.

Crews TE, Kitayama K, Fownes JH *et al.* 1995: Changes in soil phosphorus fractions and ecosystem dynamics across a long chronosequence in Hawaii. *Ecology* **76,** 1407-1424.

Dalal R 1977: Soil Organic Phosphorus. *Peer Reviewed Journa* **29,** 83.

Dalal R 1997: Long-term phosphorus trends in Vertisols under continuous cereal cropping. *Soil Research* **35,** 327-340.

Damon PM, Bowden B, Rose T & Rengel Z 2014: Crop residue contributions to phosphorus pools in agricultural soils: A review. *Soil Biology and Biochemistry* **74,** 127-137.

Dao TH 2004: Ligands and phytase hydrolysis of organic phosphorus in soils amended with dairy manure. *Agronomy Journal* **96,** 1188-1195.

DeLuca TH, Glanville HC, Harris M *et al.* 2015: A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. *Soil Biology and Biochemistry* **88,** 110-119.

Dodd R, McDowell R & Condron L 2013: Changes in soil phosphorus availability and potential phosphorus loss following cessation of phosphorus fertiliser inputs. *Soil Research* **51,** 427-436.

Doolette A, Smernik R & Dougherty W 2009: Spiking improved solution phosphorus-31 nuclear magnetic resonance identification of soil phosphorus compounds. *Soil Science Society of America Journal* **73,** 919-927.

Doolette A, Smernik R & Dougherty W 2010: Rapid decomposition of phytate applied to a calcareous soil demonstrated by a solution 31P NMR study. *European Journal of Soil Science* **61,** 563-575.

Doolette A, Smernik R & Dougherty W 2011a: A quantitative assessment of phosphorus forms in some Australian soils. *Soil Research* **49,** 152-165.

Doolette AL, Smernik RJ & Dougherty WJ 2011b: Overestimation of the importance of phytate in NaOH–EDTA soil extracts as assessed by 31P NMR analyses. *Organic Geochemistry* **42,** 955-964.

Doolette AL, Smernik RJ & McLaren TI 2017: The composition of organic phosphorus in soils of the Snowy Mountains region of south-eastern Australia. *Soil Research* **55,** 10-18.

Easterwood GW & Sartain J 1990: Clover residue effectiveness in reducing orthophosphate sorption on ferric hydroxide coated soil. *Soil Science Society of America Journal* **54,** 1345-1350.

Eichler-Löbermann B, Köhne S & Köppen D 2007: Effect of organic, inorganic, and combined organic and inorganic P fertilization on plant P uptake and soil P pools. *Journal of Plant Nutrition and Soil Science* **170,** 623-628.

Fox T & Comerford N 1990: Low-molecular-weight organic acids in selected forest soils of the southeastern USA. *Soil Science Society of America Journal* **54,** 1139-1144.

Franzluebbers A 2002: Soil organic matter stratification ratio as an indicator of soil quality. *Soil and Tillage Research* **66,** 95-106.

Frossard E, Condron LM, Oberson A, Sinaj S & Fardeau J 2000: Processes governing phosphorus availability in temperate soils. *Journal of Environmental Quality* **29,** 15-23.

Frossard E, Tekely P & Grimal J 1994: Characterization of phosphate species in urban sewage sludges by high-resolution solid-state 31P NMR. *European Journal of Soil Science* **45,** 403-408.

Gahoonia TS, Claassen N & Jungk A 1992: Mobilization of residual phosphate of different phosphate fertilizers in relation to pH in the rhizosphere of ryegrass. *Fertilizer Research* **33,** 229-237.

Gahoonia TS & Nielsen NE 1992: Control of pH at the soil-root interface. *Plant and Soil* **140,** 49-54.

Gahoonia TS & Nielsen NE 2004: Root traits as tools for creating phosphorus efficient crop varieties. *Plant and Soil* **260,** 47-57.

George TS, Hinsinger P & Turner BL 2016: Phosphorus in soils and plants–facing phosphorus scarcity. *Plant and Soil* **401,** 1-6.

Gil-Sotres F, Zech W & Alt H 1990: Characterization of phosphorus fractions in surface horizons of soils from Galicia (NW Spain) by 31P NMR spectroscopy. *Soil Biology and Biochemistry* **22,** 75-79.

Glendinning J 2000: *Australian soil fertility manual,* CSIRO Pub.

González Medeiros JJ, Pérez Cid B & Fernández Gómez E 2005: Analytical phosphorus fractionation in sewage sludge and sediment samples. *Analytical and Bioanalytical Chemistry* **381,** 873-878.

Griffin T, Honeycutt C & He Z 2003: Changes in soil phosphorus from manure application. *Soil Science Society of America Journal* **67,** 645-653.

Güsewell S 2004: N: P ratios in terrestrial plants: variation and functional significance. *New Phytologist* **164,** 243-266.

Harrison AF 1987: Soil organic phosphorus: a review of world literature. Commonwealth Agricultural Bureaux International.

He Z, Honeycutt CW, Zhang T & Bertsch PM 2006: Preparation and FT–IR characterization of metal phytate compounds. *Journal of Environmental Quality* **35,** 1319-1328.

He Z, Zhang H, Toor GS *et al.* 2010: Phosphorus distribution in sequentially extracted fractions of biosolids, poultry litter, and granulated products. *Soil Science* **175,** 154-161.

Hedley AK & Bernstein ML 1982: External fixation as a secondary procedure. *Current Concepts of External Fixation of Fractures,* pp 263-272. Springer.

Hedley M & Stewart J 1982: Method to measure microbial phosphate in soils. *Soil Biology and Biochemistry* **14,** 377-385.

Hill JE, Richardson AE, Turner B, Richardson A & Mullaney E 2007: Isolation and assessment of microorganisms that utilize phytate. *Inositol phosphates: Linking Agriculture and The Environment***,** 61-77.

Hinsinger P, Gobran GR, Gregory PJ & Wenzel WW 2005: Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. *New Phytologist* **168,** 293-303.

Hoch WA, Zeldin EL & McCown BH 2001: Physiological significance of anthocyanins during autumnal leaf senescence. *Tree Physiology* **21,** 1-8.

Holford I 1997: Soil phosphorus: its measurement, and its uptake by plants. *Soil Research* **35,** 227-240.

Hollings PE, Dutch ME & Stout JD 1969: Bacteria of four tussock grassland soils on the old man range, Central Otago, New Zealand. *New Zealand Journal of Agricultural Research* **12,** 177-192.

Huang X-L, Chen Y & Shenker M 2008: Chemical fractionation of phosphorus in stabilized biosolids. *Journal of Environmental Quality* **37,** 1949-1958.

Hue N, Craddock G & Adams F 1986: Effect of organic acids on aluminum toxicity in subsoils. *Soil Science Society of America Journal* **50,** 28-34.

Hutchens E 2009: Microbial selectivity on mineral surfaces: possible implications for weathering processes. *Fungal Biology Reviews* **23,** 115-121.

Illmer P, Barbato A & Schinner F 1995: Solubilization of hardly-soluble AlPO 4 with P-solubilizing microorganisms. *Soil Biology and Biochemistry* **27,** 265-270.

Iyamuremye F, Dick R & Baham J 1996: Organic amendments and phosphorus dynamics: I. Phosphorus chemistry and sorption. *Soil Science* **161,** 426-435.

Jansa J, Mozafar A & Frossard E 2005: Phosphorus acquisition strategies within arbuscular mycorrhizal fungal community of a single field site. *Plant and Soil* **276,** 163-176.

Jing J, Christensen JT, Sørensen P, Christensen BT & Rubæk GH 2019: Long-term effects of animal manure and mineral fertilizers on phosphorus availability and silage maize growth. *Soil Use and Management*.

Johnson AH, Frizano J & Vann DR 2003: Biogeochemical implications of labile phosphorus in forest soils determined by the Hedley fractionation procedure. *Oecologia* **135,** 487-499.

Jones DL & Darrah PR 1994: Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant and Soil* **166,** 247-257.

Jones MD & Smith SE 2004: Exploring functional definitions of mycorrhizas: are mycorrhizas always mutualisms? *Canadian Journal of Botany* **82,** 1089-1109.

Kanabo I & Gilkes R 1987: The role of soil pH in the dissolution of phosphate rock fertilizers. *Fertilizer Research* **12,** 165-173.

Khan KS & Joergensen RG 2009: Changes in microbial biomass and P fractions in biogenic household waste compost amended with inorganic P fertilizers. *Bioresource Technology* **100,** 303-309.

Khan MS, Zaidi A & Wani PA 2007: Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. *Agronomy for Sustainable Development* **27,** 29-43.

Khan MS, Zaidi A & Wani PA 2009: Role of phosphate solubilizing microorganisms in sustainable agriculture-a review. *Sustainable Agriculture,* pp 551-570. Springer.

Khasawneh F & Doll E 1979: The use of phosphate rock for direct application to soils. *Advances in Agronomy,* pp 159-206. Elsevier.

Kladivko EJ & Unger P 1994: Residue effects on soil physical properties. *Managing Agricultural Residues. Lewis Publishers, Boca Raton, FL***,** 123-141.

Koopmans G, Chardon W, Dolfing J, Oenema O, Van der Meer P & Van Riemsdijk W 2003: Wet chemical and phosphorus-31 nuclear magnetic resonance analysis of phosphorus speciation in a sandy soil receiving long-term fertilizer or animal manure applications. *Journal of Environmental Quality* **32,** 287-295.

Kouno K, Tuchiya Y & Ando T 1995: Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. *Soil Biology and Biochemistry* **27,** 1353-1357.

Laboski CA & Lamb JA 2003: Changes in soil test phosphorus concentration after application of manure or fertilizer. *Soil Science Society of America Journal* **67,** 544-554.

Lambers H & Chapin III E 1998: III & Pons, TL (1998) Plant Physiological Ecology. Springer-Verlag, New York.

Lewis D & Quirk J 1967: Phosphate diffusion in soil and uptake by plants. *Plant and Soil* **26,** 99-118.

Li X-L, George E & Marschner H 1991: Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil. *Plant and Soil* **136,** 41-48.

Lindsay W & Moreno E 1960: Phosphate phase equilibria in soils. *Soil Science Society of America Journal* **24,** 177-182.

Lobo TF, Grassi Filho H, Büll LT & Moreira LLQ 2013: Management of sewage sludge and mineral nitrogen in soil fertility over time. *Semina: Ciências Agrárias* **34,** 2705-2726.

Ma Z, Bielenberg D, Brown K & Lynch J 2001: Regulation of root hair density by phosphorus availability in Arabidopsis thaliana. *Plant, Cell & Environment* **24,** 459-467.

Maguire RO, Sims JT, Dentel SK, Coale FJ & Mah JT 2001: Relationships between biosolids treatment process and soil phosphorus availability. *Journal of Environmental Quality* **30,** 1023-1033.

Maier NA, McLaughlin MJ, Heap M, Butt M & Smart MK 2002: Effect of current-season application of calcitic lime and phosphorus fertilization on soil pH, potato growth, yield, dry matter content, and cadmium concentration. *Communications in Soil Science and Plant Analysis* **33,** 2145-2165.

Marschner H, Römheld V & Cakmak I 1987: Root-induced changes of nutrient availability in the rhizosphere. *Journal of Plant Nutrition* **10,** 1175-1184.

Marschner P 2008: The role of rhizosphere microorganisms in relation to P uptake by plants. *The Ecophysiology of Plant-Phosphorus Interactions,* pp 165-176. Springer.

Marschner P & Rengel Z 2012: Nutrient availability in soils. *Marschner's mineral nutrition of higher plants,* pp 315-330. Elsevier.

Marschner P, Yang C-H, Lieberei R & Crowley D 2001: Soil and plant specific effects on bacterial community composition in the rhizosphere. *Soil Biology and Biochemistry* **33,** 1437-1445.

Martin J & Cunningham R 1973: Factors controlling the release of phosphorus from decomposing wheat roots. *Australian Journal of Biological Sciences* **26,** 715-728.

Martin T, Oswald O & Graham IA 2002: Arabidopsis seedling growth, storage lipid mobilization, and photosynthetic gene expression are regulated by carbon: nitrogen availability. *Plant Physiology* **128,** 472-481.

Mason S, Hamon R, Zhang H & Anderson J 2008: Investigating chemical constraints to the measurement of phosphorus in soils using diffusive gradients in thin films (DGT) and resin methods. *Talanta* **74,** 779-787.

Mason S, McNeill A, McLaughlin MJ & Zhang H 2010: Prediction of wheat response to an application of phosphorus under field conditions using diffusive gradients in thin-films (DGT) and extraction methods. *Plant and Soil* **337,** 243-258.

Mason SD, McLaughlin MJ, Johnston C & McNeill A 2013: Soil test measures of available P (Colwell, resin and DGT) compared with plant P uptake using isotope dilution. *Plant and Soil* **373,** 711-722.

McBeath T, McLaughlin MJ, Armstrong R *et al.* 2007: Predicting the response of wheat (Triticum aestivum L.) to liquid and granular phosphorus fertilisers in Australian soils. *Soil Research* **45,** 448-458.

McBride M 2003: Toxic metals in sewage sludge-amended soils: has promotion of beneficial use discounted the risks? *Advances in Environmental Research* **8,** 5-19.

McKercher R & Anderson G 1989: Organic phosphate sorption by neutral and basic soils. *Communications in Soil Science & Plant Analysis* **20,** 723-732.

McLaren R, Clucas L, Taylor M & Hendry T 2004: Leaching of macronutrients and metals from undisturbed soils treated with metal-spiked sewage sludge. 2. Leaching of metals. *Soil Research* **42,** 459-471.

McLaren TI, Simpson RJ, McLaughlin MJ *et al.* 2015a: An assessment of various measures of soil phosphorus and the net accumulation of phosphorus in fertilized soils under pasture. *Journal of Plant Nutrition and Soil Science* **178,** 543-554.

McLaren TI, Smernik RJ, Guppy CN, Bell MJ & Tighe MK 2014: The organic P composition of Vertisols as determined by 31 P NMR spectroscopy. *Soil Science Society of America Journal* **78,** 1893-1902.

McLaren TI, Smernik RJ, McLaughlin MJ *et al.* 2015b: Complex forms of soil organic phosphorus–A major component of soil phosphorus. *Environmental Science & Technology* **49,** 13238-13245.

McLaren TI, Smernik RJ, McLaughlin MJ *et al.* 2019: Soil phosphorus pools with addition of fertiliser phosphorus in a long-term grazing experiment. *Nutrient Cycling in Agroecosystems***,** 1-14.

McLaughlin MJ, Alston A & Martin J 1986: Measurement of phosphorus in the soil microbial biomass: a modified procedure for field soils. *Soil Biology and Biochemistry* **18,** 437-443.

McLaughlin MJ, Alston A & Martin J 1988: Phosphorus cycling in wheat-pasture rotations. III. Organic phosphorus turnover and phosphorus cycling. *Soil Research* **26,** 343-353.

Menzies NW, Kusumo B & Moody PW 2005: Assessment of P availability in heavily fertilized soils using the diffusive gradient in thin films (DGT) technique. *Plant and Soil* **269,** 1-9.

Miethling R, Wieland G, Backhaus H & Tebbe C 2000: Variation of microbial rhizosphere communities in response to crop species, soil origin, and inoculation with Sinorhizobium meliloti L33. *Microbial Ecology* **40,** 43-56.

Mitsuhashi N, Ohnishi M, Sekiguchi Y *et al.* 2005: Phytic acid synthesis and vacuolar accumulation in suspension-cultured cells of Catharanthus roseus induced by high concentration of inorganic phosphate and cations. *Plant Physiology* **138,** 1607-1614.

Müller R, Nilsson L, Nielsen LK & Hamborg Nielsen T 2005: Interaction between phosphate starvation signalling and hexokinase-independent sugar sensing in Arabidopsis leaves. *Physiologia Plantarum* **124,** 81-90.

Nelson W, Mehlich A & Winters E 1953: The development, evaluation, and use of soil tests for phosphorus availability. *Agronomy* **4,** 153-188.

Noack SR, McBeath TM, McLaughlin MJ, Smernik RJ & Armstrong RD 2014: Management of crop residues affects the transfer of phosphorus to plant and soil pools: Results from a dual-labelling experiment. *Soil Biology and Biochemistry* **71,** 31-39.

Noack SR, McLaughlin MJ, Smernik RJ, McBeath TM & Armstrong RD 2012: Crop residue phosphorus: speciation and potential bio-availability. *Plant and Soil* **359,** 375-385.

Nziguheba G, Merckx R, Palm CA & Rao MR 2000: Organic residues affect phosphorus availability and maize yields in a Nitisol of western Kenya. *Biology and Fertility of Soils* **32,** 328-339.

Nziguheba G, Palm CA, Buresh RJ & Smithson PC 1998: Soil phosphorus fractions and adsorption as affected by organic and inorganic sources. *Plant And Soil* **198,** 159-168.

Oberson A & Joner EJ 2005: Microbial turnover of phosphorus in soil. *Organic Phosphorus In The Environment***,** 133-164.

Oberson A, Joner EJ, Turner B, Frossard E & Baldwin D 2005: Microbial turnover of phosphorus in soil. *Organic Phosphorus In The Environment***,** 133-164.

Olsen S, Cole C, Watanabe F & Dean L 1954: Estimation of available phosphorus in soils by extraction with NaHCO3. *US Department of Agriculture Circular* **939**.

Parfitt R, Fraser A & Farmer V 1977: Adsorption on hydrous oxides. III. Fulvic acid and humic acid on goethite, gibbsite and imogolite. *Journal of Soil Science* **28,** 289-296.

Paul K, Polglase P, O'connell A, Carlyle J, Smethurst P & Khanna P 2003: Defining the relation between soil water content and net nitrogen mineralization. *European Journal of Soil Science* **54,** 39-48.

Perrott K, Sarathchandra S & Waller J 1990: Seasonal storage and release of phosphorus and potassium by organic matter and the microbial biomass in a high producing pastoral soil. *Soil Research* **28,** 593-608.

Pierzynski GM, McDowell RW & Thomas Sims J 2005: Chemistry, cycling, and potential movement of inorganic phosphorus in soils. *Phosphorus: Agriculture and The Environment* **46,** 51-86.

Potter R & Benton T 1916: The Organic Phosphorus of Soil 1. *Soil Science* **2,** 291.

Potter R, Jordan C, Guedes R, Batmanian G & Han X 1991: Assessment of a phosphorus fractionation method for soils: problems for further investigation. *Agriculture, Ecosystems & Environment* **34,** 453-463.

Raghothama K & Karthikeyan A 2005: Phosphate acquisition. *Root Physiology: from Gene to Function,* pp 37-49. Springer.

Rajan S, Brown M, Boyes M & Upsdell M 1992: Extractable phosphorus to predict agronomic effectiveness of ground and unground phosphate rocks. *Fertilizer Research* **32,** 291-302.

Rajan S, Watkinson J & Sinclair A 1996: Phosphate rocks for direct application to soils. *Advances in Agronomy* **57,** 77-159.

Read D & Perez-Moreno J 2003: Mycorrhizas and nutrient cycling in ecosystems–a journey towards relevance? *New Phytologist* **157,** 475-492.

Redecker D & Raab P 2006: Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia* **98,** 885-895.

Rice CW, White P, Fabrizzi K & Wilson G 2004: Managing the microbial community for soil carbon management. *SuperSoil* **2004,** 3rd.

Richardson A, Pankhurst C, Doube B, Gupta V & Grace P 1994: Soil microorganisms and phosphorus availability. *Soil biota: management in sustainable farming systems.***,** 50-62.

Richardson AE 2001: Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Functional Plant Biology* **28,** 897-906.

Richardson AE, Hocking PJ, Simpson RJ & George TS 2009: Plant mechanisms to optimise access to soil phosphorus. *Crop and Pasture Science* **60,** 124-143.

Richardson AE, Lynch JP, Ryan PR *et al.* 2011: Plant and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant and Soil* **349,** 121-156.

Ryan P, Delhaize E & Jones D 2001: Function and mechanism of organic anion exudation from plant roots. *Annual Review Of Plant Biology* **52,** 527-560.

Savant N & Racz G 1973: Reaction products of applied pyrophosphates in some Manitoba soils. *Canadian Journal of Soil Science* **53,** 111-117.

Schachtman DP, Reid RJ & Ayling SM 1998: Phosphorus uptake by plants: from soil to cell. *Plant Physiology* **116,** 447-453.

Schefe CR, Barlow KM, Robinson NJ *et al.* 2015: 100 Years of superphosphate addition to pasture in an acid soil—current nutrient status and future management. *Soil Research* **53,** 662-676.

Schilling G, Gransee A, Deuhel A, Ležoviž G & Ruppel S 1998: Phosphorus availability, root exudates, and microbial activity in the rhizosphere. *Zeitschrift für Pflanzenernährung und Bodenkunde* **161,** 465-478.

Schmidt IK, Jonasson S & Michelsen A 1999: Mineralization and microbial immobilization of N and P in arctic soils in relation to season, temperature and nutrient amendment. *Applied Soil Ecology* **11,** 147-160.

Schnepf A, Roose T & Schweiger P 2008: Impact of growth and uptake patterns of arbuscular mycorrhizal fungi on plant phosphorus uptake—a modelling study. *Plant and Soil* **312,** 85-99.

Sharma S & Prasad R 2003: Yield and P uptake by rice and wheat grown in a sequence as influenced by phosphate fertilization with diammonium phosphate and Mussoorie rock

phosphate with or without crop residues and phosphate solubilizing bacteria. *The Journal of Agricultural Science* **141,** 359-369.

Sharpley A, Foy B & Withers P 2000: Practical and innovative measures for the control of agricultural phosphorus losses to water: An Overview. *Journal of Environmental Quality* **29,** 1-9.

Sharpley A & Tunney H 2000: Phosphorus research strategies to meet agricultural and environmental challenges of the 21st century. *Journal of Environmental Quality* **29,** 176-181.

Shen J, Yuan L, Zhang J *et al.* 2011: Phosphorus Dynamics: from soil to plant. *Plant Physiology* **156,** 997-1005.

Shen Y, Ström L, Jönsson J-Å & Tyler G 1996: Low-molecular organic acids in the rhizosphere soil solution of beech forest (Fagus sylvatica L.) cambisols determined by ion chromatography using supported liquid membrane enrichment technique. *Soil Biology and Biochemistry* **28,** 1163-1169.

Sibanda H & Young S 1986: Competitive adsorption of humus acids and phosphate on goethite, gibbsite and two tropical soils. *Journal of Soil Science* **37,** 197-204.

Simpson RJ, Stefanski A, Marshall DJ, Moore AD & Richardson AE 2015: Management of soil phosphorus fertility determines the phosphorus budget of a temperate grazing system and is the key to improving phosphorus efficiency. *Agriculture, Ecosystems & Environment* **212,** 263-277.

Singh B & Jones J 1976: Phosphorous sorption and desorption characteristics of soil as affected by organic residues. *Soil Science Society of America Journal* **40,** 389-394.

Singh J, Raghubanshi A, Singh R & Srivastava S 1989: Microbial biomass acts as a source of plant nutrients in dry tropical forest and savanna. *Nature* **338,** 499-500.

Smernik RJ & Dougherty WJ 2007: Identification of phytate in phosphorus-31 nuclear magnetic resonance spectra: The need for spiking. *Soil Science Society of America Journal* **71,** 1045-1050.

Smyth T & Sanchez P 1982: Phosphate Rock Dissolution and Availability in Cerrado Soils as Affected by Phosphorus Sorption Capacity 1. *Soil Science Society of America Journal* **46,** 339-345.

Solaiman MZ, Ezawa T, Kojima T & Saito M 1999: Polyphosphates in intraradical and extraradical hyphae of an arbuscular mycorrhizal fungus, Gigaspora margarita. *Applied and Environmental Microbiology* **65,** 5604-5606.

Sumann M, Amelung W, Haumaier L & Zech W 1998: Climatic effects on soil organic phosphorus in the North American Great Plains identified by phosphorus-31 nuclear magnetic resonance. *Soil Science Society of America Journal* **62,** 1580-1586.

Syers J, Johnston A & Curtin D 2008: Efficiency of soil and fertilizer phosphorus use. *FAO Fertilizer and Plant Nutrition Bulletin* **18**.

Takanishi I, Ohtomo R, Hayatsu M & Saito M 2009: Short-chain polyphosphate in arbuscular mycorrhizal roots colonized by Glomus spp.: A possible phosphate pool for host plants. *Soil Biology and Biochemistry* **41,** 1571-1573.

Tian J, Boitt G, Black A, Wakelin S, Condron LM & Chen L 2017: Accumulation and distribution of phosphorus in the soil profile under fertilized grazed pasture. *Agriculture, Ecosystems & Environment* **239,** 228-235.

Tibbett M & Sanders F 2002: Ectomycorrhizal symbiosis can enhance plant nutrition through improved access to discrete organic nutrient patches of high resource quality. *Annals of Botany* **89,** 783-789.

Tiessen H 2008: Phosphorus in the global environment. *The ecophysiology of plant-phosphorus interactions,* pp 1-7. Springer.

Truog E 1930: The determination of the readily available phosphorus of soils. *Agronomy Journal* **22,** 874-882.

Tunney H, Carton O, Brookes P & Johnston A 1997: *Phosphorus loss from soil to water,* CAB international.

Turner BL, McKelvie ID & Haygarth PM 2002a: Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. *Soil Biology and Biochemistry* **34,** 27-35.

Turner BL, Papházy MJ, Haygarth PM & McKelvie ID 2002b: Inositol phosphates in the environment. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* **357,** 449-469.

van Hees PA, Jones DL & Godbold DL 2002: Biodegradation of low molecular weight organic acids in coniferous forest podzolic soils. *Soil Biology and Biochemistry* **34,** 1261-1272.

Van Meeteren M, Tietema A & Westerveld J 2007: Regulation of microbial carbon, nitrogen, and phosphorus transformations by temperature and moisture during decomposition of Calluna vulgaris litter. *Biology and Fertility of Soils* **44,** 103-112.

Van Straaten P 2002: *Rocks for crops: agrominerals of sub-Saharan Africa,* Citeseer.

Vance CP, Uhde-Stone C & Allan DL 2003: Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist* **157,** 423-447.

Vincent AG, Schleucher J, Gröbner G *et al.* 2012: Changes in organic phosphorus composition in boreal forest humus soils: the role of iron and aluminium. *Biogeochemistry* **108,** 485-499.

Waigwa M, Othieno C & Okalebo J 2003: Phosphorus availability as affected by the application of phosphate rock combined with organic materials to acid soils in western Kenya. *Experimental Agriculture* **39,** 395-407.

Wang X, Lester D, Guppy C, Lockwood P & Tang C 2007a: Changes in phosphorus fractions at various soil depths following long-term P fertiliser application on a Black Vertosol from south-eastern Queensland. *Soil Research* **45,** 524-532.

Wang X, Lester D, Guppy C, Lockwood PV & Tang C 2007b: Changes in phosphorus fractions at various soil depths following long-term P fertiliser application on a Black Vertosol from south-eastern Queensland. *Soil Research* **45,** 524-532.

Wilson MA & Ellis B 1984: Influence of calcium solution activity and surface area on the solubility of selected rock phosphates. *Soil Science* **138,** 354-359.

Yan N, Marschner P, Cao W, Zuo C & Qin W 2015: Influence of salinity and water content on soil microorganisms. *International Soil and Water Conservation Research* **3,** 316-323.

Yang C-H & Crowley DE 2000: Rhizosphere microbial community structure in relation to root location and plant iron nutritional status. *Applied and Environmental Microbiology* **66,** 345-351.

Yang X & Post WM 2011: Phosphorus transformations as a function of pedogenesis: A synthesis of soil phosphorus data using Hedley fractionation method. *Biogeosciences* **8,** 2907-2916.

Ye G, Lin Y, Kuzyakov Y *et al.* 2019: Manure over crop residues increases soil organic matter but decreases microbial necromass relative contribution in upland Ultisols: Results of a 27-year field experiment. *Soil Biology and Biochemistry*.

Yeates G 1997: Soil microbial C, N, P, and microfaunal populations under Pinus radiata and grazed pasture land-use systems. *Pedobiologia* **41,** 549-565.

Zebarth B, Neilsen G, Hogue E & Neilsen D 1999: Influence of organic waste amendments on selected soil physical and chemical properties. *Canadian Journal of Soil Science* **79,** 501-504.

Zech W, Alt HG, Haumaier L & Blasek R 1987: Characterization of phosphorus fractions in mountain soils of the Bavarian Alps by 31P NMR spectroscopy. *Zeitschrift für Pflanzenernährung und Bodenkunde* **150,** 119-123.

Zhang T & MacKenzie A 1997: Changes of soil phosphorous fractions under long-term corn monoculture. *Soil Science Society of America Journal* **61,** 485-493.

Chapter 2

Plant and microbial-induced changes in P pools in soil amended with straw and inorganic P

Khuyen Thi Kim Hoang[1,2] and Petra Marschner[1]

[1]School of Agriculture, Food and Wine, The University of Adelaide, Adelaide SA 5005, Australia.

[2]Chemical Engineering Faculty, Industrial University of Ho Chi Minh, HCMC 7000, Vietnam

The work contained in this chapter is published in Journal of Soil Science and Plant Nutrition

Hoang, K. K. T., & Marschner, P. (2017). Plant and microbial-induced changes in P pools in soil amended with straw and inorganic P. *Journal of Soil Science and Plant Nutrition, 17*(4), 1088-1101.

Statement of Authorship

Title of Paper	Plant and microbial-induced changes in P pools in soil amended with straw and inorganic P
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted w ork w ritten in manuscript style
Publication Details	Khuyen Thi Kim Hoang and Petra Marschner

Principal Author

Name of Principal Author (Candidate)	Khuyen Thi Kim Hoang		
Contribution to the Paper	Performed experiment, analysed soil samples, analysed and interpreted data and wrote the manuscript		
Overall percentage (%)			
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	18/09/2019

Co-Author Contributions

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.

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of work, data interpretation, manuscript evaluation and correction. She also acted as corresponding author		
Signature		Date	18/09/2019

Journal of Soil Science and Plant Nutrition, 2017, 17 (4), 1088-1101
RESEARCH ARTICLE

Plant and microbial-induced changes in P pools in soil amended with straw and inorganic P

Khuyen Thi Kim Hoang[1,2] and Petra Marschner[1*]

*[1]School of Agriculture, Food and Wine, The University of Adelaide, Adelaide SA 5005, Australia. [2]Chemical Engineering Faculty, Industrial University of Ho Chi Minh, HCMC 7000, Vietnam. *Corresponding author: petra.marschner@adelaide.edu.au*

Abstract

The aim of this study was to determine the effect of soil amendments on P pools and their depletion by plants and microbes. The experiment was divided into two parts. In Part A, barley straw (C/P 255) was added alone or with inorganic P to reduce the C/P ratio to 127 or 25 (straw treatments). In three other treatments, the same amount of P was added as in the straw treatments as inorganic P (fertilizer treatments). The soil was incubated for three weeks. Then (Part B), wheat was grown for five weeks in one set of soils. Barley straw was added to another set to increase microbial growth and incubated moist for three weeks. P pools were measured at the end of Part A and Part B. At the end of Part A all P pools increased with P addition rate. P pools were greater in the fertilizer than the straw treatments except for microbial biomass P (MBP) which was greater in the straw treatments. After wheat growth, HCl-P, phosphatase-P, citrate-P, $CaCl_2$-P were depleted compared to Part A, MBP was higher. Addition of barley straw in Part B induced depletion of HCl-P, and citrate-P, but increased phosphatase-P, $CaCl_2$-P and MBP. It is concluded that the size of the P pools is mainly influenced by P addition rate not form in which P is added. The impact of growing plants on P pools differs from that of microbes stimulated by high C/P straw addition.

Keywords: Accumulation, C/P ratio, depletion, P pools, straw

1. Introduction

Phosphorus is an essential nutrient for all living organisms (Tiessen *et al.*, 2011), but P fertilizer resources are finite, therefore P management in cropping systems is important for future food security. To optimize P management and improve P use efficiency an understanding of P pools and their changes in soil is necessary (Balemi and Negisho, 2012). In soils, P is present in different inorganic and organic pools with varying availability. Changes in P pools in the soil-plant system are a function of P transformations and utilisation which are affected by soil properties (Alamgir and Marschner, 2016, Chatterjee *et al*., 2014) rhizosphere, and plant processes (Pierzynski *et al.*, 1990, Shen *et al.*, 2011, Ciampitti *et al*., 2011). It has been shown that the effect of crop residues on P availability and P pools depends on their C/P ratio (Dalal, 1979). Crop residues with C/P > 200 induce net P immobilisation and depletion of P pools, residues with lower C/P ratio increase P availability and P pools (Alamgir *et al*., 2012). Maltais-Landry and Frossard (2015) found that residues or water-soluble P fertilizer added at 15 mg kg^{-1} had similar effects on microbial and resin P and plant P uptake. However, it is unclear if P added at different rates as combination of crop residues and inorganic P or inorganic P alone differ in their effect on soil P pools and subsequent changes in P pools induced by plants or microbes. Organic amendments are likely to increase microbial growth and synthesis of organic P forms whereas fertilizer P may increase mainly inorganic P pools. But it is unclear if plants or microbes stimulated by straw addition differ in their influence on P pools in soil amended with organic or inorganic P amendments.

The Hedley P fractionation scheme is often used to assess soil P pools (Hedley *et al*., 1982). But it is time consuming and involves concentrations of extractants that are unlikely to occur in soils. DeLuca *et al.* (2015) recently introduced a simplified method to determine biologically available P pools. The method involves parallel extraction of soil with $CaCl_2$, citrate, phosphatase and HCl. DeLuca *et al.* (2015) consider these pools as biologically available because roots or microbes may release organic acid anions, phosphatase or protons and thus access these pools. However, it is not clear if the pools assessed by the DeLuca method are indeed available to plants and microbes.

The aims of this study were to assess (i) changes in P pools after addition of P as high C/P cereal straw, cereal straw plus inorganic P to reduce C/P ratio or inorganic P alone, and (ii) changes in these P pools by plants or microbes stimulated by high C/P straw addition. The hypotheses were (1) at a given P addition rate, the treatments with straw will induce a higher concentration of phosphatase extractable P and MBP than the treatments with inorganic P only, and (2) subsequent plant growth will induce depletion of all P pools whereas stimulation of microbes by straw addition will lead to build-up of microbial and phosphatase extractable P.

2. Materials and Methods

A loamy sand was collected from 0 to 10 cm on Waite Campus, The University of Adelaide, South Australia (Longitude 138° 38'E, Latitude 35° 6'S) which had been under permanent pasture over 80 years. The soil was used because previous studies had shown that P pools in this soil changed after amendment with organic materials (Elmajdoub, personal communication). The soil was dried at 40°C and passed through a 2 mm sieve. This temperature is not unusual because in South Australia, surface soils are often exposed to temperatures exceeding 40°C during summer. The properties of the soil are as follows: pH 6.8 (1:5 soil/water); clay

25%; sand 37%; silt 37%; total P 302 mg kg^{-1}; pH (1:5) 5.6, EC (1:5) 0.1 dS m^{-1}, total organic C 17 g kg^{-1}, total organic N 1.5 g kg^{-1}, bulk density 1.3 g cm^{-3}, maximum water-holding capacity 349 g kg^{-1}. The properties of the mature barley straw (finely ground and sieved to particle size of 0.25–2 mm) used in this experiment were: total P 1.6 g kg^{-1}; total N 4.3 g kg^{-1}; total C 408 g kg^{-1}; C/P ratio 255 and C/N ratio 95.

2.1. Experimental design

The soil was pre-incubated for 10 days at 50% water-holding capacity. Previous studies with this soil had shown that at this water content soil respiration as indicator of microbial activity is maximal (Marschner *et al.*, 2015) and wheat grows well (Xue *et al*., 2016). The experiment included seven treatments with four replicates each. The control was unamended. There were three treatments with barley straw (20 g kg^{-1}): only straw (C/P 255, P added with straw 32 mg kg^{-1}), straw + KH_2PO_4 to adjust the C/P ratio to 127 or 25. No straw was added in the three fertilizer treatments, which received only KH_2PO_4 at the same amount of P as in the straw treatments (straw + inorganic P). For treatment names and inorganic P addition rates see Table 1. To provide sufficient N for straw decomposition, all treatments received 1.3 g KNO_3 kg^{-1} soil to adjust the C/N ratio of the straw treatments to 30. Residues and inorganic nutrients were mixed thoroughly into the soil.

Table 1. Treatment names, C/P ratio, barley straw and inorganic P added (mg kg^{-1}).

Control		-	0
S255	255	+	0
S127	127	+	32
S25	25	+	288
P255		-	32
P127		-	64
P25		-	320

*P added with straw 32 mg kg^{-1}

Then, two experimental units were set up. One unit in pots (400 g soil per pot) to assess changes in P pools after plant growth and a second unit in cores (30 g core) to determine changes in P pools by soil microbes. Cores were used because they have a mesh bottom to ensure adequate aeration.

Soil (400 g dry weight equivalent) was placed in 1L pots lined with plastic bags. PVC cores with 3.7 cm diameter, 5 cm height and a nylon mesh base (7.5 µm, Australian Filter Specialist) were filled with 30 g soil (dry weight equivalent, bulk density adjusted to 1.3 g cm^{-3}). Pots and cores were incubated in the dark at 22-25 °C for 21 days to allow formation of P pools. During incubation, soil moisture was maintained at 50% of WHC by weighing pots and cores regularly and adding water if necessary. This 21-day period is referred to as Part A. The soil was incubated for three weeks because previous studies in our lab with the

same soil (Elmajdoub, personal communication) had shown that after organic amendments, the size of soil P pools increases for three weeks, but then remains stable. At the start of the following Part B, 10 pre-germinated wheat seeds (*Triticum aestivum* L., variety Axe) were planted in the pots and then grown for five weeks in a glasshouse. The temperature in the glasshouse during the experiment ranged from 25 to 35 °C. For the cores, barley straw (10 g kg^{-1}) was thoroughly mixed into the soil at the start of Part B to stimulate microbial growth. The soil was incubated for 3 weeks. This period is referred to as Part B. Soil water content was maintained at 50% WHC by weight with reverse osmosis water. During wheat growth (Part B), pots were watered daily. In Part A and in the cores in Part B, soil water content changed more slowly and was adjusted every 2-3 days.

2.2. Analyses

Soil texture was determined by the hydrometer method (Gee and Or, 2002). Soil maximum water holding capacity was measured using a sintered glass funnel connected to a 1 m water column (Wilke, 2005). Soil pH was measured after 1 h end-over-end shaking in a 1:5 soil:water ratio. Total organic carbon of residues was determined by $K_2Cr_2O_7$ and H_2SO_4 oxidation by titration with acidified $(NH_4)_2Fe(SO_4)_2.6H_2O$ (Walkley and Black, 1934). Total P in soil was determined by the phosphovanado molybdate method (Hanson, 1950) after acid digestion with nitric acid-perchloric acid at a 4:1 ratio (Olsen *et al*., 1982). Total P of shoot and plant residues was digested with nitric acid and H_2O_2 at a 4:1 ratio and also determined by the phosphovanado molybdate method (Hanson, 1950). Total N in straw was measured by a modified Kjeldahl method (Bremner and Breitenbeck, 1983).

At the end of Part A and Part B the following properties were determined. Soil available N (ammonium and nitrate) concentration was measured after 1h end-over-end shaker with 2 M KCl at 1:5 soil extractant ratio. Ammonium-N was determined by Willis *et al.* (1996) and nitrate-N as described in Miranda *et al.* (2001). Available P in soil was extracted by the anion exchange resin method (Kouno *et al*., 1995). This method was also used to determine MBP, but using hexanol instead of chloroform as fumigant. The P concentration was determined colorimetrically (Murphy and Riley, 1962). MBP was calculated as the difference between available P and P extracted with hexanol. Soil P pools were determined according to Deluca *et al*. (2015) using four extractants in parallel, including 10 mM citric acid, 1M HCl, 10 mM $CaCl_2$ and 0.2 enzyme unit phosphatase (from wheat germ) extractable P. Moist soil equivalent to 0.5 g dry soil were shaken with 10 ml of each extractant separately for 3 hrs (DeLuca *et al*., 2015). P in the extracts was determined by the malachite-green method (Ohno and Zibilske, 1991).

2.3. Statistical analysis

Data were analysed separately for pots and cores by one-way ANOVA for each sampling time with Genstat 15th edition (VSN Int. Ltd., UK). Tukey's multiple comparison tests at 95% confidence interval was used to determine significant differences among treatments.

3. Results

At the end of the 3-week incubation (Part A), the size of all P pools increased with P addition rate (Figures 1, 2; for treatment names and details see Table 1). HCl-P was the largest pool, followed by citrate-P and resin P. Phosphatase P, $CaCl_2$-P and microbial P were up to 10-fold lower than HCl-P. At the lowest P addition rate (S255 and P255), most P pools were similar as the control, except for MBP in S255 which

was about two-fold higher than the control. Most P pools (except MBP) were greater in P127 and P25 than the respective straw treatments (S127, S25). In contrast, MBP at a given P addition rate was about two-fold higher in the straw than the fertilizer treatments.

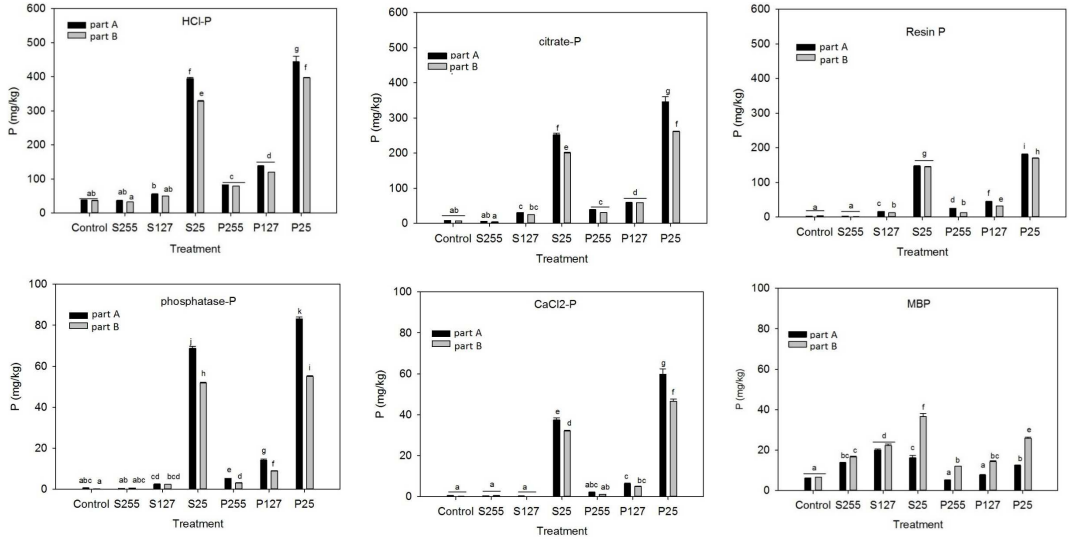

Figure 1. Concentration of P pools at the end of the three-week incubation (part A) and after five weeks growth of wheat (part B) in soil amended at the start of Part A with straw (S255, S127, S25) or inorganic P (P255, P127, P25). For treatments see Table 1. For each part, bars with different letters are significantly different (n=4, P≤0.05). Vertical lines indicate standard errors.

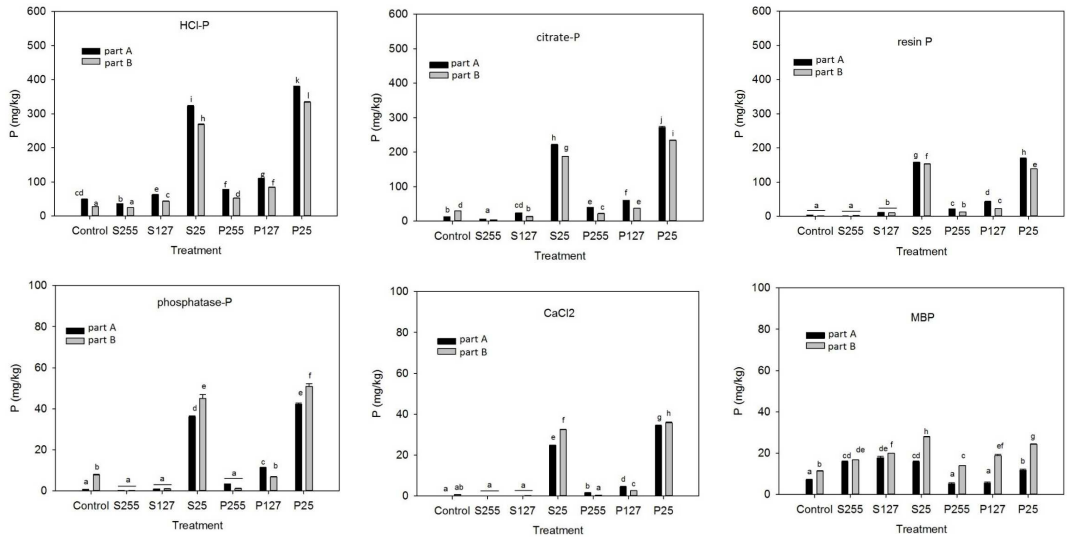

Figure 2. Concentration of P pools at the end of the first 3-week incubation (part A) and after the second 3-week incubation with straw (part B). In soil amended at the start of Part A with straw (S255, S127, S25) or inorganic P (P255, P127, P25). For treatments see Table 1. For each part, bars with different letters are significantly different (n=4, P≤0.05). Vertical lines indicate standard errors.

After growing wheat for five weeks (Part B), HCl-P, citrate-P, phosphatase P, CaCl2-P in S25 and P25 were 10-25% lower than at the end of part A with the largest difference in phosphatase P (Figure 1). This was also the case for phosphatase P in P255 and P127. Resin P was lower at the end of Part B than Part A in all fertilizer treatments. In contrast, MBP increased from Part A to Part B, in S25 and all fertilizer treatments. In S25 and P25 MBP was two-fold higher in Part B than Part A. P pools did not change from Part A to Part B in the control, S255 and S127.

Shoot dry weight was about 50% lower in straw treatments than the control whereas it was about 30% higher in the fertilizer treatments (Table 2). Root dry weight was lower in amended treatments than the control, particularly at the two highest P rates. The shoot/root ratio was about one in the control and was 0.5 ¬– 0.8 and 2.0 – 2.5 in the straw and fertilizer treatments, respectively (data not shown). The shoot/root ratio increased with P addition rate. Shoot P concentration was lowest in the control and S255. It increased with P addition rate and at a given P addition rate was higher in the straw than the fertilizer treatments. Shoot P uptake increased with P addition rate (Figure 3). At a given P addition rate, shoot P uptake was two to three-fold higher in fertilizer than the straw treatments. Compared to the control, shoot P uptake was lower in S255 and S127, 30% higher in S25 and about two-fold higher in P255 and P127, and five-fold higher in P25.

Table 2. Shoot and root biomass (g dry weight pot^{-1}) and shoot P concentration in wheat after five weeks growth in soil amended at the start of Part A with straw (S255, S127, S25) or inorganic P (P255, P127, P25). For treatments see Table 1.

	Shoot biomass ($g\ pot^{-1}$)	Root biomass ($g\ pot^{-1}$)	Shoot P concentration ($g\ kg^{-1}$)
Control	$0.73^{\pm 0.06}$ b	$0.64^{\pm 0.05}$ b	$2.32^{\pm 0.03}$ a
S255	$0.28^{\pm 0.01}$ a	$0.61^{\pm 0.02}$ b	$2.25^{\pm 0.06}$ a
S127	$0.34^{\pm 0.02}$ a	$0.55^{\pm 0.05}$ ab	$3.49^{\pm 0.09}$ b
S25	$0.36^{\pm 0.00}$ a	$0.55^{\pm 0.01}$ ab	$8.02^{\pm 0.13}$ d
P255	$1.06^{\pm 0.03}$ c	$0.52^{\pm 0.00}$ ab	$3.35^{\pm 0.06}$ b
P127	$1.03^{\pm 0.02}$ c	$0.52^{\pm 0.01}$ ab	$3.62^{\pm 0.03}$ b
P25	$1.14^{\pm 0.01}$ c	$0.46^{\pm 0.02}$ a	$7.09^{\pm 0.05}$ c

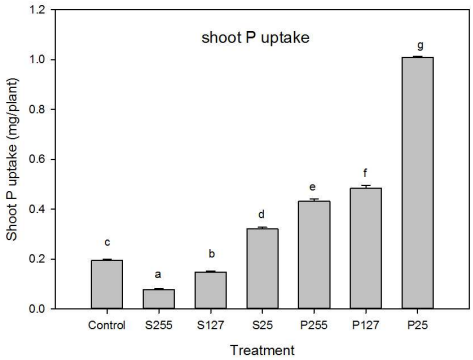

Figure 3. Wheat shoot P uptake (mg/plant) after five weeks growth in soil amended at the start of Part A with straw (S255, S127, S25) or inorganic P (P255, P127, P25). For treatments see Table 1. Bars with different letters are significantly different (n=4, P≤0.05). Vertical lines indicate standard errors.

In the cores, where microbial growth was stimulated by addition of barley straw at the start of Part B, HCl-P and citrate-P were about 10% lower at the end of Part B than in Part A (Figure 2). For HCl-P this was the case for all treatments, for citrate-P only for the fertilizer treatments, S127 and S25. In the fertilizer treatments, resin P was about 10% lower in Part B than Part A. MBP was higher in Part B than Part A with a two-fold increase in all fertilizer treatments and S25. However, in contrast to the pots, phosphatase-P and $CaCl_2$-P in S25 and P25 were up to 20% higher in Part B than Part A. In the control, HCl-P decreased from Part A to Part B whereas citrate-P, phosphatase-P and MBP increased.

Available N in Part A and Part B was lower in the straw treatments (0.3-0.4 g kg^{-1}) than the control and the fertilizer treatments (0.8-0.9 g kg^{-1}). The pH in soil with straw (6.3-6.9) was higher than pH in soil with fertilizer (5.8-6.3), and it was about 0.3-0.5 units higher in Part A compared to Part B.

4. Discussion

This study showed that P addition rate had a stronger effect on soil P pools at the end of Part A than the form in which P was added. In general, the lowest P addition rate did not influence P pools compared to the unamended control except MBP in S255 which was higher. This indicates that the straw provided enough P for limited microbial uptake compared to the unamended soil. The size of most P pools was greater in P127 and P25 than S127 and S25 except for MBP which was smaller. Greater microbial P uptake in S127 and S25 compared to P127 and P25 can only explain a small proportion of the difference between P127 and S127 in the other P pools. The greater P pools in the fertilizer treatments are likely due to the high solubility of KH_2PO_4 compared to P in straw. Although plant residues contain inorganic P (Noack *et al.*, 2012), a large proportion is in organic form and has to be mineralised before it can enter the HCl-P, citrate-P, $CaCl_2$-P and resin P pools. Therefore, the first hypothesis (at a given P addition rate, the treatments with straw will induce a higher concentration of phosphatase extractable P and MBP than the treatments with P fertilizer only) can only be confirmed for MBP. The higher phosphatase P concentration in the fertilizer treatments indicates that inorganic P addition stimulated synthesis of organic P. Both $CaCl_2$-P and resin P are considered to represent plant available P. But in this study, resin P was about four-fold higher than $CaCl_2$-P. This is likely due the removal of P from the extracting solution by the resin which induced release of labile P from soil particles. When the soil is shaken with $CaCl_2$, P will remain in solution and not result in a concentration gradient-induced release of labile P.

The first part of the second hypothesis (subsequent plant growth will induce depletion of all P pools whereas stimulation of microbes by straw addition will lead to build-up of microbial and phosphatase extractable P) cannot be confirmed because plant growth did not deplete resin P and increased MBP. However, the second part of the hypothesis can be confirmed. In the cores, where straw was added at the start of both Part A and Part B, phosphatase P and MBP where higher at the end of Part B than Part A. This can be explained by the second straw addition at the start of Part B which stimulated microbial growth resulting in higher microbial P uptake and phosphatase labile P (likely microbial metabolites).

Wheat growth induced a decrease of about 10% in HCl-P, citrate-P and phosphatase P which can be explained by plant P uptake and transformation into other pools. Resin P did not change and $CaCl_2$-P changed little from Part A to Part B. These P pools are considered to be plant-available (DeLuca *et al*., 2015, Hedley *et al*., 1982) and therefore likely to be depleted by plants and microbes. The depletion was probably compensated by release of P from HCl-P, citrate-P and phosphatase P by root or microbial exudates (Tarafdar and Claassen, 1988). Similarly, Hassan *et al.* (2012) reported that various legumes depleted P pools considered to be not readily available, probably by inducing transformation into available P pools. The strong increase in MBP from Part A to Part B particularly in the fertilizer treatments can be explained by C supply by roots (Jiang-shan *et al*., 2005). The increase was greater in fertilizer than straw treatments because in the latter, MBP was already high at the end of Part A.

In the cores, microbial growth was stimulated by straw addition. We had expected that MBP would increase more in the cores than in the pots where roots supplied the C. However, this was not the case, suggesting that supply of readily available C by roots and straw was similar. Mature wheat straw contains a high proportion of structural C which is only slowly decomposable and may not have been decomposed during the three weeks in Part B. In contrast to the pot experiment, resin P, phosphatase-P and $CaCl_2$-P increased from Part A to Part B in the cores. In the pots, P from these pools would be taken up by plants and thus removed from the soil. The increase in resin P and $CaCl_2$-P in the cores suggests that P released from HCl-P and citrate-P remained available in absence of plants. The increase in phosphatase-P indicates formation of phosphatase-labile organic P by microbes.

In the Hedley P fractionation soil is shaken sequentially with different extractants and it is assumed that P released by the previous extractant is removed before the next extractant is added (Hedley *et al.*, 1982). In the DeLuca method used here, soil is shaken simultaneously with the different extractants (DeLuca *et al.*, 2015). Therefore HCl-P is likely to include P extractable with citrate, phosphatase or $CaCl_2$. As weakest extractant, $CaCl_2$-P will include little or no P detectable with the other extractants. Unlike the original DeLuca method, we also measured MBP. It is a small pool (5-40 mg kg^{-1}), but our study shows that it is a highly dynamic pool that responds to P addition as well as C input in form of rhizodeposits or straw. The strong response of MBP to C input by plants or residues is in agreement with other studies (Bradford *et al*., 2013, Malik *et al.*, 2013). Although MBP increased during plant growth in our study, it is considered to be a potentially plant available P pool (Balota *et al*., 2003). MBP in the rhizosphere has been shown to correlate with plant P uptake (Solaiman *et al*., 2007).Therefore it should be included in methods for determining biologically available P pools.

5. Conclusions

The study showed that P pools assessed with the DeLuca method are influenced by P addition rate and can be depleted by plants and microbes. Our findings highlight the importance of MBP as dynamic P pool particularly when microbial growth is stimulated by root exudates or residue addition. Future studies could employ 33P labelled fertilizer or residues to trace amended P into P pools and plants.

Acknowledgements

Khuyen Thi Kim Hoang thanks the Vietnam International Education Development (VIED) for providing the postgraduate scholarship.

References

Alamgir, M., Marschner, P. 2016. Changes in P pools over three months in two soils amended with legume residues. J. Soil Sci. Plant Nutr. 16, 76-87.

Alamgir, M., McNeill, A., Tang, C., Marschner, P. 2012. Changes in soil P pools during legume residue decomposition. Soil Biol. Biochem. 49, 70-77.

Balemi, T., Negisho, K. 2012. Management of soil phosphorus and plant adaptation mechanisms to phosphorus stress for sustainable crop production: a review. J. Soil Sci. Plant Nutr. 12, 547-562.

Balota, E.L., Colozzi-Filho, A., Andrade, D.S., Dick, R.P. 2003. Microbial biomass in soils under different tillage and crop rotation systems. Biol. Fertil. Soils. 38, 15-20.

Bradford, M.A., Keiser, A.D., Davies, C.A., Mersmann, C.A., Strickland, M.S. 2013. Empirical evidence that soil carbon formation from plant inputs is positively related to microbial growth. Biogeochemistry. 113, 271-281.

Bremner, J., Breitenbeck, G.A. 1983. A simple method for determination of ammonium in semimicro-Kjeldahl analysis of soils and plant materials using a block digester. Comm. Soil Sci. Plant Anal. 14, 905-913.

Chatterjee, D., Datta, S., Manjaiah, K. 2014. Fractions, uptake and fixation capacity of phosphorus and potassium in three contrasting soil orders. J. Soil Sci. Plant Nutr. 14, 640-656.

Ciampitti, I.A., Picone, L.I., Rubio, G., García, F.O. 2011. Pathways of Phosphorous Fraction Dynamics in Field Crop Rotations of the Pampas of Argentina. Soil Sci. Soc. Am. J. 75, 918-926.

Dalal, R.C. 1979. Mineralization of Carbon and Phosphorus from Carbon-14 and Phosphorus-32 Labelled Plant Material Added to Soil. Soil Sci. Soc. Am. J. 43, 913-916.

DeLuca, T., Glanvile, H., Harris, M., Emmett, B., Pingree, M., de Sosa, L., Cerda-Moreno, C., Jones, D. 2015. A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. Soil Biol. Biochem. 88, 110-119.

Gee, G.W., Or, D. 2002. 2.4 Particle-size analysis. Methods of soil analysis. Part 4, 255-293.

Hanson, W. 1950. The photometric determination of phosphorus in fertilizers using the phosphovanado-molybdate complex. J. Sci. Food Agric. 1, 172-173.

Hassan, H.M., Marschner, P., McNeill, A., Tang, C. 2012. Growth, P uptake in grain legumes and changes in rhizosphere soil P pools. Biol. Fertil. Soils. 48, 151-159.

Hedley, M.J., Stewart, J., Chauhan, B. 1982. Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci. Soc. Am. J. 46, 970-976.

Jiang-shan, Z., Jian-fen, G., Guang-shui, C., Wei, Q. 2005. Soil microbial biomass and its controls. J. For. Res. 16, 327-330.

Kouno, K., Tuchiya, Y., Ando, T. 1995. Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biol. Biochem. 27, 1353-1357.

Malik, M., Khan, K., Marschner, P. 2013. Microbial biomass, nutrient availability and nutrient uptake by wheat in two soils with organic amendments. J. Soil Sci. Plant Nutr. 13, 955-966.

Maltais-Landry, G., Frossard, E. 2015. Similar phosphorus transfer from cover crop residues and water-soluble mineral fertilizer to soils and a subsequent crop. Plant Soil. 393, 193-201.

Marschner, P., Hatam, Z., Cavagnaro, T. 2015. Soil respiration, microbial biomass and nutrient availability after the second amendment are influenced by legacy effects of prior residue addition. Soil Biol. Biochem. 88, 169-177.

Miranda, K.M., Espey, M.G., Wink, D.A. 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide. 5, 62-71.

Murphy, J., Riley, J.P. 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta. 27, 31-36.

Noack, S.R., McLaughlin, M.J., Smernik, R.J., McBeath, T.M., Armstrong, R.D. 2012. Crop residue phosphorus: speciation and potential bio-availability. Plant Soil. 359, 375-385.

Ohno, T., Zibilske, L.M. 1991. Determination of low concentrations of phosphorus in soil extracts using malachite green. Soil Sci. Soc. Am. J. 55, 892-895.

Olsen, S., Sommers, L., Page, A. 1982. Methods of soil analysis. Part 2. Chemical and microbiological properties of Phosphorus. ASA Monograph. 403-430.

Pierzynski, G.M., Logan, T.J., Traina, S.J., Bigham, J.M. 1990. Phosphorus Chemistry and Mineralogy in Excessively Fertilized Soils: Quantitative Analysis of Phosphorus-rich Particles. Soil Sci. Soc. Am. J. 54, 1576-1583.

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., Zhang, F. 2011. Phosphorus dynamics: from soil to plant. Plant Physiol. 156, 997-1005.

Solaiman, Z., Marschner, P., Wang, D., Rengel, Z. 2007. Growth, P uptake and rhizosphere properties of wheat and canola genotypes in an alkaline soil with low P availability. Biol. Fertil. Soils. 44, 143.

Tarafdar, J., Claassen, N. 1988. Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. Biol. Fertil. Soils. 5, 308-312.

Tiessen, H., Ballester, M.V., Salcedo, I. 2011. Phosphorus and Global Change. In: Phosphorus in Action. Bünemann E., Oberson A. and Frossard E. (eds), pp 459-471. Springer Berlin Heidelberg.

Walkley, A., Black, I.A. 1934. An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29-38.

Wilke, B.M. 2005. Determination of chemical and physical soil properties. In: Monitoring and Assessing Soil Bioremediation. Margesin R. and Schinner F. (eds) pp 47-95. Springer Berlin Heidelberg.

Xue, R., Shen, Y., Marschner, P. 2016. Low soil water content during plant growth influences soil respiration and microbial biomass after plant removal and rewetting. J. Soil Sci. Plant Nutr. 16, 955-966.

Chapter 3

P Pools After Seven-Year Fertiliser Application Are Influenced by Wheat Straw Addition and Wheat Growth

Khuyen Thi Kim Hoang[1,2] and Petra Marschner[1]

[1]School of Agriculture, Food and Wine, The University of Adelaide, Adelaide SA 5005, Australia.

[2]Chemical Engineering Faculty, Industrial University of Ho Chi Minh, HCMC 7000, Vietnam

The work contained in this chapter is published in Journal of Soil Science and Plant Nutrition

Hoang, K. T. K., & Marschner, P. (2019). P Pools After Seven-Year P Fertiliser Application Are Influenced by Wheat Straw Addition and Wheat Growth. *Journal of Soil Science and Plant Nutrition*, 1-8.

Statement of Authorship

Title of Paper	P pools after Seven- year P Fertiliser Application are influenced by Wheat straw Addition and Wheat Growth
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Khuyen Thi Kim Hoang and Petra Marschner

Principal Author

Name of Principal Author (Candidate)	Khuyen Thi Kim Hoang		
Contribution to the Paper	Performed experiment, analysed soil samples, analysed and interpreted data and wrote the manuscript		
Overall percentage (%)			
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	18/09/2019

Co-Author Contributions

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.

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of work, data interpretation, manuscript evaluation and correction. She also acted as corresponding author		
Signature		Date	18/09/2019

Journal of Soil Science and Plant Nutrition
https://doi.org/10.1007/s42729-019-00059-2

ORIGINAL PAPER

P Pools After Seven-Year P Fertiliser Application Are Influenced by Wheat Straw Addition and Wheat Growth

Khuyen Thi Kim Hoang [1,2] · Petra Marschner [1]

Received: 11 January 2019 / Accepted: 19 April 2019

Abstract
Little is known about the effect of long-term P fertilisation on soil P pools and how they are influenced by straw addition and plant growth. Two experiments were carried out with soil which had been amended with 0, 10 and 20 kg P ha^{-1} for 7 years (referred to 0P, 10P, 20P). In experiment 1, soil was incubated moist for 4 months without or with 5 g kg^{-1} barley straw (C/P 255). Then wheat was grown for 4 weeks. In experiment 2, wheat was grown for 5, 10 and 15 weeks. HCl P, citrate P, resin P and microbial biomass P (MBP) were two-fold higher with 20P and 10P than 0P. After 4 months compared to 10 days, HCl P and citrate P were 25% lower with straw, but unchanged without straw. Resin P and MBP were two-fold higher. With 10P and 20P after 4-week wheat growth, citrate P was 25% higher and HCl P 25% lower. Plant P uptake was higher with straw, In experiment 2 after 10 and 15 weeks compared to the start at 10P and 20P, HCl P and citrate P were reduced by 20%. Plant P uptake was smaller than the decrease in HCl and citrate P. The greater P uptake by wheat in soil with straw indicates that microbial biomass P is an important P source for plants. In P amended soil, wheat mobilised HCl and citrate P, but only took up a fraction of the mobilised P.

Keywords P pools · P uptake · Straw

1 Introduction

When P is added to soil, for example as fertiliser and manure, only 20–30% is taken up by the plant in the first year; the rest is fixed or precipitated into less labile P forms (Richardson et al. 2001) through sorption and complexation in the soil. Therefore, P concentration in the soil solution and P diffusion rate are low (Barber 1962, 1995; Hinsinger et al. 2005; Lewis and Quirk 1967). Due to the low P fertiliser efficiency, often more P is added with fertilisers than crops take up resulting in a build-up of stable soil P forms which can be leached or removed in runoff and pollute waterways (Sharpley 1995). In soil, P can be found in various pools that can be either organic (Po, 20% to 80% of total P) or inorganic (Pi, 35% to 70% of total P) (Brady and Weil 2002; Harrison 1987). The predominant forms of Pi in acidic soils are Fe or Al phosphates, in alkaline soils Ca phosphates. The main forms of Po are esters, phytate and soil microbial biomass (Turner et al. 2002). The available P concentration in the soil solution is low, ranging from 0.01 to 1 mg/l in fertile soils. Therefore, microorganisms and plants have developed a wide range of strategies to enhance P availability (Jones and Oburger 2011). Microorganisms and plant roots can increase P availability by acidification or exudation of organic acid anions and phosphatases. Plant roots may also form mycorrhizal associations (Ryan et al. 2001; Iyamuremye and Dick 1996).

The effect of plants on soil P pools has been studied (Guo et al. 2000; Nuruzzaman et al. 2005; Nziguheba et al. 2000). For example, legumes can mobilise stable soil P, which is thought to be due to organic acid release by roots (Hassan et al. 2012). We showed recently that wheat growth induced the depletion of various P pools in a soil to which inorganic P was added (Hoang and Marschner 2017). Long-term studies can be used to investigate nutrient cycling and understand turnover of nutrients. There have been many field-based long-term studies on soil P fractions using Hedley fractionation, yield or P uptake (Zhang and MacKenzie 1997; Laboski and Lamb 2003; Wang et al. 2007; Eichler-Löbermann et al. 2007). Hedley et al. (1982) reported that 52% of stable P and 22% of total Po was lost after 65 years of wheat-fallow

✉ Petra Marschner
petra.marschner@adelaide.edu.au

[1] School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5005, Australia

[2] Chemical Engineering Faculty, Industrial University of Ho Chi Minh, HCMC 7000, Vietnam

Published online: 02 May 2019

cropping without fertiliser addition. Organic amendments such as crop residues affect the transformation of P pools and P availability based on their C/P ratio. Crop residues with $C/P > 200$ induce net P immobilisation and depletion of P pools, whereas residues with lower C/P ratio increase P availability and P pools (Alamgir et al. 2012). There may be a period of several months between harvest of one crop and sowing of the next, but little is known about P pool transformations during this time particularly in soil from long-term fertiliser trials. Further, little is known about P pools during cereal growth. Farmers usually apply fertilisers over many years. Compared to short-term P amendment experiments (one or two crop growth periods), long-term trials are likely to particularly stimulate more stable inorganic and organic P pools and better reflect P availability to crops.

The aim of this study is to assess P pools in soil from a long-term trial where P was added for 7 years (i) after 4-month moist incubation with and without straw compared to 10 days and after subsequent plant growth and (ii) after 4-month plant growth.

The hypotheses were as follows: (1) changes in P pools will be greater in soil with long-term P addition than in the unfertilised control; (2) incubation with straw will increase microbial biomass P, reduce particularly resin P, but also citrate P and HCl P compared to the treatment without straw; (3) P pools will be depleted during plant growth.

2 Materials and Methods

2.1 Soil

The trial was set up at BCG Farming Systems Site, Jil Jil, 22 km north of Birchip, Victoria, Australia (latitude 35° 58′ S, longitude 142° 54′ E). The soil is classified as Calcarosol in Australian Soil Classification. Three rates of P (0, 10 and 20 kg P ha^{-1}) were applied annually to the same plots from 2003 to 2011 (referred to as 0P, 10P and 20P hereafter). Each year, a base rate (5 kg P ha^{-1}) of mono-ammonium phosphate (N10:P22:K0:S1) was applied to 10P and 20P. Triple superphosphate (N0:P22:K0:S1) was then applied to adjust the P rate to 10 and 20 kg P ha^{-1} without adding further N. To ensure the N rate was the same for all treatments, urea was applied at 5 kg N ha^{-1} to 0P. The trial began in 2003 after 2-year fallow, since then has followed a typical Mallee rotation for this soil type (Gabb et al. 2011). Yield was about 30% higher in 10P and 20P than 0P, and in some years, yield was higher in 20P than 10P.

Soil samples were collected in 2010 and 2011. Ten samples per treatment were collected randomly, using a 2.5-cm diameter soil corer to a depth of 10 cm. The soil was air-dried and stored in the dark. Samples from both years were pooled and then used for the experiment. The soil has the following properties clay 1%, silt 50%, sand 49%; pH 8.2 (1:5 soil:water); total organic C 1.3 g kg^{-1}, maximum water–holding capacity 357 g kg^{-1}.

The properties of the mature barley straw (dried, finely ground and sieved to particle size of 0.25–2 mm) used in this experiment were as follows: total P 1.6 g kg^{-1}, total N 4.3 g kg^{-1}, total C 408 g kg^{-1}, C/P ratio 255.

2.2 Experimental Design

The soil from each P rate was incubated for 10 days at 50% maximum water–holding capacity after which baseline P pools and available N were measured. Then two experiments were carried out. The aim of experiment 1 was to evaluate P pools in long-term fertiliser soil after 4-month incubation without or with barley straw and subsequent plant growth. The soil was incubated at room temperature for 4 months at 50% water–holding capacity without or with 5 g kg^{-1} barley straw. After 4 months, P pools and available N were measured and soil was placed in pots (400 g dry soil equivalent per pot) and planted with 10 pre-germinated wheat seeds (*Triticum aestivum* L., var. Axe). Wheat was grown for 5 weeks in a glasshouse. The aim of experiment 2 was to assess the impact of plant growth to maturity on soil P pools. Wheat was grown in un-amended soil for 5, 10 and 15 weeks. The temperature in the glasshouse during the experiment ranged from 25 to 35 °C. Throughout both experiments, soil water content was maintained at 50% WHC by weight and adding reverse osmosis water if necessary. During wheat growth, pots were watered daily. Soils were analysed for N and P pools after 10 days and after 5, 10 and 15 weeks.

2.3 Analyses

Soil analyses were carried out as described in Hoang and Marschner (2017). Briefly, soil texture was measured by the hydrometer method (Gee and Or 2002). Soil maximum water–holding capacity was determined using a sintered glass funnel connected to a 1-m water column (Wilke 2005). Soil pH was measured after shaking soil in a 1:5 soil:water ratio for 1 h. Resin P was determined using anion-exchange resin membrane converted to bicarbonate form; microbial biomass P (MBP) was measured with the same method by adding hexanol as fumigant (Kouno et al. 1995). The P concentration was determined colorimetrically (Murphy and Riley 1962). MBP was calculated as the difference between anion exchange P without hexanol (resin P) and P extracted with hexanol. Ammonium-N was determined by Willis et al. (1996) and nitrate-N as described in Miranda et al. (2001). Available N is the sum of nitrate and ammonium-N.

Soil P pools were determined according to Deluca et al. (2015) using four extractants in parallel, including 10 mM citric acid, 1 M HCl, 10 mM $CaCl_2$ and 0.2 enzyme unit

phosphatase (from wheat germ) extractable P. Moist soil equivalent to 0.5 g dry soil were shaken with 10 ml of each extractant separately for 3 h (DeLuca et al. 2015). P in the extracts was determined by the malachite-green method (Ohno and Zibilske 1991).

2.4 Statistical Analysis

Data were analysed by one-way ANOVA separately for each sampling time with Genstat 15th edition (VSN Int. Ltd., UK). Tukey's multiple comparison tests at 95% confidence interval was used to determine significant differences among treatments.

3 Results

Throughout both experiments, $CaCl_2$ P and phosphatase P were below detection limit.

3.1 Experiment 1

After 10 days, HCl P was twofold and citrate P was threefold higher with 20P than 0P (Table 1). HCl P was about 10-fold higher than citrate P. Resin P was not detectable in 0P and similar in 10P and 20P. MBP was higher than resin P and about two-fold higher in 10P and 20P than 0P. Available N was about twofold higher in 10P and 20P than 0P (Table 2).

After 4-month incubation compared to 10 days, citrate P and HCl P without straw were unchanged, but lower with straw (Fig. 1). Both pools were higher in 10P and 20P than 0P, about threefold for citrate P and 40% higher for HCl P. HCl P was lower with than without straw, about 10% in 0P and 25% in 10P and 20P. Citrate P was about 30% lower with than without straw in 0P and 10P, but was not affected by the straw treatment in 20P. Resin P was still undetectable in 0P, but about 10-fold higher than after 10 days in 10P and 20P (Fig. 1). Without straw, resin P was two-fold higher than with straw in 10P and threefold higher in 20P. MBP after 4 months was about two-fold higher than after 10 days and highest in 10P. In 0P and 10P, MBP was higher with than without straw, 25% higher in 0P and twofold higher in 10P. Straw addition had no effect on MBP in 20P. Available N was similar as after 10 days without straw, but lower with straw (Table 2). With straw, it was about 50% lower in 0P and 20% lower in 10P and 20P. With and without straw, available N was highest in 10P.

After 5-week wheat growth, HCl P was about 30% lower than after 4 months (Fig. 1). It was lower in 0P than 10P and 20P. Straw addition reduced HCl P only in 20P where it was about 20% lower than without straw. Citrate P was about two-fold higher than after 4 months in 0P, 30% higher in 10P, but did not change in 20P (Fig. 1). Compared to 0P, citrate P was about two-fold higher in 10P and 20P. Straw addition reduced citrate P by about 5% in 10P and 20P, but had no effect in 0P. Resin P was similar as after 4 months and not detectable in 0P; it was highest in 10P. Resin P was two-fold higher without than with straw in 10P and threefold higher in 20P. MBP in 0P was similar as after 4 months without straw, but about 20% lower with straw. In 10P, MBP was lower than after 4 months, 30% without straw and 50% with straw. In 20P, MBP was similar as after 4 months without straw, but 20% higher with straw. P treatments did not differ in MBP without straw. But with straw, MBP was higher in 10P and 20P than 0P. Available N without straw was about 30% lower than after 4 months (Table 2). With straw, it was eight-fold lower in 0P, 30% lower in 10P and 50% lower in 20P. Available N was always lower with than without straw, with greatest differences between straw treatments in 0P. Available N was highest in 10P.

Shoot and root biomass differed little among P or straw treatments (data not shown). Shoot P concentration was lower in 0P than the other two P treatments (data not shown). Shoot P uptake was lowest in 0P (Fig. 2). Straw addition did not affect shoot P uptake in 0P but increased P uptake in 10P and slightly also in 20P.

Table 1 P pools of Birchip soil after 10-dayincubation

P rates	P pool (mg kg^{-1}) after 10 days			
	HCl	Citrate	Resin P	MBP
0P	68.3[a]	4.3[a]	0.0[a]	0.8[a]
10P	114.5[b]	14.1[b]	0.4[b]	1.4[b]
20P	153.3[c]	13.0[b]	0.4[b]	1.7[b]

Table 2 Available N (mg kg^{-1}) at 0P, 10P and 20P with/without straw addition after 10-day and 4-month moist incubation and after 5 weeks of plant growth (experiment 1)

Treatment	Available N (mg kg^{-1})			
	P rates	10 days	4 months	After 5-week plant growth
No straw	0P	99.7[a]	98.3[b]	55.1[b]
	10P	198.9[c]	199.2[e]	179.4[f]
	20P	168.0[b]	163.5[d]	125.5[e]
With straw	0P		47.0[a]	6.8[a]
	10P		159.1[d]	103.2[d]
	20P		142.1[c]	78.2[c]

3.2 Experiment 2

Before plant growth compared to 0P, HCl P and MBP were was about twofold higher and citrate P about three-fold higher in 10P and 20P (Table 1). At all sampling times during plant

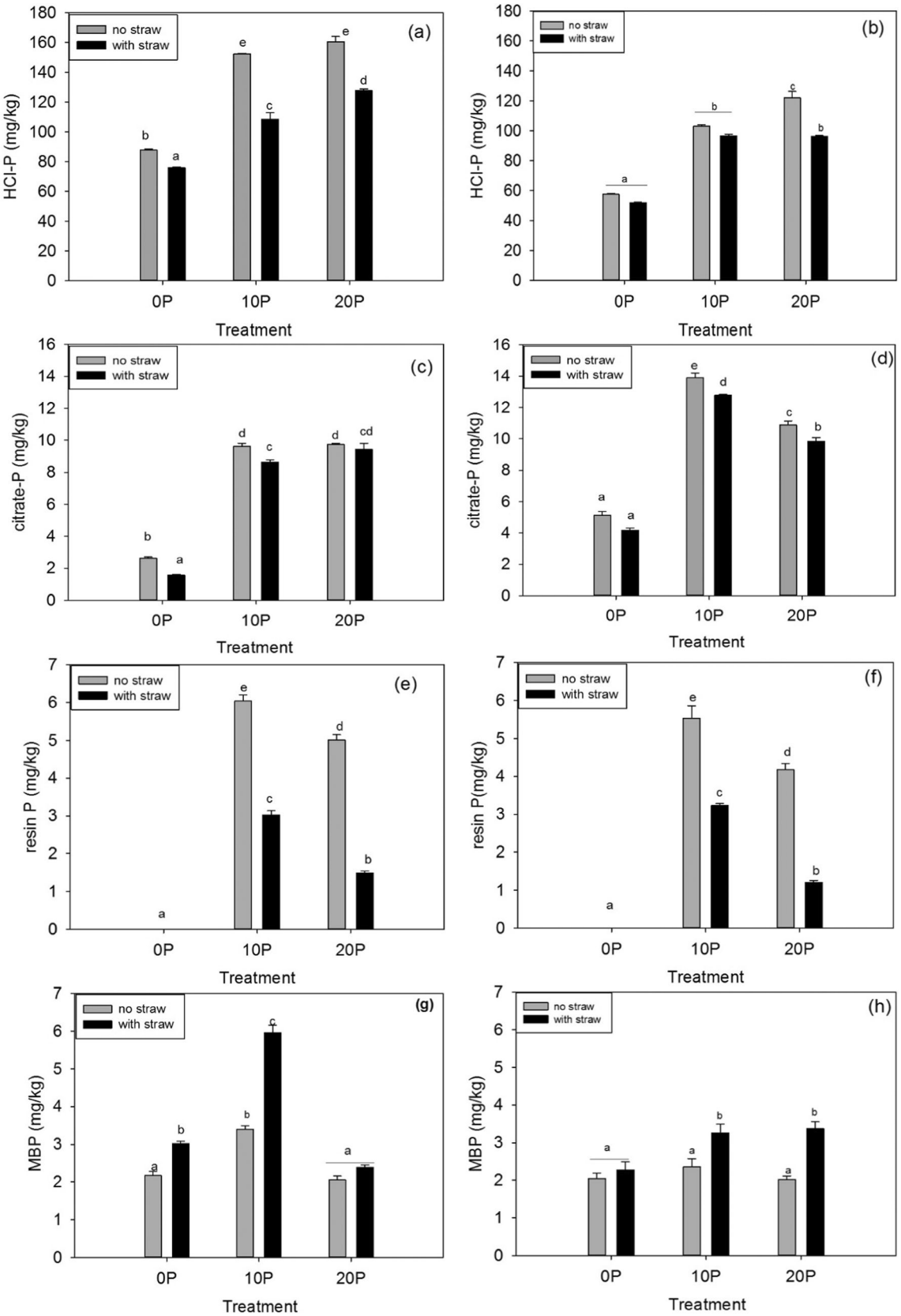

Fig. 1 HCl P (**a**, **b**), citrate P (**c**, **d**), resin P (**d**, **e**) and microbial biomass P (**f**, **g**) after 4-month moist incubation before wheat growth (**a**, **c**, **e**, **g**) and after 5-week growth of wheat (**b**, **d**, **f**, **h**) (experiment 1). Vertical lines indicate standard errors. For each period, bars with different letters are significantly different ($n = 4$, $P \leq 0.05$)

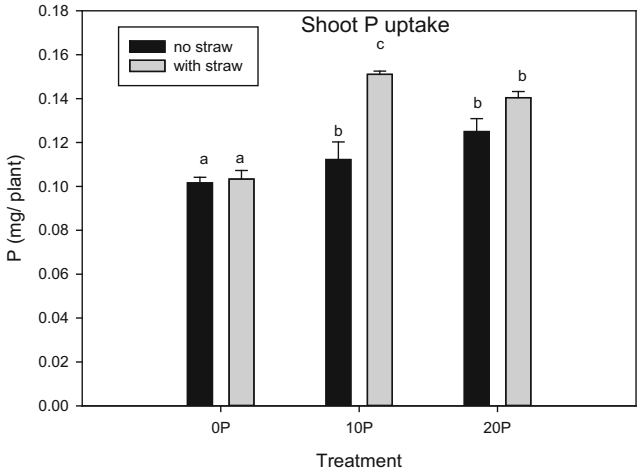

Fig. 2 Shoot P uptake (mg/plant) after 5 weeks (experiment 1). Bars with different letters are significantly different ($n = 4$, $P \leq 0.05$)

growth, HCl P was lowest in 0P (Fig. 3). HCl P decreased with length of plant growth, particularly in 20P. Citrate P was three or more fold higher with 10P and 20P than in 0P and was lowest after 15-week wheat growth. Resin P was very low in 0P and lower in 20P than 10P. In 10P and 20P, it was more than two-fold lower after 10 weeks than after 5 weeks and not detectable after 15 weeks. After 10 days and 5 weeks, MBP was highest in 0P, but after 10 and 15 weeks, it was very low in 0P and much higher in 10P and 20P. In 10P and 20P, MBP was higher after 15 weeks than at the earlier sampling times. Available N was lowest in 0P at all sampling times (Table 3). In all treatments, it was lowest after 10 weeks. Shoot and root biomass differed little among P rates (data not shown). Shoot P concentration in 0P was highest after 5 weeks and lowest after 15 weeks (data not shown). After 5 weeks, shoot P uptake was about 30% lower in 0P than with added P (Fig. 4). But after 10 weeks, shoot P uptake was lowest with 20P, where it was about 25% lower than 0P and 10P. After 15 weeks, shoot P uptake was 30–40% in 0P higher than with added P.

4 Discussion

The experiments confirmed the first hypothesis (changes in P pools will be greater in soil with long-term P addition than in the unfertilised control). However, the second hypothesis (incubation with straw will increase microbial biomass P, reduce particularly resin P, but also citrate P and HCl P compared to the treatment without straw) and third hypothesis (P pools will be depleted during plant growth) can only be confirmed for certain P pools and sampling times, e.g., citrate P was depleted by plants after more than 10 weeks compared to 5 weeks.

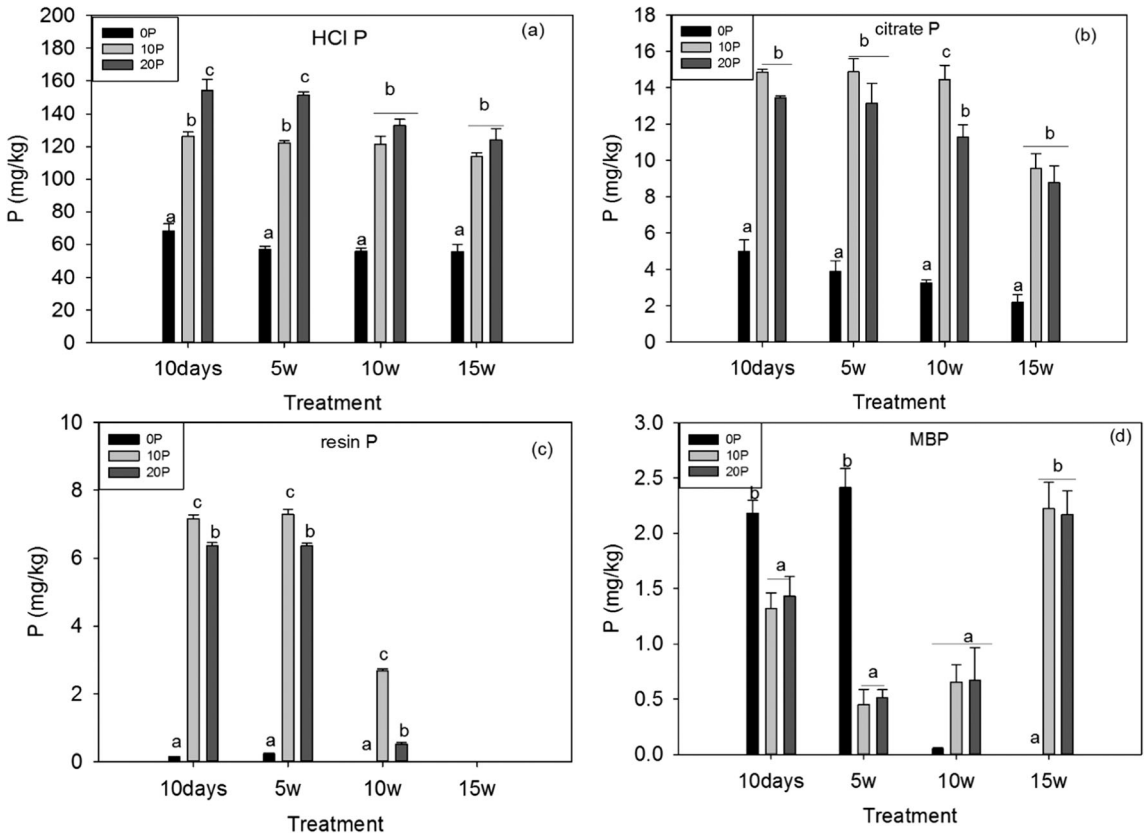

Fig. 3 HCl P (**a**), citrate P (**b**), resin P (**c**) and microbial biomass P (**d**) after 10 days and after 5-, 10- and 15-week growth of wheat (experiment 2). Vertical lines indicate standard errors. For each sampling time, bars with different letters are significantly different ($n = 4$, $P \leq 0.05$)

Table 3 Available N (mg kg^{-1}) in soil at 0P, 10P and 20P after 10 days and after 5, 10 and 15-week plant growth (experiment 2). Values with different letters are significantly different ($n = 4$, $P \leq 0.05$)

Time	Available N (mg kg^{-1})			
P rates	10 days	5 weeks	10 weeks	15 weeks
0P	21.8^{a}	5.61^{a}	0.10^{a}	25.93^{a}
10P	94.27^{b}	90.00^{b}	77.14^{c}	79.11^{b}
20P	116.60^{c}	105.93^{c}	55.62^{b}	116.25^{c}

In general, P pools, available N and shoot P concentration were lower in 0P than when P was added during the field trial. This suggests that 9 years lack of P addition results in depletion of P pools, particularly resin P. There was little difference between 10P and 20P, indicating that the twofold higher P addition rate in 20P did not strongly increase P pools compared to 10P. A possible reason for the lack of difference between the two P rates is the greater plant P uptake and thus removal of P from the soil in 20P. Between 2003 and 2011, yield was about 30% higher in 10P and 20P than 0P, and in some years, yield, and therefore P uptake was higher in 20P than 10P (Table 1). This is in agreement with a 10-year study of annual fertiliser P rates with maize using four P rates (11, 22, 45 and 90 kg ha^{-1}) (Richards et al. 1995). In that study, labile P (resin P and $NaHCO_3$-Pi) increased from 22 to 90 kg ha^{-1}, but there was little difference between 11 and 22 kg ha^{-1}. In a short-term study of soil P pools in a loamy sand (Alamgir and Marschner 2013) with five rates of P (0, 3, 10, 30 and 100 mg P kg^{-1} as KH_2PO_4), P pools after 42 days of wheat growth in the control were lower than in treatments with P addition with little difference in P pools between rates 3, 10 and 30 mg P kg^{-1}. However, shoot P uptake in their study did not differ between control and 3 mg kg^{-1} and was higher at the higher P rates.

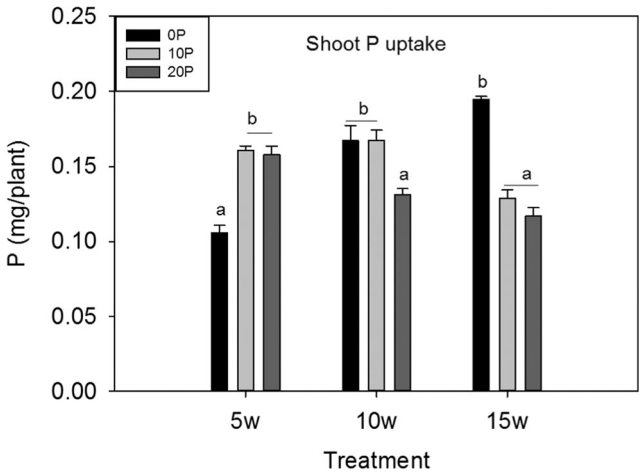

Fig. 4 Shoot P uptake (mg/plant) after 5, 10 and 15 weeks (experiment 2). Bars with different letters are significantly different ($n = 4$, $P \leq 0.05$)

Compared to 10 days, the 4-month incubation without straw had no effect on HCl P and citrate P, but had higher resin P and MBP. The increase in resin P and MBP without reduction in other measured P pools indicates mobilisation of organic and inorganic P not assessed by the Deluca method. It is also possible that the increase in resin P and MBP was due to P flux from HCl P, but was not detectable as a decrease because resin P and MBP are 10-fold smaller than HCl P.

4.1 Experiment 1

After 4-month incubation with straw, HCl P, citrate P and resin P were lower than without straw, whereas MBP was generally higher. The increase in MBP can be explained by P immobilisation triggered by the addition of high C/P wheat straw (Nguyen et al. 2016). P taken up by microbes may have come from HCl P, citrate P or resin P. But HCl P was 20–30 g kg^{-1} lower with straw, whereas MBP was only 1–2 g kg^{-1} higher. HCl P was likely converted into P pools not assessed by the Deluca method such as very stable inorganic and organic P. Plants were removed P from soil, particularly in 10P and 20P, but resin P was similar after 4 months and plant growth for 5 weeks. This suggests that resin P was replenished from HCl P and, in some treatments, from MBP which were both lower after than before plant growth. Citrate P increased during plant growth, likely due to flux from HCl P. Roots and rhizosphere microbes may have mobilised HCl P through release of protons (Tarafdar and Claassen 1988).

Straw addition had less effect on HCl P, resin P and MBP after plant growth than before (after 4-month incubation), likely because roots supplied microbes with carbon through exudates and root turnover (Jiang-shan et al. 2005). Shoot P uptake with straw was higher than without straw as after long-term incubation which was not expected. However, P uptake by the microbial biomass can reduce P binding to soil particles and biomass P can be released after biomass turnover (Oberson et al. 2001). Although MBP is a small pool, it turns over quickly and can therefore be a continuous source of P for plants.

4.2 Experiment 2

In P amended soils (10P and 20P) compared to before plant growth (after 10-day moist incubation), HCl P and citrate P were lower after 15 weeks of wheat growth and resin P was not detectable. This indicates the ability of wheat to access less available P pools increases with plant age, possibly by depleting resin P and thereby inducing release from HCl and citrate P. MBP in 10P and 20P was higher after 15 than 5 and 10 weeks which may be due to root death after and therefore increased carbon availability. Plant P uptake was about 10-fold lower than the decrease in HCl P and citrate P during plant growth. This suggests that root and microbial exudates

have mobilised HCl P and citrate P which was only partly taken up by the plants, and the rest may have been strongly adsorbed to soil particles and thereby becoming unavailable to plants.

Shoot and root biomass did not differ between P rates, but shoot P concentration after 5 weeks was higher with added P than 0P, whereas the reverse was true after 15 weeks. A possible explanation is that plants matured more quickly in 10P and 20P than in 0P due to better nutrient supply. Therefore after 15 weeks, they had shed leaves in the former treatments, whereas they were still actively growing in 0P.

It should be noted that P diffusion in soil is slow. Therefore, soil exploitation by roots is important for plant P uptake. However, in this study, roots were found throughout the soil in the pots and biomass did not differ among treatments, likely because of the limited soil volume. Therefore, root growth is unlikely to have limited P uptake. In the field on the other hand, root penetration plays an important role for P uptake (Bates and Lynch 1996; Gahoonia and Nielsen 2003; Bhat and Nye 1973).

5 Conclusion

The study showed that although straw addition reduced resin P, citrate P and HCl P after 4-month incubation, plant P uptake after 4-week growth was greater with straw than without. This indicates that microbial biomass P can be an important P source for plants. Long-term P amendment resulted in an increase in HCl P, citrate P and resin P. These pools decreased during wheat growth confirming that they can be considered to be bioavailable. However, the decrease in HCl and citrate P was greater than plant P uptake. This indicates that plants only took up a proportion of the P mobilised by root exudates. The remaining P was likely converted into less available P forms.

Acknowledgements Khuyen Thi Kim Hoang received a postgraduate scholarship from Vietnamese International Education Development. We thank Dr. Sean Mason for supplying the soil from the fertiliser trial.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

Alamgir M, Marschner P (2013) Short-term effects of application of different rates of inorganic P and residue P on soil P pools and wheat growth. J Plant Nutr Soil Sci 176:696–702

Alamgir M, McNeill A, Tang C, Marschner P (2012) Changes in soil P pools during legume residue decomposition. Soil Biol Biochem 49: 70–77

Barber SA (1962) A diffusion and mass-flow concept of soil nutrient availability. Soil Sci 93:39–49

Barber SA (1995) Soil nutrient bioavailability: a mechanistic approach. Wiley, Hoboken

Bates T, Lynch J (1996) Stimulation of root hair elongation in Arabidopsis thaliana by low phosphorus availability. Plant Cell Environ 19:529–538

Bhat K, Nye P (1973) Diffusion of phosphate to plant roots in soil. Plant Soil 38:161–175

Brady N, Weil R (2002) The nature and properties of soils. Prentice Hall, Upper Saddle River

DeLuca TH, Glanville HC, Harris M, Emmett BA, Pingree MR, de Sosa LL, Cerdá-Moreno C, Jones DL (2015) A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. Soil Biol Biochem 88:110–119

Eichler-Löbermann B, Köhne S, Köppen D (2007) Effect of organic, inorganic, and combined organic and inorganic P fertilization on plant P uptake and soil P pools. J Plant Nutr Soil Sci 170:623–628

Gabb S, Harm VR, Sean M (2011) Long-term phosphorous (P) trial and evaluation of the DGT soil phosphorous test. Season Res Res 2011:95–100

Gahoonia T, Nielsen N (2003) Phosphorus (P) uptake and growth of a root hairless barley mutant (bald root barley, brb) and wild type in low-and high-P soils. Plant Cell Environ 26:1759–1766

Gee GW, Or D (2002) Particle-size analysis. In: Dane JH, Topp CG (Eds.) Methods of soil analysis Part 4 Physical Methods. Soil Science Society of America, Madison, pp 255–293

Guo F, Yost R, Hue N, Evensen C, Silva J (2000) Changes in phosphorus fractions in soils under intensive plant growth. Soil Sci Soc Am J 64: 1681–1689

Harrison AF (1987) Soil organic phosphorus: a review of world literature. CAB International, Wallingford

Hassan HM, Marschner P, McNeill A, Tang C (2012) Growth, P uptake in grain legumes and changes in rhizosphere soil P pools. Biol Fertil Soils 48:151–159

Hedley MJ, Stewart J, Chauhan B (1982) Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. Soil Sci Soc Am J 46:970–976

Hinsinger P, Gobran GR, Gregory PJ, Wenzel WW (2005) Rhizosphere geometry and heterogeneity arising from root-mediated physical and chemical processes. New Phytol 168:293–303

Hoang KKT, Marschner P (2017) Plant and microbial-induced changes in P pools in soil amended with straw and inorganic P. J Soil Sci Plant Nutr 17:1088–1101

Iyamuremye F, Dick R (1996) Organic amendments and phosphorus sorption by soils. Adv Agron 56:139–185

Jiang-shan Z, Jian-fen G, Guang-shui C, Wei Q (2005) Soil microbial biomass and its controls. J For Res 16:327–330

Jones DL, Oburger E (2011) Solubilization of phosphorus by soil microorganisms. In: Phosphorus in action. Springer, Berlin, pp 169–198

Kouno K, Tuchiya Y, Ando T (1995) Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biol Biochem 27:1353–1357

Laboski CA, Lamb JA (2003) Changes in soil test phosphorus concentration after application of manure or fertilizer. Soil Sci Soc Am J 67: 544–554

Lewis D, Quirk J (1967) Phosphate diffusion in soil and uptake by plants. Plant Soil 26:99–118

Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5:62–71

Murphy J, Riley JP (1962) A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta 27: 31–36

Nguyen TT, Cavagnaro TR, Ngo HTT, Marschner P (2016) Soil respiration, microbial biomass and nutrient availability in soil amended

with high and low C/N residue–influence of interval between residue additions. Soil Biol Biochem 95:189–197
Nuruzzaman M, Lambers H, Bolland MD, Veneklaas EJ (2005) Phosphorus uptake by grain legumes and subsequently grown wheat at different levels of residual phosphorus fertiliser. Aust J Agric Res 56:1041–1047
Nziguheba G, Merckx R, Palm CA, Rao MR (2000) Organic residues affect phosphorus availability and maize yields in a nitisol of western Kenya. Biol Fertil Soils 32:328–339
Oberson A, Friesen DK, Rao IM, Buehler S, Frossard E (2001) Phosphorus transformations in an oxisol under contrasting land-use systems: the role of the soil microbial biomass. Plant Soil 237: 197–201
Ohno T, Zibilske LM (1991) Determination of low concentrations of phosphorus in soil extracts using malachite green. Soil Sci Soc Am J 55:892–895
Richards JE, Bates TE, Sheppard S (1995) Changes in the forms and distribution of soil phosphorus due to long-term corn production. Can J Soil Sci 75:311–318
Richardson A, Hadobas P, Hayes J, O'hara C, Simpson R (2001) Utilization of phosphorus by pasture plants supplied with myo-inositol hexaphosphate is enhanced by the presence of soil micro-organisms. Plant Soil 229:47–56
Ryan P, Delhaize E, Jones D (2001) Function and mechanism of organic anion exudation from plant roots. Ann Rev Plant Biol 52:527–560
Sharpley AN (1995) Soil phosphorus dynamics: agronomic and environmental impacts. Ecol Eng 5:261–279
Tarafdar J, Claassen N (1988) Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. Biol Fertil Soils 5: 308–312
Turner BL, McKelvie ID, Haygarth PM (2002) Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. Soil Biol Biochem 34:27–35
Wang X, Lester D, Guppy C, Lockwood P, Tang C (2007) Changes in phosphorus fractions at various soil depths following long-term P fertiliser application on a Black Vertosol from south-eastern Queensland. Soil Res 45:524–532
Wilke B-M (2005) Determination of chemical and physical soil properties. Monitoring and assessing soil bioremediation. Springer, Berlin, pp 47–95
Willis RB, Montgomery ME, Allen PR (1996) Improved method for manual, colorimetric determination of total kjeldahl nitrogen using salicylate. J Agric Food Chem 44(7):1804–1807
Zhang T, MacKenzie A (1997) Changes of soil phosphorous fractions under long-term corn monoculture. Soil Sci Soc Am J 61:485–493

Chapter 4

Effect of combined sewage sludge and inorganic P application on soil P pools and subsequent changes induced by plants

Chapter 4

Effect of combined sewage sludge and inorganic P application on soil P pools and subsequent changes induced by plants

Khuyen Thi Kim Hoang, Petra Marschner, Ashlea Doolette

School of Agriculture, Food and Wine, The University of Adelaide, Australia.

Abstract

Using sewage sludge as soil amendment to provide nutrients to plants is an attractive alternative to inorganic fertilisers. However, the slow release of nutrients by sewage sludge can limit plant growth. Combined application of sewage sludge and inorganic N and P may provide a sustained nutrient source for plants. In this experiment in a sandy loam, the same amount of N and P (43 mg P kg^{-1} soil and 60 mg N kg^{-1} soil) was added in all amended treatments which varied in proportion of sewage sludge and inorganic N and P. Sewage sludge or inorganic N and P were added either alone (100S and 100F), or 75% sludge+25% inorganic fertiliser, 50% sludge+50% inorganic fertiliser and 25% sludge+75% inorganic fertiliser. Unamended soil was used as control. After amendment, wheat was grown for 5 weeks. HCl P, citrate P, resin P, microbial biomass P, available N and inorganic N and P in leachate were determined before planting and after harvest. Before planting, HCl P was about four-fold higher in amended treatments than the control. Compared to the control, citrate P was five to seven-fold higher in amended treatments. Resin P was lowest in the control, about five-fold higher in 100S and increased with proportion of F. Leachate N was higher in amended than unamended soils. In amended soils, it increased with proportion of F. Only in treatments

with >50% S, leachate P was higher than in the control. Shoot P uptake was higher in amended treatments than the control, about 50% higher in 100S and 75S25F and two to three-fold higher in treatments with ≥50% F. After harvest, HCl P in amended treatments was lowest in 100F. Citrate P was higher in treatments with ≥50% S than in 100F. Available N and leachate inorganic N were very low after harvest. Leachate inorganic P was lowest in 100S and increased with proportion of F. It can be concluded that combinations of sludge with inorganic fertiliser, particularly those with ≥50% N and P from sludge may be optimal because they increased plant P uptake but also maintained P pools while reducing potential P leached.

Keywords: inorganic fertiliser, nutrient leaching, P pools, sewage sludge.

4.1. Introduction

Phosphorus (P) is essential for plant growth and crop production (Marschner, 1995), but fertiliser efficiency is low due to fixation of applied P (Bertrand et al., 2003). Plant available P includes P in the soil solution which is immediately available; and potentially available P such as microbial biomass P, exchangeable inorganic P and labile organic P (Bünemann & Condron, 2007). Rising global demand for food is expected to increase the demand for P fertilisers (Torri et al., 2017). However, rock phosphate reserves used for P fertiliser production are declining. Therefore, the recycling of nutrients from organic waste products such as farmyard manure, compost and sewage sludge should be considered (Scherer & Sharma, 2002). Sewage sludge has been used as source of P in agriculture, forestry and to improve soil properties (Frossard et al., 1996, Pritchard et al., 2010). It is the by-product of waste water treatment and contains C, N, P and other nutrients (Tian et al., 2009, Haynes et al., 2009). Phosphorus in the sewage sludge originates from the removal of P from water by precipitation as Fe- or Al-bound phosphates (Ahmad et al., 2019, González Medeiros et al., 2005).

However, sludge can also contain contaminants such as metals and organic pollutants (pesticides, polychlorinated biphenyls, polycyclic aromatic hydrocarbons) which can have adverse effects on fauna and flora (Dean & Suess, 1985, Harrison et al., 2006). In Australia, the use of sewage sludge is controlled by guidelines which specify safe concentrations of metals, such as zinc, copper and cadmium.

Amendment of soil with sewage sludge can increase P availability by releasing P and by decreasing soil P sorption capacity (Siddique & Robinson, 2003). Inorganic P in sewage sludge may be soluble or bound to oxides and hydroxides of Al, Fe, Mn (González Medeiros et al., 2005). The organic P forms in sewage sludge include inositol phosphate, phospholipids, nucleic acids, phosphoproteins and various sugar phosphates (Maguire et al., 2001, Frossard et al., 1994). Huang et al. (2008) found that the main P fraction was HCl-P (35%) followed by $NaHCO_3$-P (20%), water soluble P (12%) and residual P (11%). He et al. (2010) reported that organic P accounted for 24% of total P and 45% in HCl fraction. In a study of P leaching in soil amended with fertiliser and sewage sludge, Siddique et al. (2000) found that the cumulative amount of P leached from soils amended with fertilisers was higher than with sewage sludge. This can be explained by the slower release of nutrients from organic amendments than inorganic fertiliser (Lu et al., 2012, Ahmad et al., 2008). To improve nutrient availability to crops and plant growth as well as to promote the proper recycling of renewable organic waste (Meade et al., 2011), organic amendments can be combined with inorganic fertiliser (Suge et al., 2011, Schulz & Glaser, 2012, Meade et al., 2011). However, little research has been carried out with sewage sludge combined with inorganic fertiliser. Therefore the aim of this experiment was to assess the effect of the combination of sewage sludge and inorganic fertiliser on soil P pools, P leaching and plant P uptake. We hypothesised that (i) compared to fertiliser addition, most P pools (except MBP) and N and P leaching will be lower with sludge

amendment, (ii) in sludge treatments, pools will change less after planting of wheat than with fertiliser only because sludge P is continuously released during decomposition, and (iii) in the combined treatments, P pools, P leaching and wheat P uptake will increase with proportion of fertiliser.

4.2. Materials and methods

4.2.1. Soil and sewage sludge

Sandy loam was collected at 0-10 cm from Monarto, SA (35^{o} 04' S 139^{o} 07' E), air-dried at 40^{o}C and sieved to > 2 mm prior to the experiment. The properties of the soil are: sand 75%, silt 17%, clay 9%, pH (1:5) 7.5, total P 127.9 mg kg^{-1}, total organic C 6 g kg^{-1}, total N 0.5 g kg^{-1}, maximum water-holding capacity (WHC) 118 g kg^{-1}. Dried and crushed sewage sludge obtained from Bolivar Waste Water Treatment Plant was sieved to 0.25-2 mm particle size. It had the following properties: total P 5.7 mg kg^{-1}, total organic C 6.4 g kg^{-1}, total N 7.8 g kg^{-1}. Other properties of the sewage sludge are listed in Table 1. The sludge meets EPA guideline on land and groundwater issues.

4.2.2. Experimental design

The experiment included six treatments with four replicates each. The sewage sludge application rate of 10,000 kg ha^{-1}, equivalent to 7.7 g kg^{-1} soil, was selected because it is commonly used by Australian farmers. The amount of P and N added in all amended treatments was 43 mg P kg^{-1} soil and 60 mg N kg^{-1} soil. The control was unamended soil. The amended treatments included sewage sludge alone, inorganic N and P alone (KH_2PO_4 + KNO_3) and the same rate of P and N added as sludge + inorganic N and P at different proportions: 75% sludge+25% inorganic fertiliser (75S25F), 50% sludge+50% inorganic fertiliser (50S50F) and 25% sludge+75% inorganic fertiliser (25S75F). See also Table 2.

Sewage sludge and inorganic fertilisers were mixed thoroughly into the dry soil (400 g dry weight equivalent). Then, reverse osmosis (RO) water was added to adjust the soil water content to 75% of maximum WHC which is the optimum water content for microbial activity in soils of this texture according to Alamgir et al. (2012) . Soil (400 g) was placed into 1 L pots lined with plastic bags. Ten germinated wheat seeds (*Triticum aestivum* L., variety Mace) were planted in each pot and grown in the glasshouse for 5 weeks. After 1 week, the plants were thinned to 9 plants per pot. The temperature in the glasshouse during the experiment ranged from 25 to 35^{o}C. Soil moisture was maintained at 75% of maximum WHC by weight with RO water.

To determine N and P leaching immediately after amendment and after plant growth, 30 g soil was placed into PVC cores with a mesh bottom (3.7 cm diameter, 5 cm height and a 7.5 µm nylon net base) and packed to a bulk density of 1.3 g cm^{-3}. Then 20 ml of RO water was added in 5 ml aliquots which stimulated rainfall of 18.6 mm. Among additions, the water was allowed to drain from the surface before the next aliquot was added. Sufficient water was added to generate leachate out of the lower end of the cores which was filtered before measurement of inorganic N and P.

4.2.3. Analyses

Soil analyses were carried out as described in Hoang and Marschner (2017). Briefly, soil texture was measured by the hydrometer method (Gee & Or, 2002). Soil maximum water-holding capacity was determined after (Wilke, 2005). Soil pH was measured after shaking soil in a 1:5 soil:water ratio for 1 h. Total organic C in sewage sludge was determined by wet oxidation and titration (Walkley & Black, 1934). For total P, shoot and sewage sludge were digested with nitric acid and H_2O_2 at a 4:1 ratio, P was measured by the phosphovanado

molybdate method (Hanson, 1950). Total N in soil and sludge was measured by a modified Kjeldahl method (Bremner & Breitenbeck, 1983).

Soil P pools were determined based on DeLuca et al. (2015) with some modifications. Soil was extracted by shaking a 0.5 g dry soil for 3 h separately with 10 ml of 1 M HCl or 10 mM citric acid. Instead of using 10 mM $CaCl_2$, available P was measured with anion exchange resin method (Kouno et al., 1995). Microbial biomass P (MBP) was measured with the same method by adding hexanol as fumigant (Kouno et al., 1995). MBP was calculated as the difference between available P and P extracted with hexanol. In previous experiments, phosphatase P concentration was low and variable therefore it was not measured it in this study. P concentration in the extracts was measured colorimetrically (630 nm) using the malachite-green method as described in Ohno and Zibilske (1991).

Available N (ammonium and nitrate) was measured after 1h end-over-end shaker with 2 M KCl at 1:5 soil extractant ratio. Ammonium-N was determined by Willis et al. (1996) and nitrate-N as described in Miranda et al. (2001).

Inorganic N and P in the leachate were measured using the same analytical methods as for available N and available P. Leachate N and P are expressed in mg kg^{-1} soil which was calculated as [P/N concentration in leachate x leachate volume]/g soil in the column multiplied by 1000.

4.2.4. Statistical analysis

There were four replicates per treatment. Normal distribution of the data was tested by the Shapiro- Wilk test. The data of P pools was log10 transformed to achieve normal distribution. One-way ANOVA was used to analyse the data for each sampling time separately with Genstat 15^{th} edition (VSN Int. Ltd., UK). Tukey's multiple comparison test at 95% confidence interval

was used to determine significant differences among treatments. For each treatment, two sample t-test was carried out to determine significant differences of between the two sampling times.

4.3. Results

Before planting, HCl P was about four-fold higher in amended treatments than the control (Fig. 1a). Compared to the control, citrate P was about five-fold higher in amended treatments (Fig. 1c). Resin P was lowest in the control, about five-fold higher in 100S and increased with proportion of F; it was 15-fold higher than the control in 50S50F and 25-fold higher in 100F. MBP was lowest in 100F (Fig. 2a) where it was 30% lower than the control. MBP was 75% higher than the control in 100S and 75S25F. Available N was not detectable in the control, in the amended treatments it was lowest in 100S, increased with proportion of F and was about four-fold higher in 100F than 100S (Table 4).

Compared to the control, inorganic N in leachate was about 30% higher in 100S, two-fold higher in 75S25F and 50S50F, and four-fold higher in 25S75F and 100F (Fig. 2c). Inorganic P in leachate which was two orders of magnitude lower than leachate inorganic N differed little between control and treatments with ≥50% S (Fig. 2e). Compared to the control, inorganic P in the leachate was about two-fold higher in 25S75F and five-fold higher in 100F.

Shoot biomass was highest in 50S50F and 25S75F where it was about 50% higher than the control (Table 3). It differed little among the other treatments. Root biomass was up to two-fold higher than the control in treatments with ≥50% F. Shoot P concentration was higher than the control in all amended treatments, with the greatest increase in 25S75F where it was nearly two-fold higher than the control. Shoot P uptake was also higher in amended

treatments than the control, about 50% higher in 100S and 75S25F and two to three-fold higher in treatments with ≥50% F.

After harvest, HCl P was lowest in the control (Fig. 1b). Among amended treatments, HCl P was lowest in 100F, two-fold higher in 25S75F and about three-fold higher in treatments with ≥50% S. Compared to before planting, HCl P after harvest was 10% higher in 75S25F and 50S50F, but two-fold lower in 100F and four-fold lower in the control. Citrate P after harvest was lowest in the control and four to five-fold higher in the amended treatments (Fig. 1d). Among amended treatments, it was about 20% higher with ≥50% S than 25S75F and 100F. Compared to before planting, citrate P after planting remained unchanged in the control, but generally decreased in the amended treatments by about 10%. After harvest, resin P was lowest in the control and was between five and twenty-fold higher in the amended treatments (Fig. 1f). Compared to 100S, resin P was two-fold higher in 75S25S, three-fold higher in 50S50F and four-fold higher in 25S75F and 100F. Compared to before planting, resin P after harvest was 20% lower in 100F and 75S25S, but had changed little in the other treatments. MBP after harvest was lowest in 25S75F and about five-fold higher in the other treatments (Fig. 2b). It was 20-30% lower than before planting in most treatments except 100F where it was 30% higher. Available N and leachate inorganic N after harvest were very low (Table 4, Fig. 2d). Leachate inorganic P after harvest was lowest in 100S and about two-fold higher in control and 75S25F, four-fold higher in 50S50F and more than six-fold higher in 25S75F and 100F (Fig. 2f). Leachate inorganic P differed little between before planting and after harvest in control, 100S and 75S25F, but it increased compared to before planting by 75% in 50S50F and two-fold in 25S75F. It decreased by 25% in 100F.

4.4. Discussion

The experiment showed that sewage sludge alone increased soil P pools, but only slightly increased plant growth and P uptake compared to the unamended control. This is in contrast to field experiments where sewage sludge increased plant growth, yield and nutritional quality (Sharma et al., 2017, Singh & Agrawal, 2008). The lack of sewage sludge effect on plant growth may be due to the short duration of the present experiment or the sewage sludge rate.

Combinations with inorganic N and P and ≥50% N and P from sludge increased P pools as well as plant P uptake compared to sludge alone while reducing potential N and P leaching compared to inorganic N and P alone.

Before planting

The first hypothesis (compared to fertiliser addition, most P pools (except MBP) and N and P leaching will be lower with sludge amendment) has to be declined for HCl P and citrate P because all amendments increased HCl P and citrate P compared to the control to the same extent. The increase of HCl P and citrate P by sewage sludge can be explained by two factors. The measured HCl P and citrate P may come in part from sewage sludge itself as it contains P bound to Fe and Al (Ahmad et al., 2019, González Medeiros et al., 2005). This is likely the reason for the high HCl and citrate P measured shortly after amendment. Over time, P release from sludge as inorganic P may have been bound to soil particles and thus become HCl and citrate extractable P. However, this release seems to have been small as HCl and citrate P changed little over time in the treatments with high proportions of sewage sludge and resin P remained low. This is in agreement with previous studies which also showed that available P was low after sewage sludge amendment (Houben et al., 2019, Pokhrel et al., 2018).

The higher MBP before planting in all treatments with sludge than 100F can be explained by the addition of microbial biomass with the sludge (Sanchez-Monedero et al., 2004). Other studies also showed that sewage sludge amendment increased MBP (Andriamananjara et al., 2016a, Houben et al., 2019).

Inorganic N and P in leachate were highest with 100F because of the addition of soluble N and P in 100F. Leachate N and available N decreased with proportion sludge in the combined treatments because soluble N was added with the fertiliser whereas a large proportion of N in the sludge has to be mineralised before it becomes available or can be leached.

After harvest of wheat

The second hypothesis (in sludge treatments, pools will change less after planting of wheat than with fertiliser only because sludge P is continuously released during decomposition) can be confirmed.

HCl P changed little between before planting and after plant growth in treatments with sludge, but decreased by about 50% in 100F. The decrease of HCl P in 100F can be explained by direct mobilisation of HCl P by protons released by roots (Tarafdar & Claassen, 1988) or depletion of resin P which would trigger release of HCl P. Another possible reason for the decrease is the conversion of HCl P into P pools not assessed by the Deluca method. The lack of change in HCl P in treatments with high proportions of sewage sludge indicates that little of HCl P contained in the sludge was released during the experiment.

The reduction of citrate and resin P after plant growth, particularly in treatments with high proportions of fertiliser P. However even in these treatments, the reduction in resin P and citrate P over time was much greater than P uptake by plants. This indicates that most of the citrate P and resin P was converted into P pools not assessed with the Deluca method. In

treatments with high proportion of sewage sludge, the decrease in citrate P and resin P was smaller than in 100F which can be explained by the poorer plant growth and continuous release of small amounts of P from the sewage sludge. Uptake by soil microbes of P released from sewage sludge over time (Torri et al., 2017) may explain why MBP did not change over time in 100S. Another reason could be that microbial biomass in the sludge remained stable because the sewage sludge provided a continuous source of available OC. However, in the other treatments with sewage sludge, MBP was lower after plant growth than before indicating that turnover of microbes in the sewage sludge was not compensated by P uptake by soil microbes, possibly due to the lower OC availability in these treatments compared to 100S. The roots of the growing wheat plants apparently could not supply sufficient C to maintain a high microbial biomass, possibly due to the relatively low root density.

The third hypothesis (in the combined treatments, P pools, P leaching and wheat P uptake will increase with proportion of fertiliser) can only be confirmed for resin P and leached inorganic P. Shoot P concentration and P uptake were highest in 25S75F which suggests that a combined treatment where a small proportion of N and P are supplied by sludge is beneficial for plant P uptake. This may be because the fertiliser supplied large amounts of N and P for initial growth as evident in the high resin P and leachate P before planting. However, later the sludge provided a longer lasting P source as suggested by the higher HCl P and resin P in 25S75F than 100F after plant growth. The low MBP in 25S75F after plant growth indicates high microbial biomass turnover which may also supply P for plants. Another possible explanation for the low MBP is the high plant P uptake which could limit uptake of P by microbes. In treatments with $\geq$50% sludge, insufficient supply of plant available P from the amendment was apparently limiting plant P uptake. With 100F, the initially soluble P may have been rapidly converted into less available P pools.

Before planting and after wheat growth, inorganic P in the leachate was higher in 100F and 25S75F than treatments with ≥50% S which shows that even after plant growth some fertiliser P was still soluble. In the combined treatments, leachate inorganic P was up to two-fold higher after wheat growth than before planting which indicates that a proportion of P released from sludge during plant growth was water soluble. However, leachate P concentration was very low which explains why in treatments with ≥50% S the increase in leachate P concentration did not affect resin P and why plant P uptake was low.

The very low available N and leachate inorganic N after wheat growth can be explained by plant N uptake. In the treatments with sludge, mineralisation of N in the sludge was apparently too low to provide more inorganic N than plants could take up.

4.5. Conclusion

The experiment showed that sewage sludge alone may increase labile P pools, but has little effect on plant growth. Inorganic N and P addition on the other hand, increased available P and plant P uptake, but also resulted in high potential for P leaching. Combinations of sludge with inorganic fertiliser, particularly those with ≥50% N and P from sludge may be optimal because they increased plant P uptake but also maintained P pools while reducing potential P leached.

Acknowledgement

Khuyen Thi Kim Hoang received a postgraduate scholarship from Vietnamese International Education Development. We greatly thank Garry Smith and the All Waters Company for the sludge.

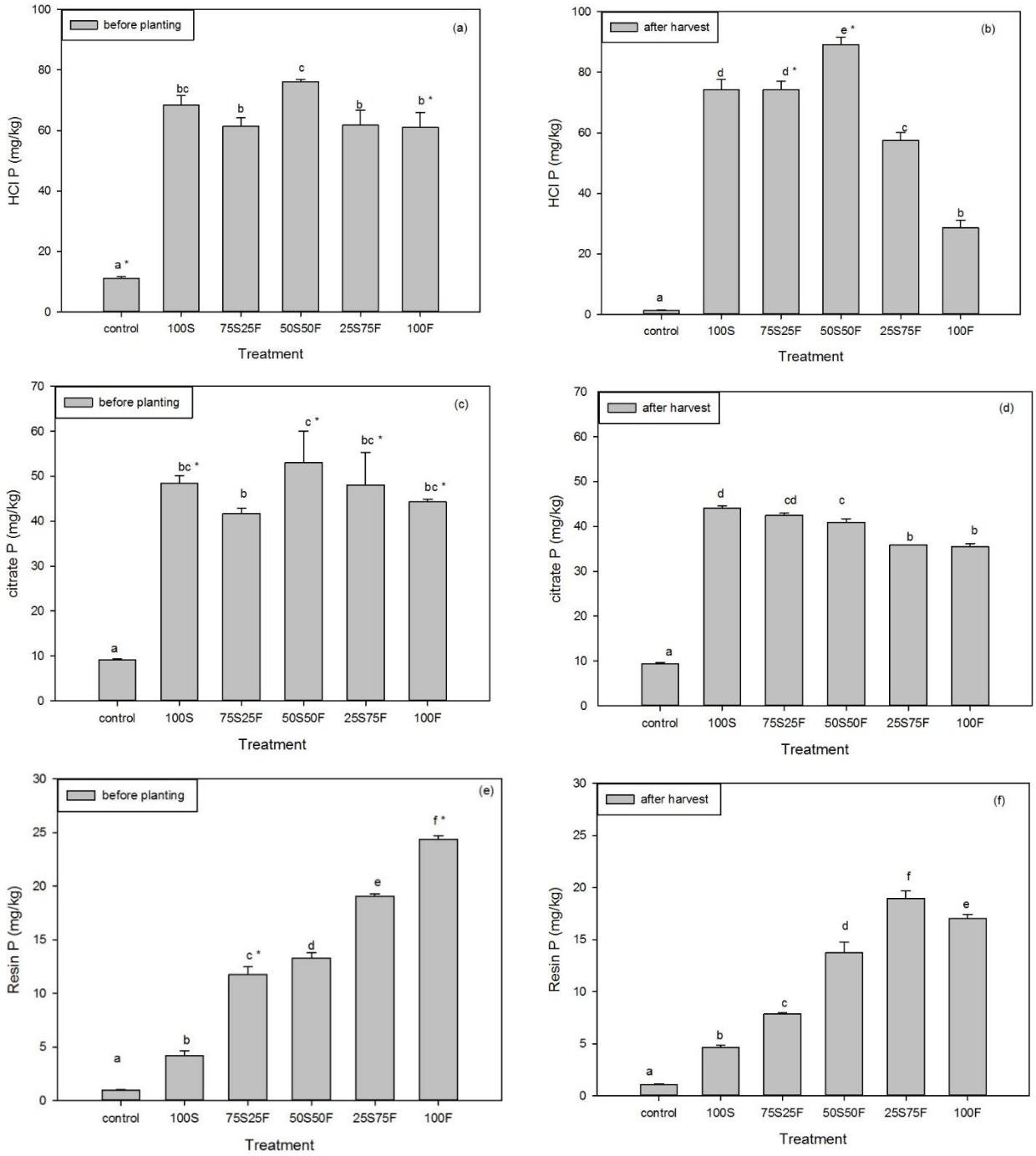

Figure 1. HCl P (a, b), citrate P (c, d), resin P (e, f) before planting and after plant growth in unamended soil or soil amended with P using different proportions of sewage sludge (S) and inorganic fertiliser (F). Vertical lines indicate standard errors. For each sampling time, bars with different letters are significantly different (n=4, P ≤0.05).

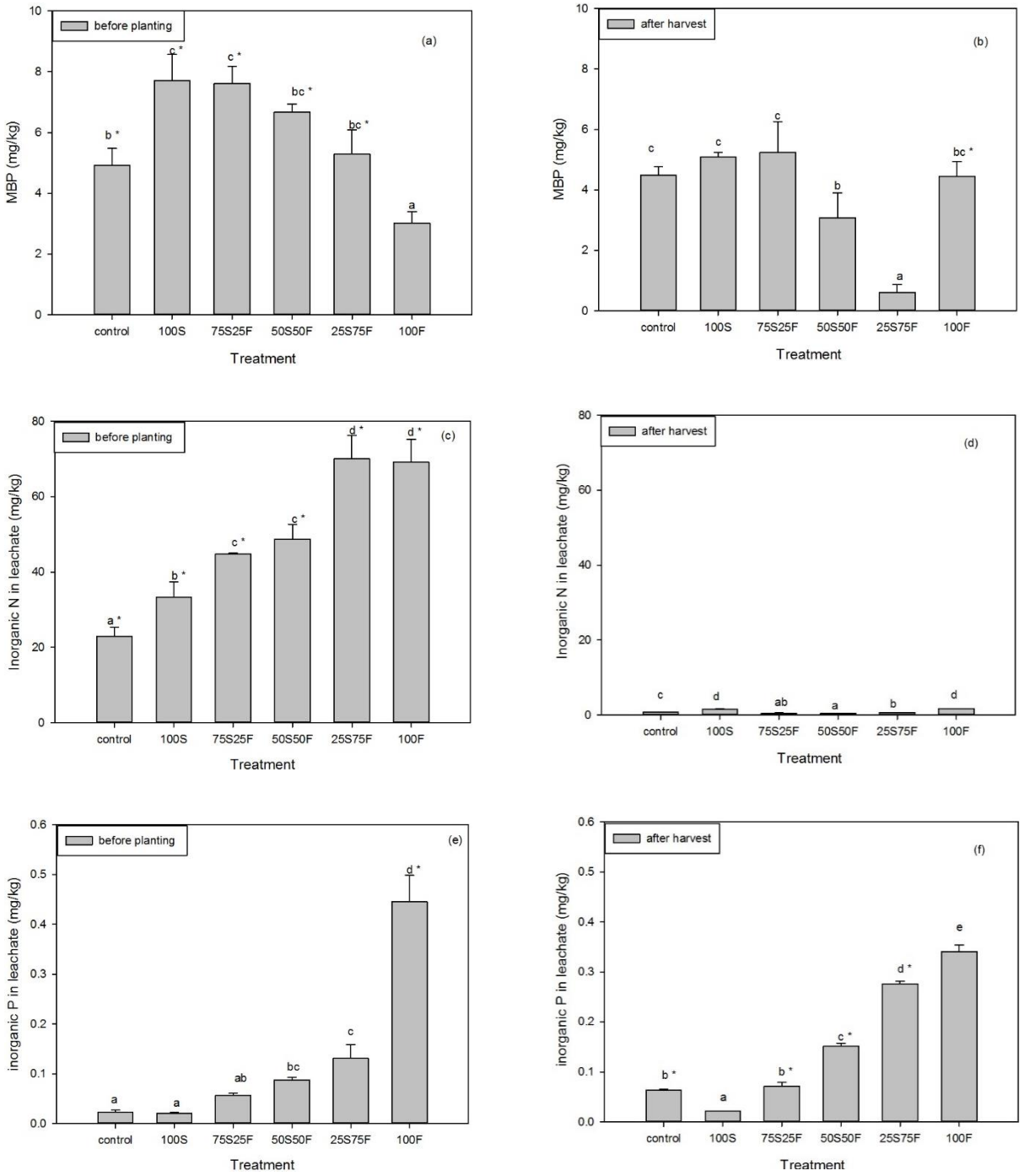

Figure 2. Microbial biomass P (a, b), inorganic N (c, d) and inorganic P (e, f) in leachate before planting and after plant growth in unamended soil or in soil amended with P using different proportions of sewage sludge (S) and inorganic fertiliser (F). Vertical lines indicate standard errors. For each sampling time, bars with different letters are significantly different (n=4, P ≤0.05).

Table 1. Properties of the sewage sludge

Property	mg kg^{-1}
Chromium (VI)	<0.5
Total Cd	3.4
Total Cu	517
Total Zn	675
Total N	4310
Total P	2060
Nitrate + nitrite	45.6
Total Organic C	6420

Table 2. Amount of P and N (mg kg^{-1}) and sewage sludge added in the experiment.

		Total P (mg kg^{-1})	Total N (mg kg^{-1})		
	sludge added (g kg^{-1})	added with sludge	added with sludge	Pi added (mg kg^{-1})	Ni added (mg kg^{-1})
Control	0.0	0.0	0.0	0.0	0.0
100S	7.5	43.3	60.0	0.0	0.0
75S25F	5.5	32.5	45.0	10.8	15.0
50S50F	3.7	21.6	30.0	21.6	30.0
25S75F	1.8	10.8	15.0	32.5	45.0
100F	0.0	0.0	0.0	43.3	60.0

Table 3. Shoot biomass, root biomass (g dry weight pot^{-1}), shoot P concentration and shoot p uptake (mg $plant^{-1}$) in wheat after five weeks growth in unamended soil and soil amended with different proportions of sewage sludge and inorganic N and P fertiliser. Values with different letters are significantly different (n=4, P ≤0.05).

Treatment	Shoot biomass (g pot^{-1})	Root biomass (g pot^{-1})	Shoot P concentration (g kg^{-1})	Shoot P uptake (mg $plant^{-1}$)
Control	$0.72^{\pm0.05}$ a	$0.87^{\pm0.08}$ b	$0.83^{\pm0.02}$ a	$0.06^{\pm0.00}$a
100S	$0.80^{\pm0.03}$ a	$0.80^{\pm0.11}$ a	$1.27^{\pm0.03}$ cd	$0.10^{\pm0.00}$b
75S25F	$1.01^{\pm0.02}$ b	$1.00^{\pm0.03}$ c	$1.04^{\pm0.04}$ b	$0.10^{\pm0.00}$b
50S50F	$1.15^{\pm0.11}$ c	$1.53^{\pm0.03}$ d	$1.14^{\pm0.12}$ bc	$0.12^{\pm0.00}$c
25S75F	$1.17^{\pm0.07}$ c	$1.32^{\pm0.09}$ cd	$1.55^{\pm0.08}$ e	$0.19^{\pm0.01}$d
100F	$0.84^{\pm0.03}$ a	$1.20^{\pm0.08}$ bcd	$1.37^{\pm0.06}$ d	$0.13^{\pm0.01}$c

Table 4. Available N concentration (mg kg^{-1}) before planting and after plant growth in unamended soil and soil amended with different proportions of sewage sludge and inorganic N and P fertiliser. Values with different letters are significantly different (n=4, P ≤0.05).n.d stands for not detectable.

Treatment	Available N (mg kg^{-1}) before planting	Available N (mg kg^{-1}) after plant growth
Control	$0.0^{\pm 0.0}$ a	n.d
100S	$26.7^{\pm 0.5}$ b	n.d
75S25F	$36.7^{\pm 3.0}$ c	n.d
50S50F	$48.0^{\pm 2.5}$ d	n.d
25S75F	$59.4^{\pm 2.6}$ e	n.d
100F	$90.3^{\pm 2.9}$ f	n.d

References

Ahmad, A.M., Ugya, A.Y., Isah, H.A. & Imam, T.S. (2019). Mineralization and mobilization of biosolids phosphorus in soil: A concise review. *Journal of Applied Biology & Biotechnology Vol, 7*, 98-106.

Ahmad, R., Arshad, M., Khalid, A. & Zahir, Z.A. (2008). Effectiveness of organic-/bio-fertilizer supplemented with chemical fertilizers for improving soil water retention, aggregate stability, growth and nutrient uptake of maize (Zea mays L.). *Journal of Sustainable Agriculture, 31*, 57-77.

Alamgir, M., McNeill, A., Tang, C. & Marschner, P. (2012). Changes in soil P pools during legume residue decomposition. *Soil Biology and Biochemistry, 49*, 70-77.

Andriamananjara, A., Rabeharisoa, L., Prud'homme, L. & Morel, C. (2016). Drivers of plant-availability of phosphorus from thermally conditioned sewage sludge as assessed by isotopic labeling. *Frontiers In Nutrition, 3*, 19.

Bertrand, I., Holloway, R., Armstrong, R. & McLaughlin, M. (2003). Chemical characteristics of phosphorus in alkaline soils from southern Australia. *Soil Research, 41*, 61-76.

Bremner, J. & Breitenbeck, G.A. (1983). A simple method for determination of ammonium in semimicro-Kjeldahl analysis of soils and plant materials using a block digester 1. *Communications in Soil Science & Plant Analysis, 14*, 905-913.

Bünemann, E.K. & Condron, L.M. 2007. Phosphorus and sulphur cycling in terrestrial ecosystems. *Nutrient Cycling in Terrestrial Ecosystems,* pp 65-92. Springer.

Dean, R.B. & Suess, M.J. (1985). The risk to health of chemicals in sewage sludge applied to land. *Waste Management & Research, 3*, 251-278.

DeLuca, T.H., Glanville, H.C., Harris, M. *et al.* (2015). A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. *Soil Biology and Biochemistry, 88*, 110-119.

Frossard, E., Sinaj, S., Zhang, L. & Morel, J.-L. (1996). The fate of sludge phosphorus in soil-plant systems. *Soil Science Society of America Journal, 60*, 1248-1253.

Frossard, E., Tekely, P. & Grimal, J. (1994). Characterization of phosphate species in urban sewage sludges by high-resolution solid-state 31P NMR. *European Journal of Soil Science, 45*, 403-408.

Gee, G.W. & Or, D. (2002). 2.4 Particle-size analysis. *Methods of soil analysis. Part, 4*, 255-293.

González Medeiros, J.J., Pérez Cid, B. & Fernández Gómez, E. (2005). Analytical phosphorus fractionation in sewage sludge and sediment samples. *Analytical and Bioanalytical Chemistry, 381*, 873-878.

Hanson, W. (1950). The photometric determination of phosphorus in fertilizers using the phosphovanado-molybdate complex. *Journal of the Science of Food and Agriculture, 1*, 172-173.

Harrison, E.Z., Oakes, S.R., Hysell, M. & Hay, A. (2006). Organic chemicals in sewage sludges. *Science of The Total Environment, 367*, 481-497.

Haynes, R., Murtaza, G. & Naidu, R. (2009). Inorganic and organic constituents and contaminants of biosolids: implications for land application. *Advances In Agronomy, 104*, 165-267.

He, Z., Zhang, H., Toor, G.S. *et al.* (2010). Phosphorus distribution in sequentially extracted fractions of biosolids, poultry litter, and granulated products. *Soil Science, 175*, 154-161.

Hoang, K.K.T. & Marschner, P. (2017). Plant and microbial-induced changes in P pools in soil amended with straw and inorganic P. *Journal of Soil Science and Plant Nutrition, 17***,** 1088-1101.

Houben, D., Michel, E., Nobile, C., Lambers, H., Kandeler, E. & Faucon, M.-P. (2019). Response of phosphorus dynamics to sewage sludge application in an agroecosystem in northern France. *Applied Soil Ecology, 137***,** 178-186.

Huang, X.-L., Chen, Y. & Shenker, M. (2008). Chemical fractionation of phosphorus in stabilized biosolids. *Journal of Environmental Quality, 37***,** 1949-1958.

Kouno, K., Tuchiya, Y. & Ando, T. (1995). Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. *Soil Biology and Biochemistry, 27***,** 1353-1357.

Lu, Q., He, Z.L. & Stoffella, P.J. (2012). Land application of biosolids in the USA: a review. *Applied and Environmental Soil Science, 2012*.

Maguire, R.O., Sims, J.T., Dentel, S.K., Coale, F.J. & Mah, J.T. (2001). Relationships between biosolids treatment process and soil phosphorus availability. *Journal of Environmental Quality, 30***,** 1023-1033.

Marschner, H. (1995). Mineral nutrition of higher plants. 2nd. *Edn. Academic Pres*.

Meade, G., Lalor, S. & Mc Cabe, T. (2011). An evaluation of the combined usage of separated liquid pig manure and inorganic fertiliser in nutrient programmes for winter wheat production. *European Journal of Agronomy, 34***,** 62-70.

Miranda, K.M., Espey, M.G. & Wink, D.A. (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide, 5***,** 62-71.

Ohno, T. & Zibilske, L.M. (1991). Determination of low concentrations of phosphorus in soil extracts using malachite green. *Soil Science Society of America Journal, 55***,** 892-895.

Pokhrel, S., Milke, M., Bello-Mendoza, R., Buitrón, G. & Thiele, J. (2018). Use of solid phosphorus fractionation data to evaluate phosphorus release from waste activated sludge. *Waste Management, 76***,** 90-97.

Pritchard, D., Penney, N., McLaughlin, M., Rigby, H. & Schwarz, K. (2010). Land application of sewage sludge (biosolids) in Australia: risks to the environment and food crops. *Water Science and Technology, 62***,** 48-57.

Sanchez-Monedero, M., Mondini, C., De Nobili, M., Leita, L. & Roig, A. (2004). Land application of biosolids. Soil response to different stabilization degree of the treated organic matter. *Waste Management, 24***,** 325-332.

Scherer, H. & Sharma, S. (2002). Phosphorus fractions and phosphorus delivery potential of a luvisol derived from loess amended with organic materials. *Biology and Fertility of Soils, 35***,** 414-419.

Schulz, H. & Glaser, B. (2012). Effects of biochar compared to organic and inorganic fertilizers on soil quality and plant growth in a greenhouse experiment. *Journal of Plant Nutrition and Soil Science, 175***,** 410-422.

Sharma, B., Sarkar, A., Singh, P. & Singh, R.P. (2017). Agricultural utilization of biosolids: A review on potential effects on soil and plant grown. *Waste Management, 64***,** 117-132.

Siddique, M.T. & Robinson, J.S. (2003). Phosphorus sorption and availability in soils amended with animal manures and sewage sludge. *Journal of Environmental Quality, 32***,** 1114-1121.

Siddique, M.T., Robinson, J.S. & Alloway, B.J. (2000). Phosphorus reactions and leaching potential in soils amended with sewage sludge. *Journal of Environmental Quality, 29***,** 1931-1938.

Singh, R. & Agrawal, M. (2008). Potential benefits and risks of land application of sewage sludge. *Waste management, 28***,** 347-358.

Suge, J., Omunyin, M. & Omami, E. (2011). Effect of organic and inorganic sources of fertilizer on growth, yield and fruit quality of eggplant (Solanum Melongena L). *Archives of Applied Science Research, 3***,** 470-479.

Tarafdar, J. & Claassen, N. (1988). Organic phosphorus compounds as a phosphorus source for higher plants through the activity of phosphatases produced by plant roots and microorganisms. *Biology and Fertility of Soils, 5***,** 308-312.

Tian, G., Granato, T., Cox, A., Pietz, R., Carlson, C. & Abedin, Z. (2009). Soil carbon sequestration resulting from long-term application of biosolids for land reclamation. *Journal of Environmental Quality, 38***,** 61-74.

Torri, S.I., Correa, R.S. & Renella, G. (2017). Biosolid application to agricultural land—A contribution to global phosphorus recycle: A review. *Pedosphere, 27***,** 1-16.

Walkley, A. & Black, I.A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science, 37***,** 29-38.

Wilke, B.-M. 2005. Determination of chemical and physical soil properties. *Monitoring and Assessing Soil Bioremediation,* pp 47-95. Springer.

Chapter 5

Conclusion and Future Research

Conclusions and Future Research

The experiments of this thesis showed how form of P amendment, P rate, organic amendments and plant growth influence soil P pools. The main findings are summarized in Figure 1.

The effect of increasing P addition rates in combinations of straw and inorganic P was investigated in the experiment described in Chapter 2. All P pools except MBP increased with P addition rate and the relative increase was similar. This shows that added P is converted in various P pools, but the absolute increase was greater for HCl and citrate P than the other pools. Thus, P was predominately converted into these pools. MBP did not increase with P rate because in soils, microbial growth is mainly limited by organic C availability (Soong et al., 2019, Tian et al., 2016, Drenovsky et al., 2004). Therefore it was higher in treatments with straw than with only inorganic P.

The depletion of P pools by plants was shown in the experiments in Chapters 2 and 3, confirming that they are bioavailable (DeLuca et al., 2015, Hedley et al., 1982b). Plants strongly reduced HCl and citrate P which shows that these P pools are available to plants. Plants may directly access HCl and citrate P by release of protons or organic acid anions from roots ((Wang & Lambers, 2019)). However, they may also access these pools indirectly by taking up available P forms such as resin and $CaCl_2$ P. Depletion of these pools would trigger the release of P from HCl P and citrate P. This latter process is indicated by the finding that resin P was little affected by plant growth although it is considered as readily available P form (DeLuca et al., 2015, Hedley et al., 1982a). Plants also reduced phosphatase-labile P, possibly

by release of phosphatase from roots or indirectly by increasing microbial activity and thereby microbial mineralisation of organic matter.

In Chapter 2, straw was added to stimulate microbial growth (Takeda et al., 2009) and thereby investigate the effect of microbes on P pools. Similar to plants, microbes depleted HCl P and citrate P. However, in contrast to the reduction of phosphatase P by plants, microbial growth stimulation by straw addition increased phosphatase P. This increase in phosphatase-labile P may be due to microbial metabolites or possibly partial mineralisation of organic matter which could make organic P more labile or accessible to the phosphatase enzyme. MBP was a small P pool, but microbial uptake could still reduce plant P availability (Richardson & Simpson, 2011). However, this immobilisation is transient as microbes die when organic C availability is low, e.g. after decomposition of added straw (Guppy et al., 2005, Jalali & Ranjbar, 2009, Polglase et al., 1992).

The experiment described in Chapter 4 showed that sewage sludge alone is a poor P source for plants although it increased HCl and citrate P to the same extent compared to the control as inorganic P addition alone. The finding that these pools changed little during plant growth with only sewage sludge shows that these pools are not accessible to plants. In contrast, plants depleted HCl and citrate P with soluble inorganic P addition alone. It is likely that HCl and citrate P measured in the soil after sewage sludge addition were in the sludge itself whereas after inorganic P addition the added soluble P was converted into HCl and citrate P. These newly formed P forms may be more accessible to plants than the aged HCl and citrate P in the sludge. Nevertheless, amendment with sludge alone has environmental benefits as it

only slightly increased P leaching compared to the control whereas inorganic P addition increased P leaching more than ten-fold. The experiment showed that combination of sewage sludge and inorganic P with ≥50% of N and P from sludge increased plant growth and P uptake but also maintained low P leaching rates. Such a combination may be particularly important in sandy soils where P loss via leaching can be substantial (Shober et al., 2006, Andriamananjara et al., 2016b). The HCl P and citrate P in the sludge may serve as a long-term P source for plants and may be suitable for crops with slow growth rates.

However in the experiments described in Chapters 2 and 4, the proportion of added P taken up by plants was small. This confirms that the soils used in these experiments have a high P fixing capacity, i.e., added P is rapidly converted in poorly available P forms. Nevertheless, the capacity of plants to access HCl P and citrate P suggests that some of the less available P forms can become plant available, particularly when plants are grown for longer periods of time. This may trigger transformation of recalcitrant P pools into HCl P and citrate P. Wheat was grown in the experiments, but other crop species such as white lupin may have greater access to bioavailable as well as recalcitrant P forms because they release larger amounts of organic acid anions (Dissanayaka et al., 2015, Uhde-Stone et al., 2005).

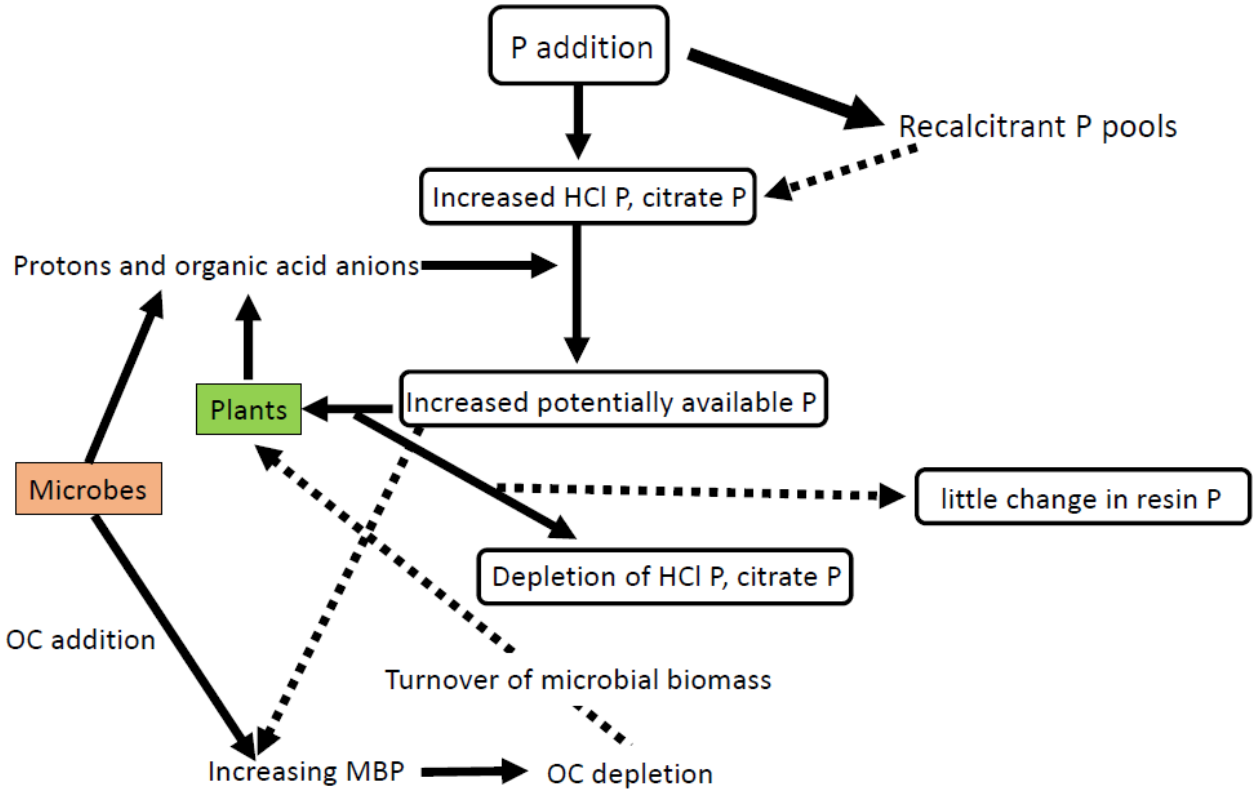

Figure 1: Effect of P amendment, plants and microbes on soil P pools. Thickness of arrows indicates flux size, hatched arrows indicates minor fluxes.

Future research

In the studies described in this thesis, the effect of the amendments on soil P pools and plant P uptake were maximised by adding the organic amendments finely ground and mixing them into to the soil, maintaining the soil moist using a small pot volume. In the field conditions are different because (i) organic amendments may be added in larger particles or left on the soil surface, (ii) soil water content may vary, and (iii) roots have access to a large soil volume and may not come close to the organic amendments.

In field experiments, organic amendments could be added in different particles sizes, mixed into the soil or left on the soil surface. P pools could be measured at different soil depths and P uptake by crops over the growing season determined.

In this thesis, soil water content was maintained at optimal level for plant growth and microbial activity. But this may not be the case in the field and it is known that soil water content influences P transformations. For example, saturation of soil can increase P availability by inducing anaerobic conditions which can mobilise Fe and thereby release P bound to Fe (Pandey & Srivastava, 2009, Ben-Gal & Dudley, 2003). However, drainage or leaching can remove P from the root zone (Carstensen et al., 2019, Sadhukhan et al., 2019). Drying of soils on the other hand, reduces P diffusion and thus P uptake by microbes and plants as well as P fluxes among pools (Jipeng et al., 2017). To assess the effect of soil water content in a field situation, there could three treatments: ambient rainfall, reduced rainfall by setting up roofs and increased water supply by irrigation. At different points during the growing season, P pools could be measured at different soil depths and P uptake by crops determined.

In the experiments described in this thesis, three soils were used, but not directly compared. Soil properties such as texture and pH influence P pools and transformations. For example, sandy soils have fewer binding sites for P than clay-rich soils (Andersson et al., 2013). Thus more P will remain plant available in sandy than clayey soils. The type of P minerals formed and their solubility are influenced by soil pH (Harrison, 1982, Marschner et al., 2005). At low pH, Fe/Al phosphates occur whereas at high pH, Ca phosphates are formed (Penn et al., 2018) The stability of these minerals depends on pH, increasing the pH in acid soils or reducing it in alkaline soils releases P from minerals and thereby increases P availability (Li et al., 2010,

Hinsinger, 2001). To better understand P transformations, future experiments could include addition of the same P rates and P forms to a range of soils differing in texture and pH.

The effect of root access could be investigated by installing root barriers at different distance from the planted rows but applying the same amount of amendments. P pools could be measured at different soil depths and P uptake by crops over the growing season determined.

In this thesis, the source of P in P pools and plants could not be determined. Future experiments could use ^{33}P labelled fertilisers together with the organic amendments and then measure ^{33}P in plants and soil P pools.

Farmers often apply sewage sludge several times over the years. A long-term field experiment could be set up with annual sewage sludge application where crop P uptake and soil P pools are measured annually. Further treatments would include an unamended control and addition of inorganic fertilisers with P and N at the same rate as in sludge treatments. Additionally, farmer's soils with known history of sewage sludge application could be sampled. In such a field experiments, parameters such as soil organic matter content, aggregate stability and crop metal uptake could also be measured.

References

Andersson, H., Bergström, L., Djodjic, F., Ulén, B. & Kirchmann, H. (2013). Topsoil and subsoil properties influence phosphorus leaching from four agricultural soils. *Journal of Environmental Quality, 42***,** 455-463.

Andriamananjara, A., Rabeharisoa, L., Prud'homme, L. & Morel, C. (2016). Drivers of Plant-Availability of Phosphorus from Thermally Conditioned Sewage Sludge as Assessed by Isotopic Labeling. *Frontiers in Nutrition, 3*.

Ben-Gal, A. & Dudley, L.M. (2003). Phosphorus availability under continuous point source irrigation. *Soil Science Society of America Journal, 67***,** 1449-1456.

Carstensen, M.V., Børgesen, C.D., Ovesen, N.B., Poulsen, J.R., Hvid, S.K. & Kronvang, B. (2019). Controlled Drainage as a Targeted Mitigation Measure for Nitrogen and Phosphorus. *Journal of Environmental Quality, 48***,** 677-685.

DeLuca, T.H., Glanville, H.C., Harris, M. *et al.* (2015). A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. *Soil Biology and Biochemistry, 88***,** 110-119.

Dissanayaka, D.M.S.B., Maruyama, H., Masuda, G. & Wasaki, J. (2015). Interspecific facilitation of P acquisition in intercropping of maize with white lupin in two contrasting soils as influenced by different rates and forms of P supply. *Plant and Soil, 390***,** 223-236.

Drenovsky, R., Vo, D., Graham, K. & Scow, K. (2004). Soil water content and organic carbon availability are major determinants of soil microbial community composition. *Microbial Ecology, 48***,** 424-430.

Guppy, C.N., Menzies, N.W., Blamey, F.P.C. & Moody, P.W. (2005). Do Decomposing Organic Matter Residues Reduce Phosphorus Sorption in Highly Weathered Soils? *Soil Science Society of America Journal, 69***,** 1405-1411.

Harrison, A. (1982). Labile organic phosphorus mineralization in relationship to soil properties. *Soil Biology and Biochemistry, 14***,** 343-351.

Hedley, M.J., Stewart, J. & Chauhan, B. (1982a). Changes in inorganic and organic soil phosphorus fractions induced by cultivation practices and by laboratory incubations. *Soil Science Society of America Journal, 46***,** 970-976.

Hedley, M.J., Stewart, J. & Chauhan, B. (1982b). Changes in Inorganic and Organic Soil Phosphorus Fractions Induced by Cultivation Practices and by Laboratory Incubations 1. *Soil Science Society of America Journal, 46***,** 970-976.

Hinsinger, P. (2001). Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review. *Plant and Soil, 237***,** 173-195.

Jalali, M. & Ranjbar, F. (2009). Rates of decomposition and phosphorus release from organic residues related to residue composition. *Journal of Plant Nutrition and Soil Science, 172***,** 353-359.

Jipeng, W., Yanhong, W., Jun, Z. *et al.* (2017). Air-drying changes the distribution of Hedley phosphorus pools in forest soils. *Pedosphere*.

Li, H., Shen, J., Zhang, F., Marschner, P., Cawthray, G. & Rengel, Z. (2010). Phosphorus uptake and rhizosphere properties of intercropped and monocropped maize, faba bean, and white lupin in acidic soil. *Biology and Fertility of Soils, 46***,** 79-91.

Marschner, P., Solaiman, Z. & Rengel, Z. (2005). Growth, phosphorus uptake, and rhizosphere microbial-community composition of a phosphorus-efficient wheat cultivar in soils differing in pH. *Journal of Plant Nutrition and Soil Science, 168***,** 343-351.

Pandey, C. & Srivastava, R. (2009). Plant available phosphorus in homegarden and native forest soils under high rainfall in an equatorial humid tropics. *Plant and Soil, 316***,** 71-80.

Penn, C.J., Rutter, E.B., Arnall, D.B., Camberato, J., Williams, M. & Watkins, P. (2018). A discussion on mehlich-3 phosphorus extraction from the perspective of governing chemical reactions and phases: Impact of soil pH. *Agriculture, 8***,** 106.

Polglase, P., Jokela, E. & Comerford, N. (1992). Nitrogen and phosphorus release from decomposing needles of southern pine plantations. *Soil Science Society of America Journal, 56***,** 914-920.

Richardson, A.E. & Simpson, R.J. (2011). Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiology, 156***,** 989-996.

Sadhukhan, D., Qi, Z., Zhang, T.-Q., Tan, C.S. & Ma, L. (2019). Modeling and Mitigating Phosphorus Losses from a Tile-Drained and Manured Field Using RZWQM2-P. *Journal of Environmental Quality, 48***,** 995-1005.

Shober, A.L., Hesterberg, D.L., Sims, J.T. & Gardner, S. (2006). Characterization of phosphorus species in biosolids and manures using XANES spectroscopy. *Journal of Environmental Quality, 35***,** 1983-1993.

Shober, A.L., Hesterberg, D.L., Sims, J.T. & Gardner, S. (2006). Characterization of phosphorus species in biosolids and manures using XANES spectroscopy. *Journal of Environmental Quality, 35*, 1983-1993.

Soong, J.L., Fuchslueger, L., Marañon-Jimenez, S. *et al.* (2019). Microbial carbon limitation-the need for integrating microorganisms into our understanding of ecosystem carbon cycling. *Global Change Biology*.

Takeda, M., Nakamoto, T., Miyazawa, K., Murayama, T. & Okada, H. (2009). Phosphorus availability and soil biological activity in an Andosol under compost application and winter cover cropping. *Applied Soil Ecology, 42*, 86-95.

Tian, Q., Yang, X., Wang, X. *et al.* (2016). Microbial community mediated response of organic carbon mineralization to labile carbon and nitrogen addition in topsoil and subsoil. *Biogeochemistry, 128*, 125-139.

Uhde-Stone, C., Liu, J., Zinn, K.E., Allan, D.L. & Vance, C.P. (2005). Transgenic proteoid roots of white lupin: a vehicle for characterizing and silencing root genes involved in adaptation to P stress. *The Plant Journal, 44*, 840-853.

Wang, Y. & Lambers, H. (2019). Root-released organic anions in response to low phosphorus availability: recent progress, challenges and future perspectives. *Plant and Soil*, 1-22.

Appendix 1

The depletion of soil P pools by wheat in soil amended with straw and KH_2PO_4

The depletion of soil P pools by wheat in soil amended with straw and KH_2PO_4

Khuyen Thi Kim Hoang[1,2] and Petra Marschner[1]

[1]School of Agriculture, Food and Wine, The University of Adelaide, Adelaide SA 5005, Australia and [2]Chemical Engineering Faculty, Industrial University of Ho Chi Minh, HCMC 7000, Vietnam

Abstract

Aim: Cereal straw is often left in the field after harvest and then mixed into the soil before sowing of the following crop. The aim of this study was to determine how combinations of straw and inorganic P affect soil P pools and P uptake by wheat over time.

Methods: P was added at 64 mg kg^{-1} either as only inorganic P (KH_2PO_4) or as 5, 10, 15, or 20g kg^{-1} straw with inorganic P. After 3 weeks incubation, wheat was grown for five or nine weeks. P pools were measured after 3, 5 and 9 weeks

Results: Citrate P, HCl P, phosphatase P and resin P decreased with straw rate whereas microbial biomass P increased. Wheat growth and P uptake were higher with inorganic P alone than in straw treatments. Wheat depleted citrate P, HCl P, phosphatase P and resin P over time.

Conclusion: Microbial P immobilization reduced P availability for wheat throughout the experiment even though microbial biomass P decreased from week 5 to week 9. This suggests that microbial metabolites were converted into stable soil organic matter.

Keywords: C/P ratio, depletion, P pools; straw

1. Introduction

Phosphorus is an essential macronutrients for plants, but its availability in soil is low because it is converted into stable inorganic and organic P forms which are poorly plant available (Richardson, 2001). Thus, P fertiliser efficiency is low and farmers often apply P in excess of plant demand (George et al., 2016). The current deposits used for extraction of rock phosphate which is then converted into P fertilisers, are limited (Walan et al., 2014). It is therefore important to optimise management of P fertilisation. Organic amendments can be nutrient sources and improve soil properties (Noack et al., 2012). The effect of organic amendments on nutrient availability depends on their chemical composition, in case of P, on their C/P ratio (Dalal, 1979, Alamgir et al., 2012). Cereal straw is often left in the field after harvest and then mixed into the soil before sowing of the following crop. However, mature straw is low in P and can result in net P immobilisation by soil microbes due to its high C/P ratio which results in low P availability for plants (Alamgir et al., 2012). It has been suggested that turnover of the microbial biomass can lead to release of immobilised P which can then be taken up by plants (Kouno et al., 2002). It is unclear to what extent inorganic P can be replaced by P from straw and how P availability changes over time during crop growth. To better understand the fate of P in soil, the size of various P pools can be measured. Deluca et al. (2015) introduced a method that can be used to determine P pools that can be mobilised by root and microbial exudates and can therefore assess potentially available P.

The aim of this study was to assess how straw rate influences P pools and wheat growth when P is supplied at the same rate as either inorganic P alone, straw alone or combinations of straw and inorganic P. The hypotheses were (i) with increasing straw rate, microbial biomass

P will increase whereas the other P pools will decrease, and (ii) depletion of P pools by plants will increase with plant age.

2. Materials and methods

2.1. Soil

A loamy sand was collected from 0 to 10 cm on Waite Campus, The University of Adelaide, South Australia (Longitude 138° 38'E, Latitude 35° 6'S) which had been under permanent pasture over 80 years. The soil was dried at 40°C and passed through a 2 mm sieve. The properties of the soil are as follows: pH 6.8 (1:5 soil/water); clay 25%; sand 37%; silt 37%; total P 302 mg kg^{-1}; pH (1:5) 5.6, EC (1:5) 0.1 dS m^{-1}, total organic C 17 g kg^{-1}, total organic N 1.5 g kg^{-1}, bulk density 1.3 g cm^{-3}, maximum water-holding capacity 349 g kg^{-1}. The properties of the mature barley straw (finely ground and sieved to particle size of 0.25-2 mm) used in this experiment were: total P 1.6 g kg^{-1}; total N 4.3 g kg^{-1}; total C 408 g kg^{-1}; C/P ratio 255.

2.2. Experimental design

The soil was pre-incubated for 3 weeks at 50% WHC. The experiment included 5 treatments with four replicates each. The control was soil with 64 mg kg^{-1} inorganic P only and 4 straw treatments (5, 10, 15, 20 g kg^{-1} soil) with 400g soil per pot. Then inorganic P was used to adjust the total P to 64 P mg kg^{-1} (table 1). All treatments received 80 mg N kg^{-1} to provide enough N for plants and again to those plants harvested in week 9. Residues and inorganic nutrients were mixed thoroughly into the soil.

Table 1. Treatment names and barley straw and inorganic P added (mg kg^{-1}).

Treatment	Straw rate (g kg^{-1})	Total P added with straw (mg kg^{-1})	Pi added with fertiliser (mg kg^{-1})
Control	0	0	64
Straw 5	5	8	56
Straw 10	10	16	48
Straw 15	15	24	40
Straw 20	20	32	32

Soil at 400g dry weight equivalent was placed in 500 ml pots lined with plastic bags then incubated in the dark at 22-25^{o}C for 21 days to allow the formation of P pools. During incubation, soil moisture was maintained at 50% WHC by weight. Then, 10 and 5 pre-germinated wheat seeds (*Triticum aestivum L., variety Axe)* were planted, 10 seedlings for the harvest after 5 weeks, 5 for the harvest after 9 weeks. The different plant number was used to ensure sufficient plant biomass, but also to minimize plant competition in the treatment where wheat was grown for 9 weeks. The pots were placed in a glasshouse with natural light and temperature from 25 to 30^{o}C.

2.3. Analyses

Soil texture was determined by the hydrometer method (Gee & Or, 2002). Soil maximum water holding capacity was measured using a sintered glass funnel connected to a 1 m water column (Wilke, 2005). Soil pH was measured after 1 h end-over-end shaking in a 1:5 soil:water ratio. Total organic carbon of residues was determined by $K_2Cr_2O_7$ and H_2SO_4 oxidation by titration with acidified $(NH_4)_2Fe(SO_4)_2.6H_2O$ (Walkley & Black, 1934). Total P in soil was determined by the phosphovanado molybdate method (Hanson, 1950) after acid digestion with nitric acid-perchloric acid at a 4:1 ratio (Olsen et al., 1982). For total P, wheat shoots and straw were digested with nitric acid and H_2O_2 at a 4:1 ratio followed by P determination using

the phosphovanado molybdate method (Hanson, 1950). Total N in straw was measured by a modified Kjeldahl method (Bremner & Breitenbeck, 1983)

At the end of 5 and 9 weeks the following properties were determined. Soil available N (ammonium and nitrate) concentration was measured after 1 h end-over-end shaker with 2M KCl at 1:5 soil extractant ratio. Ammonium-N was determined after Willis et al. (1996) and nitrate-N as described in Miranda et al. (2001). Available P in soil was extracted by the anion exchange resin method (Kouno et al., 1995). This method was also used to determine microbial biomass P (MBP), but using hexanol instead of chloroform as fumigant. The P concentration was determined colorimetrically (Murphy & Riley, 1962). MBP was calculated as the difference between available P and P extracted with hexanol.

Soil P pools were determined according to Deluca et al. (2015) using four extractants in parallel, including 10 mM citric acid, 1M HCl, 10 mM $CaCl_2$ and 0.2 enzyme unit phosphatase (from wheat germ) extractable P. Moist soil equivalent to 0.5 g dry soil were shaken with 10 ml of each extractant separately for 3 hrs (DeLuca et al., 2015). P in the extracts was determined by the malachite-green method (Ohno & Zibilske, 1991).

2.4. Statistical analysis

Data were analysed separately for each sampling time by one-way ANOVA with Genstat 15th edition (VSN Int. Ltd., UK). Tukey's multiple comparison tests at 95% confidence interval was used to determine significant differences among treatments.

3. Results

HCl-P was the largest P pool (e), followed by citric acid P (b), resin P (c), MBP (a) and phosphatase P (d). $CaCl_2$ P in week 0 (after three weeks incubation) was detectable only in the control (only inorganic P added) and Straw 5 (f).

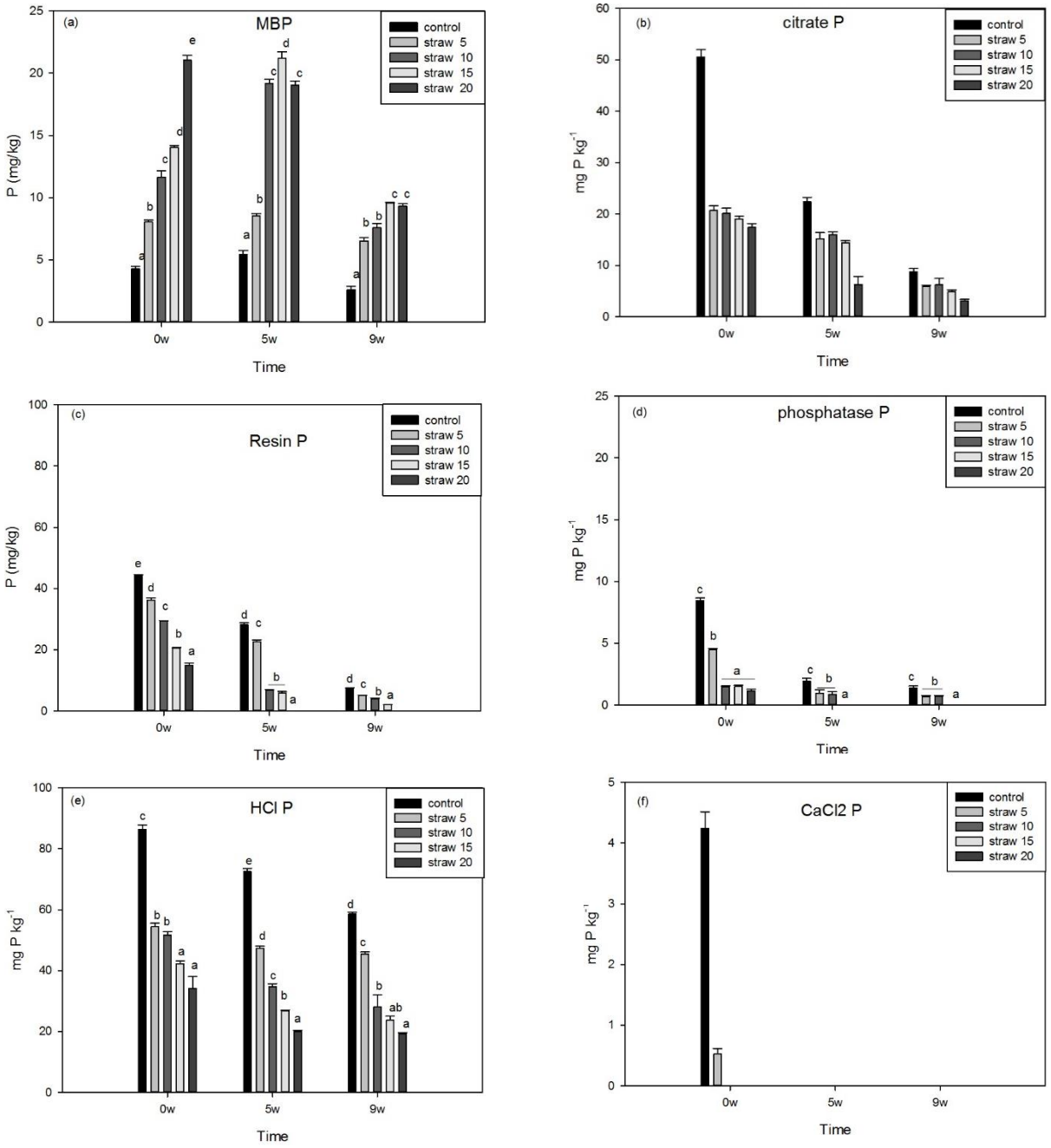

Figure 1. Concentration of P pools 3 weeks incubation, after 5 weeks and 9 weeks growth of wheat in unamended soil (control) and soil amended with straw at 5, 10, 15 and 20 g kg^{-1} and inorganic P to a total P addition of 64 mg P kg^{-1}. Vertical lines indicate standard errors. For each sampling date, bars with different letters are significantly different (n=4, P≤0.05)

In week 0, all P pools except MBP were highest in the control and lowest in Straw 20. In the straw treatments, P pool concentrations decreased with straw addition rate. Compared to the control, HCl, citric acid, phosphatase and resin P were between two and seven-fold lower in Straw 20. $CaCl_2$ P was not detectable in Straw 10, 15 and 20. MBP increased with straw addition rate, it was nearly five-fold higher in Straw 20 than the control.

After 5 weeks growth of wheat, the concentrations of HCl, citric acid, phosphatase and resin P were lower than before plant growth (week 0), HCl P about 20%, citrate P 25-60%, phosphatase and resin P 40-80%. As in week 0, HCl, citric acid, phosphatase and resin P were highest in the control and lowest in Straw 20. Differences among straw treatments in citric acid and phosphatase P were less pronounced in week 5 than week 0. However, differences in resin P were greater in week 5 than week 0. For example, resin P in Straw 15 was about half of that in the control in week 0, but about five-fold lower in week 5. Phosphatase P was not detectable in Straw 15 and Straw 20. MBP increased from week 0 to week 5 except in Straw 20 where it decreased. The increase was greater in Straw 10 and Straw 15 than Straw 5. MBP after 5 weeks was lowest in the control and did not differ between Straw 10 and 20.

The decrease in P pools from week 5 to week 9 was smaller than from week 0 to week 5 in HCl and phosphatase P in all treatments. In HCl, citrate, phosphatase and resin P the decrease was greater in the control than the straw treatments. In week 9 HCl, citrate, phosphatase and resin were highest in the control. Among straw treatments, HCl and resin P decreased with straw addition rate, but citric acid P differed little between Straw 5 and 10. MBP was about 50% lower in week 9 than week 5. It was lowest in the control and highest in Straw 15 and 20.

In week 0, available N was highest in the control. Among straw treatments, available N decreased with increasing straw addition rate. It was about 30-fold lower in Straw 20 than

the control. In week 5, available N was very low, it was highest in the control and not detectable in Straw 15 and 20. After sampling in week 5, N was applied at 80 mg N/kg to increase plant N availability since N availability in week 5 was low. In week 9, available N was higher than in week 5. It was higher in the control and Straw 10 than the other straw treatments. Among the straw treatments, available N decreased with straw rate.

In week 0, soil pH was 6.3-6.5 in the straw treatments and 5.7 in the control (data not shown). Soil pH increased in the control by about 0.6 units from week 0 to week 5 whereas it increased only by about 0.2 units in the straw treatments. Soil pH differed little between week 5 and week 9 and was similar in all treatments.

In week 5, shoot dry weight was about three-fold higher in the control than the straw treatments whereas root dry weight was only about 20% higher in the control and rate 20 than others. The shoot/root ratio was 2 in the control and about 1 in the straw treatments. Shoot dry weight per plant increased about three-fold from week 5 to week 9 in the control and Straw 5, but only about two-fold in the other straw treatments. Root dry weight per plant increased by about 30% in the control from week 5 to week 9, but remained unchanged in the straw treatments. The shoot/root ratio was 4.8 in the control, 2.7 in Straw 5 and between 1.3 and 1.5 in Straw 10-20. In both week 5 and week 9, shoot P uptake was highest in the control. In the straw treatments, it decreased with straw addition rate. Compared to the control, shoot P uptake in week 5 was 40% lower in Straw 5 and 70% lower in Straw 20. Compared to week 5, shoot P uptake in week 9 was two-fold higher in the control and Straw 5, but changed little in the other straw treatments. Compared to the control, shoot P uptake in week 9 was 40% lower in Straw 5 but about five-fold lower in Straw 20.

Table 3. Shoot biomass, root biomass (g dry weight pot^{-1}), shoot P concentration and shoot P uptake (mg $plant^{-1}$) in wheat after five weeks and nine weeks growth in soil amended with straw at different rates and inorganic N and P and unamended soil. Values with different letters are significantly different (n=4, $P \leq 0.05$).

5 weeks	Shoot biomass (g $plant^{-1}$)	Root biomass (g $plant^{-1}$)	shoot P concentration (g kg^{-1})	Shoot p uptake (mg $plant^{-1}$)
Control	0.16^{b}	0.08^{b}	3.49^{a}	0.55^{d}
Straw 5	0.06^{a}	0.06^{a}	5.37^{b}	0.33^{c}
Straw 10	0.05^{a}	0.06^{a}	5.71^{b}	0.31^{bc}
Straw 15	0.05^{a}	0.06^{a}	5.65^{b}	0.28^{b}
Straw 20	0.05^{a}	0.07^{ab}	3.36^{a}	0.18^{a}

9 weeks	Shoot biomass (g $plant^{-1}$)	Root biomass (g $plant^{-1}$)	shoot P concentration (g kg^{-1})	shoot p uptake (mg $plant^{-1}$)
Control	0.60^{c}	0.13^{b}	1.88^{a}	1.12^{e}
Straw 5	0.19^{b}	0.07^{a}	3.87^{c}	0.66^{d}
Straw 10	0.10^{a}	0.07^{a}	4.48^{c}	0.46^{c}
Straw 15	0.09^{a}	0.06^{a}	2.92^{b}	0.26^{b}
Straw 20	0.08^{a}	0.06^{a}	2.40^{ab}	0.17^{a}

4. Discussion

The hypotheses were (i) with increasing straw rate, microbial biomass P will increase whereas the other P pools will decrease, and (ii) depletion of P pools by plants will increase with plant age.

The first hypothesis (with increasing straw rate, microbial biomass P will increase whereas the other P pools will decrease) can be confirmed. The increase in MBP with straw rate can be explained by P immobilization due to the high C/P ratio of straw (Nguyen et al., 2016).

However, the increase in MBP with straw rate was smaller than the decrease in the other P pools. P may be bound to straw or P may have been taken up by microbes, but after cell death the P-containing microbial metabolites became part of the soil organic matter. Another explanation for the stronger decrease in the other P pools could be P uptake by the plants which increased with decreasing straw rate. MBP was lower in week 9 than at the earlier sampling times. This was most pronounced at straw rates ≥ 10 g kg^{-1}. The lower MBP in week 9 can be explained by depletion of decomposable C from straw. By week 5, easily decomposable C had likely been largely depleted. Roots may also provide decomposable C for microbes, but the low MBP in the control indicates that the amount of C supplied by roots is small. Thus in the control, microbes were probably C limited as indicated by the low MBP despite high concentrations of the other P pools. Available N in week 9 was lower in the control and straw 20 than in the other treatments. In the control, the low N availability can be explained by plant uptake. In straw 20, strong plant uptake is unlikely because the plants were very small. More likely is N immobilisation by microbes decomposing the straw (Cheshire et al., 1999) as it can be seen for P. This is also indicated by the decrease in available N concentration with increasing straw rate in weeks 0 and 9. The low N and P availability at straw rates ≥10 g kg^{-1} resulted in strongly reduced plant growth compared to the control which shows that soil microbes can outcompete plants when supplied with an organic C source that is low in N and P. Similar findings were reported in (Bardgett et al., 2003, Dunn et al., 2006, Marschner, 2008, Marschner et al., 2011). The fact that nutrient competition reduced plant growth for up to 9 weeks suggest that although microbial biomass P decreased, P remained poorly available to plants. This indicates that microbial P was converted into stable organic P forms (Golchin et al., 1997). Microbial metabolites are an important component of stable soil organic matter (Wei et al., 2015).

Table 2. Soil available N (mg kg^{-1}) after 3 weeks incubation (week 0) and after 5 weeks and 9 weeks growth of wheat in unamended soil (control) and soil amended with straw at 5, 10, 15 and 20 g kg^{-1} . Values with different letters are significantly different (n=4, $P \leq 0.05$).

week	0	5	9
Control	107.33^{e}±0.45	0.44^{b}±0.06	3.36^{a}±0.22
Straw 5	50.81^{d}±0.70	0.17^{a}±0.04	45.25^{d}±1.42
Straw 10	20.87^{c}±2.96	0.02^{a}±0.00	23.74^{c}±2.92
Straw 15	12.34^{b}±1.66	0.00^{a}±0.00	13.12^{b}±1.12
Straw 20	1.50^{a}±0.27	0.00^{a}±0.00	3.52^{a}±0.32

The second hypothesis (depletion of P pools by plants will increase with plant age) can also be confirmed, but P pool depletion did not increase linearly with plant age. In the control, citrate P, resin P and HCl P decreased to a similar extent from week 0 to week 5 as from week 5 to week 9 whereas in the other P pools the decrease from week 5 to week 9 was smaller than in the first 5 weeks. But whereas resin P and citrate P decreased by about 50% in each growth period, the reduction in HCl P was only about 10%. The smaller reduction in HCl P indicates that it is less available to plants than resin P and citrate P, but may also be due to the larger size of HCl P compared to resin and citrate P. Plants access resin P by taking up P from the soil solution which triggers the release of labile P (Hoang & Marschner, 2019). Organic acid anions released by roots mobilise citrate P (Jones & Darrah, 1994). In the straw treatments, citrate P decreased by about 50% in each growth period, but HCl P changed little and the reduction in resin P was greater from week 5 to week 9 than from week 0 to week 5. This suggests that plants in the straw treatments accessed primarily resin P after week 5. They may not have been able to further decrease HCl P because the remaining HCl P was too strongly bound to soil particles.

The increase in plant biomass was greater from week 5 to week 9 than in the first 5 weeks, but the increase in shoot P uptake between week 0 and week 5 matched that from week 5 to week 9. This indicates that much of the biomass increase was in structural compounds with relatively low P concentration as indicated by the lower shoot P concentration in plants harvested in week 9 compared to those harvested in week 5. Nutrient dilution by growth is common in plants (Jarrell & Beverly, 1981, Loehwing, 1953).

At straw rates ≥ 10 g kg^{-1}, depletion of P pools greater from week 0 to week 5 than from week 5 to week 9 because increase in plant biomass greater from week 0 to week 5. Plants likely to be P and N limited after 5 weeks due to N and P immobilisation by microbes decomposing the straw. Addition of N in week 5 did not cause growth increase as it did in the control and straw 5 which suggests that P limitation is the main constraint for plants. Another possible reason for the lack of growth increase is that the added N was taken up by microbes and therefore not available to plants. As with microbial P, microbial N may have been converted into stable organic N upon biomass turnover.

Conclusion

High rates of straw addition (≥ 10 g kg^{-1}) strongly reduced plant growth even in presence of some inorganic P compared to the same P rate as only inorganic P. This reduction was long-lasting although microbial biomass was lower after 9 than 5 weeks. This suggests that microbial metabolites were converted into stable organic P forms. The results indicate that addition of straw with inorganic P is not a suitable method to improve fertiliser efficiency. It is possible that at higher P addition rates and with the majority of P from inorganic fertiliser, benefits from adding straw such as improvement of soil structural stability would outweigh the negative effects of microbial nutrient competition.

Acknowledgements

Khuyen Thi Kim Hoang PhD thanks the Vietnam International Education Development (VIED) for providing the postgraduate scholarship.

References

Alamgir, M., McNeill, A., Tang, C., & Marschner, P. (2012). Changes in soil P pools during legume residue decomposition. *Soil Biology and Biochemistry, 49*, 70-77.

Bardgett, R. D., Streeter, T. C., & Bol, R. (2003). Soil microbes compete effectively with plants for organic-nitrogen inputs to temperate grasslands. *Ecology, 84*(5), 1277-1287.

Bremner, J., & Breitenbeck, G. A. (1983). A simple method for determination of ammonium in semimicro-Kjeldahl analysis of soils and plant materials using a block digester 1. *Communications in Soil Science & Plant Analysis, 14*(10), 905-913.

Cheshire, M., Bedrock, C., Williams, B., Chapman, S., Solntseva, I., & Thomsen, I. (1999). The immobilization of nitrogen by straw decomposing in soil. *European Journal of Soil Science, 50*(2), 329-341.

Dalal, R. C. (1979). Mineralization of Carbon and Phosphorus from Carbon-14 and Phosphorus-32 Labelled Plant Material Added to Soil. *Soil Sci. Soc. Am. J., 43*(5), 913-916.

DeLuca, T. H., Glanville, H. C., Harris, M., Emmett, B. A., Pingree, M. R., de Sosa, L. L., . . . Jones, D. L. (2015). A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. *Soil Biology and Biochemistry, 88*, 110-119.

Dunn, R. M., Mikola, J., Bol, R., & Bardgett, R. D. (2006). Influence of microbial activity on plant–microbial competition for organic and inorganic nitrogen. *Plant and Soil, 289*(1-2), 321-334.

Gee, G. W., & Or, D. (2002). 2.4 Particle-size analysis. *Methods of soil analysis. Part, 4*(598), 255-293.

George, T. S., Hinsinger, P., & Turner, B. L. (2016). Phosphorus in soils and plants–facing phosphorus scarcity. *Plant and Soil, 401*(1-2), 1-6.

Golchin, A., Baldock, J., & Oades, J. (1997). A model linking organic matter decomposition, chemistry, and aggregate dynamics. *Soil processes and the carbon cycle. CRC Press, Boca Raton*, 245-266.

Hanson, W. (1950). The photometric determination of phosphorus in fertilizers using the phosphovanado-molybdate complex. *Journal of the Science of Food and Agriculture, 1*(6), 172-173.

Hoang, K. T. K., & Marschner, P. (2019). P Pools After Seven-Year P Fertiliser Application Are Influenced by Wheat Straw Addition and Wheat Growth. *Journal of Soil Science and Plant Nutrition*, 1-8.

Jarrell, W., & Beverly, R. (1981). The dilution effect in plant nutrition studies *Advances in Agronomy* (Vol. 34, pp. 197-224): Elsevier.

Jones, D. L., & Darrah, P. R. (1994). Role of root derived organic acids in the mobilization of nutrients from the rhizosphere. *Plant and Soil, 166*(2), 247-257.

Kouno, K., Tuchiya, Y., & Ando, T. (1995). Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. *Soil Biology and Biochemistry, 27*(10), 1353-1357.

Kouno, K., Wu, J., & Brookes, P. (2002). Turnover of biomass C and P in soil following incorporation of glucose or ryegrass. *Soil Biology and Biochemistry, 34*(5), 617-622.

Loehwing, W. (1953). Mineral Nutrition of Plants.

Marschner, P. (2008). The role of rhizosphere microorganisms in relation to P uptake by plants *The ecophysiology of plant-phosphorus interactions* (pp. 165-176): Springer.

Marschner, P., Crowley, D., & Rengel, Z. (2011). Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis–model and research methods. *Soil Biology and Biochemistry, 43*(5), 883-894.

Miranda, K. M., Espey, M. G., & Wink, D. A. (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide, 5*(1), 62-71.

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta, 27*, 31-36.

Nguyen, T. T., Cavagnaro, T. R., Ngo, H. T. T., & Marschner, P. (2016). Soil respiration, microbial biomass and nutrient availability in soil amended with high and low C/N residue–Influence of interval between residue additions. *Soil Biology and Biochemistry, 95*, 189-197.

Noack, S. R., McLaughlin, M. J., Smernik, R. J., McBeath, T. M., & Armstrong, R. D. (2012). Crop residue phosphorus: speciation and potential bio-availability. *Plant and Soil, 359*(1-2), 375-385.

Ohno, T., & Zibilske, L. M. (1991). Determination of low concentrations of phosphorus in soil extracts using malachite green. *Soil Science Society of America Journal, 55*(3), 892-895.

Olsen, S., Sommers, L., & Page, A. (1982). Methods of soil analysis. Part 2. *Chemical and microbiological properties of Phosphorus. ASA Monograph*(9), 403-430.

Richardson, A. E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Functional Plant Biology, 28*(9), 897-906.

Walan, P., Davidsson, S., Johansson, S., & Höök, M. (2014). Phosphate rock production and depletion: Regional disaggregated modeling and global implications. *Resources, Conservation and Recycling, 93*, 178-187.

Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science, 37*(1), 29-38.

Wei, Y., Zhao, Y., Xi, B., Wei, Z., Li, X., & Cao, Z. (2015). Changes in phosphorus fractions during organic wastes composting from different sources. *Bioresource Technology, 189*, 349-356.

Wilke, B.-M. (2005). Determination of chemical and physical soil properties *Monitoring and Assessing Soil Bioremediation* (pp. 47-95): Springer.

Appendix 2

Vermicompost Influcences Soil P Pools and Available N- Effect of Placement and Combination with Inorganic Fertiliser

Juqi Li, Khuyen Thi Kim Hoang [1,2], Nazia Hassan, Petra Marschner[1]

[1]School of Agriculture, Food and Wine, The University of Adelaide, Adelaide SA 5005, Australia.

[2]Chemical Engineering Faculty, Industrial University of Ho Chi Minh, HCMC 7000, Vietnam

The work contained in this chapter is published in Journal of Soil Science and Plant Nutrition

Li, J., Hoang, K. T. K., Hassan, N., & Marschner, P. (2019). Vermicompost Influences Soil P Pools and Available N—Effect of Placement and Combination with Inorganic Fertiliser. *Journal of Soil Science and Plant Nutrition*, 1-6.

Statement of Authorship

Title of Paper	Vermicompost Influences Soil P Pools and Available N—Effect of Placement and Combination with Inorganic Fertiliser
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Juqi Li & Khuyen Thi Kim Hoang & Nazia Hassan & Petra Marschner1

Principal Author

Name of Principal Author (Candidate)	Juqi Li
Contribution to the Paper	Performed experiment, interpreted data and wrote the manuscript
Overall percentage (%)	
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 18/09/2019

Co-Author Contributions

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.

Name of Co-Author	Petra Marschner
Contribution to the Paper	Supervised development of work, data interpretation, manuscript evaluation and correction. She also acted as corresponding author
Signature	Date 18/09/2019

Name of Co-Author	Khuyen Thi Kim Hoang
Contribution to the Paper	Performed experiment, analysed soil samples, analysed data
Signature	Date 18/09/2019

Name of Co-Author	Nazia Hassan		
Contribution to the Paper	Assisted with sample analyses		
Signature		Date	18/09/2019

Journal of Soil Science and Plant Nutrition
https://doi.org/10.1007/s42729-019-00088-x

ORIGINAL PAPER

Vermicompost Influences Soil P Pools and Available N—Effect of Placement and Combination with Inorganic Fertiliser

Juqi Li[1] · Khuyen Thi Kim Hoang[1,2] · Nazia Hassan[3] · Petra Marschner[1]

Received: 1 April 2019 / Accepted: 7 August 2019

Abstract
Compost application can increase plant nutrient availability. But the effect of compost on nutrient availability may depend on a number of factors. In this study, the effect of application method (mulch layer or mixed into the soil) and combination with inorganic fertiliser on soil P pools and available N was investigated. Soil was filled in microcosm with six treatments, including control, vermicompost layer with or without fertilisers (CL, CL/F), bulk soil mixed with inorganic fertiliser alone (F), vermicompost alone (CM) and both of inorganic fertiliser and vermicompost (CM/F). The microcosms were incubated in the dark for 3 weeks. Citrate P, HCl P and resin P were the highest in F, but MBP was higher in CM and CM/F. Citrate P and HCl P were about three- and six-fold higher in CM and CM/F than in CL and CL/F. Available N was the highest in CL/F and 20% higher in CL than in CM. Vermicompost mixed into soil slightly increased soil nutrient availability compared to unamended soil but had little effect when placed on the soil surface. Vermicompost mixed into soil with inorganic N and P could be used to minimise loss of N and P after inorganic fertiliser addition and thereby provide a longer-lasting nutrient supply for plants.

Keywords Detritusphere · Inorganic fertiliser · P pools · Vermicompost

1 Introduction

The detritusphere is defined as a thin layer of soil adjacent to organic amendments usually < 5 mm and influenced by nutrients released during the decomposition of organic amendments (Liu et al. 2011). The detritusphere can occur at the soil surface under mulches or surrounding organic amendment particles mixed into the soil. It is characterised by high concentrations of easily available compounds, particularly in the early stages of decomposition of organic amendments (Poll et al. 2010). Nutrients released during decomposition can move from the organic amendment layer into the detritusphere by diffusion or via fungal hyphae (Frey et al. 2003). The high nutrient availability in the detritusphere increases the abundance of bacteria and fungi compared to bulk soil (Marschner et al. 2012; Kandeler et al. 1999). Nutrient availability and turnover have been studied in the detritusphere of a range of plant residues. However, little is known about the detritusphere of composts which are often used in horticulture or home gardens. Compost addition to soil has been shown to increase crop yield and extractable P in soil (Diacono and Montemurro 2019; Saleem et al. 2017). Compared to fresh plant residues, composts are strongly decomposed during composting and may therefore decompose more slowly after application than plant residues with similar nutrient concentration (Masunga et al. 2016).

Decomposition rate of organic amendments and nutrient release depend on their properties, particularly their C/N and C/P ratio. For example, amendment with high C/N ratio (> 20) organic amendments results in at least temporary net N immobilisation (Hadas et al. 2004), whereas organic amendments with low C/N ratio (< 20) lead to net N mineralisation (Moritsuka et al. 2004). P availability in soil amended with organic materials depends on their C/P ratio. Net P immobilisation occurs after amendment with high C:P ratio (≥ 200) organic materials because microbes need more P than supplied by the organic amendment for growth (Alamgir et al. 2012; Tian et al. 1992). On the other hand, organic amendments with

✉ Petra Marschner
petra.marschner@adelaide.edu.au

[1] School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA 5005, Australia

[2] Chemical Engineering Faculty, Industrial University of Ho Chi Minh, Ho Chi Minh City 7000, Vietnam

[3] PMAS-Arid Agriculture University Rawalpindi, Punjab 46000, Pakistan

Published online: 13 August 2019

low C:P ratio (≤ 200) induce net P mineralisation because the amount of P released from the amendment is greater than microbial P demand (Alamgir et al. 2012; Tian et al. 1992).

Conventional compost production involves a thermophilic phase in which compost piles heat up to 70 °C (Atiyeh et al. 2000). During the production of vermicompost, on the other hand, earthworms decompose organic materials such as sewage sludge, animals waste and crop residues with little heat generation (Lim et al. 2015). Digestion by earthworms produces small particles of peat-like material with high water-holding capacity and porosity, as well as a large surface area, which can adsorb nutrients (Lim et al. 2015). Compared to conventional compost, vermicompost may contain more nutrients and a larger proportion of nutrients can be present in readily available forms such as water-soluble C, nitrate and exchangeable P or K (Lim et al. 2015). Mixing vermicompost in soil can increase crop yield. For example, Gutiérrez-Miceli et al. (2007) found that tomato yield was higher in vermicompost-amended soil compared to inorganic fertilised soil. They suggested that the higher yield was due to increased microbial biomass and activity as well as plant growth-influencing substances such as plant growth hormones produced during vermicomposting. Organic amendments can be mixed into the soil or applied on the soil surface as mulch. Organic mulches are often used in horticulture particularly in dry climates to maintain soil water content, which can increase microbial activities and crop yield (Sinkevičienė et al. 2009). However, it is not clear (i) to which extent nutrient availability in the soil layer adjacent to the mulch (detritusphere) differs from unamended soil, and (ii) if nutrient availability in detritusphere of vermicompost differs from that of mixing vermicompost into the soil. It has been shown that the addition of inorganic fertilisers with organic materials may increase their mineralisation by decreasing the C/nutrient ratio (Hadas et al. 2004). Little is known about the effect of inorganic fertiliser amendment to soil on nutrient availability in soil with organic materials.

To address these questions, available N and P pools in soil after vermicompost application were determined with vermicompost applied either as layer on the soil surface or mixed into the soil. The aims of this study were to (1) determine if soil P pools and available N in 0–2 mm from the soil surface are influenced by the vermicompost application method, as layer on the soil surface or mixed into the soil, and (2) assess if the effect of vermicompost on soil P pools and available N is influenced by inorganic fertiliser addition to the soil.

The first hypothesis was that in the soil layer 0–2 mm from the surface, P pools representing readily available and potentially available inorganic P and available N will decrease in the following order: compost layer with fertiliser > compost mixed with fertiliser > fertilised no compost > compost layer without fertiliser > compost mixed without fertiliser> control without nutrient addition. The second hypothesis was that the treatment effect will be greater resin P than in the more stable P pools.

2 Materials and Methods

2.1 Soil and Vermicompost

A sandy loam was collected at 0–10 cm from Monarto, South Australia (35° 04′ S 139° 07′ E), air-dried and sieved to less than 2 mm prior to the experiment. The soil has the following properties: sand 74%, silt 17%, clay 9%, pH (1:5) 7.5, EC (1:5) 0.02 ds m^{-1}, total C 6.28 g kg^{-1}, total N 0.47 g kg^{-1}, total P 128 mg kg^{-1}, maximum water holding capacity (WHC) at pF = 2.0 (− 10 kPa) 188 g kg^{-1}, available P (resin P) 3.35 mg kg^{-1} and available N (ammonium and nitrate) 19.61 mg kg^{-1}.The properties of vermicompost (mainly earthworm casts sieved to particle size 0.25–2 mm) were the following: total C 96.7 g kg^{-1}, total N 2.8 g kg^{-1}, total P 3.9 g kg^{-1}, water-extractable P 0.38 g kg^{-1}, water-extractable N 0.20 g kg^{-1}, C/N ratio 35 and C/P ratio 25.

2.2 Experimental Design

The experiment included six treatments with four replicates each (Table 1). To activate soil microbes, the air-dry soil was rewetted with reverse osmosis (RO) water to 75% WHC which is optimal for microbial activity in this soil and then pre-incubated moist for 10 days at 20–25 °C in the dark. The microcosm setup used, consisting of two PCV caps (70 mm diameter, 20 mm height), was described in Erinle et al. (2018). Briefly, incubated soil (equivalent to 105 g dry soil) was placed into each cap and adjusted to bulk density of 1.4 g cm^{-3}. The closed ends of the caps had three holes allowing gas exchange. Then the open side of each cap was covered by fine nylon mesh (mesh size 0.1 mm × 0.8 mm) cut into circles with a diameter of 85 mm. In treatments with compost layer, a thin, uniform layer of vermicompost (4.2 g per microcosm, equivalent to 20 g kg^{-1}, 56 mg total N kg^{-1}, 78 mg total P kg^{-1}) was placed on the mesh covering one cap.

Table 1 Treatment names, compost layer (CL, detritusphere) or mixed into the soil (CM) and inorganic fertilisers mixed into the soil (F)

Treatments	Compost layer	Compost in soil	Inorganic fertilisers in soil
Control	–	–	–
CL	+	–	–
CL/F	+	–	+
F	–	–	+
CM	–	+	–
CM/F	–	+	+

This was then covered with the mesh-covered opening of another cap thereby sandwiching the compost between the two meshes. The two caps were held together with rubber bands to avoid loss of vermicompost during the experiment. In the treatments with mixed compost, 20 g kg^{-1} compost was thoroughly mixed with the soil before placing it in the caps which were covered with mesh and pressed together as described above. In treatments with inorganic fertilisers, NH_4Cl_2 and KH_2PO_4 were mixed into the soil at 20 kg P ha^{-1} and 50 kg N ha^{-1} (equivalent to 154 mg P kg^{-1} and 385 mg N kg^{-1}) before placing the soil in the caps. The N and P rates represent typical farmer rates for cereals on this soil. The control was unamended and without vermicompost between the meshes. The microcosms were incubated vertically at 20–25 °C in the dark for 3 weeks and soil moisture was maintained at 75% WHC by checking the water content daily by weight and adding reverse osmosis (RO) water between the meshes if necessary. After 3 weeks, the vermicompost layer between the meshes was collected to determine dry weight. Then the meshes were removed and soil in 0–2 mm from the mesh was collected from each cap. The soil from the 0–2 mm layers from both caps of one microcosm was combined and thoroughly mixed. Unamended soils and treatments with fertiliser and compost mixed into the soil were sampled similarly.

2.3 Analyses

Soil texture was determined by the hydrometer method (Gee and Or 2002). Soil maximum water holding capacity was measured using a sintered glass funnel connected to a 1-m water column (Wilke 2005). Total organic carbon in vermicompost and soil was determined by wet oxidation and titration (Walkley and Black 1934). Total P concentration in vermicompost was analysed by digestion with nitric acid and H_2O_2 at a 4:1 ratio followed by determination of the P concentration in the digest by the phosphovanado-molybdate method (Hanson 1950). Total N concentration in the vermicompost was measured by a modified Kjeldahl method (Bremner and Breitenbeck 1983). Water-soluble inorganic P and N in the vermicompost was extracted with RO water as described in Konieczyński and Wesołowski (2007): to 1 g manure, 30 mL of RO water was added, then shaken for 2 h and filtered. The P concentration in extracts was measured colourimetrically at 630 nm using the malachite-green method as described in Ohno and Zibilske (1991). Ammonium-N in extracts was determined according to Willis et al. (1996), nitrate-N was determined based on Miranda et al. (2001).

Soil P pools, microbial biomass N and available N were measured after pre-incubation and 3 weeks in microcosms. Soil P pools were measured using a modification of the method of DeLuca et al. (2015) which is based on how plant and microbes access available and labile P pools. $CaCl_2$ P and phosphatase P were not determined because they were very low in this soil (< 5 mg kg^{-1}, Li, unpublished). Citrate acid and HCl P mimic organic acids and protons released by roots or microbes to access inorganic P which weakly adsorbed to Fe/Al oxides, clay particles or present in inorganic precipitates (DeLuca et al. 2015). Each pool was extracted by shaking 0.5 g of dry soil for 3 h with 10 ml of 10 mM citric acid and 1 M HCl separately. In addition, available P (resin P) and microbial biomass P (MBP) were measured with the anion exchange resin method (Kouno et al. 1995). Hexanol was used as a fumigant (McLaughlin et al. 1986). MBP is the difference in P concentration between fumigated and unfumigated soil (McLaughlin et al. 1986). No correction factor was used for MBP because preliminary tests showed that the recovery of a P spike in this soil was 98% (data not shown). P concentration in the extracts was determined by the malachite-green method (Ohno and Zibilske 1991). Available N (ammonium and nitrate) was measured after extraction with 2 M KCl at a 1:5 soil extractant ratio. Ammonium-N was determined after Willis et al. (1996) and nitrate-N as described in Miranda et al. (2001).

2.4 Statistical Analyses

All statistical analyses were carried out using Genstat 17th edition (VSN Int. Ltd., UK). Data of P pools and available N was analysed by one-way analysis of variance (ANOVA) with treatment as factor. Tukey's multiple comparison tests at 95% confidence interval were used to determine significant differences among treatments.

3 Results

After the pre-incubation, citrate P was two-fold higher than HCl P and MBP and four-fold higher than resin P (Table 2). Compared to after pre-incubation (Table 2), HCl P and resin P in the control were about 50% lower after 3 weeks in the microcosms (Fig. 1). The dry weight of vermicompost per microcosm was 4.0 g in CD and CD/F (original 4.2 g per microcosm). Thus, about 5% of the compost was decomposed over 3 weeks.

Citrate P, HCl P and resin P were highest in F, but MBP was higher in CM and CM/F than the other treatments. Compared

Table 2 Citrate P, HCl P, resin P, microbial biomass P (MBP) and available N in soil in unamended soil after pre-incubation ($n = 4 \pm$ standard error)

Citrate P	HCl P	Resin P	MBP	Available N
mg kg^{-1}				
8.2 ± 0.3	4.3 ± 0.2	1.7 ± 0.1	4.1 ± 0.2	19.6 ± 0.3

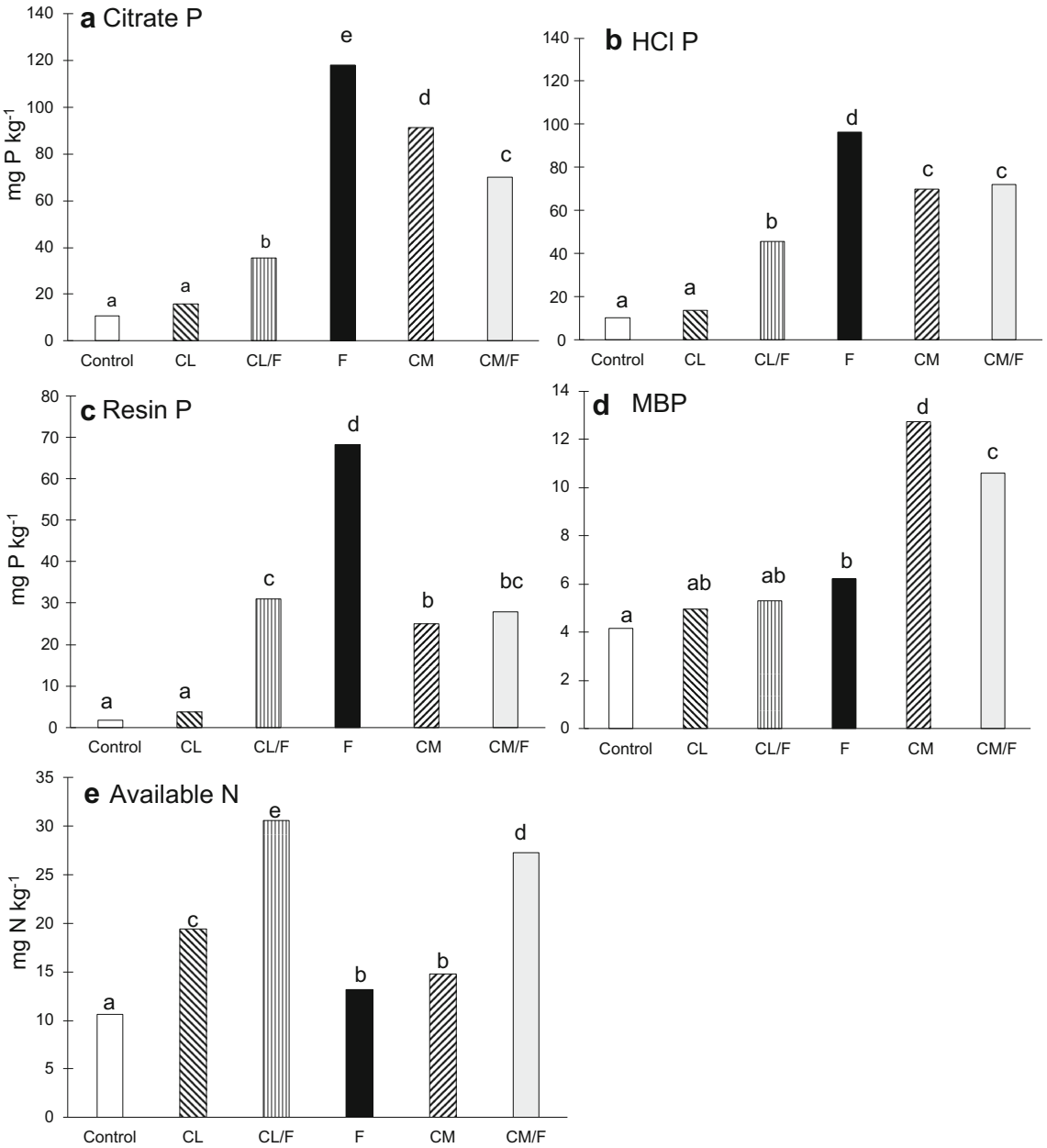

Fig. 1 **a** Citrate P, **b** HCl P, **c** resin P, **d** microbial biomass P (MBP) and **e** available N at after 3 weeks incubation in control, vermicompost layer soil without or with inorganic fertiliser mixed into the soil (CL, CL/F) or bulk soil amended with inorganic fertiliser alone (F), vermicompost alone (CM) or with both vermicompost and inorganic fertiliser (CM/F). Different letters indicate significant differences between treatments ($n = 4$, $p \leq 0.05$)

to the control and CL, citrate P and HCl P were about four-fold higher in CL/F and six to eight-fold higher in CM and CM/F and twelve-fold higher in F (Fig. 1a, b). Resin P did not differ between control and CL, but it was about 15-fold higher in CL/F, CM and CM/F and 30-fold higher in F (Fig. 1c). MBP in CL and CL/F was similar as in the control. It was about 30% higher than the control in F and three-fold higher in CM and CM/F (Fig. 1d). Available N was about 30% higher than the control in F and CM, two-fold higher in CL and three-fold higher in CL/F and CM/F (Fig. 1e). Available N was only nitrate; ammonium was below the detection limit.

4 Discussion

The first hypothesis (in the soil layer 0–2 mm from the surface, P pools representing readily available and potentially available inorganic P and available N will decrease in the following order: compost layer with fertiliser > compost mixed with fertiliser > fertilised no compost > compost layer without fertiliser > compost mixed without fertiliser > control without nutrient addition) has to be declined for the P pools because citrate P, HCl P and resin P decreased in the following order F>CM, CM/F>CL/F>CL, control. MBP was highest in

CM and CM/F. The hypothesis can be accepted for available N which was highest in CL/F.

The P addition rate with F was about two-fold higher than total P added with compost. P in the fertiliser is soluble and can therefore be quickly bound to surfaces of soil minerals (citrate P and HCl P). On the other hand, a large proportion of compost P has to be mineralised before it can be bound to mineral surfaces and only a very small proportion of compost was decomposed during the experiment (about 5%). Although more P was added in CL/F and CM/F than F, citrate P, HCl P and resin P were lower in CL/F and CM/F than F. Organic compounds released from the compost such as organic acid anions may have bound to soil particles and thereby decreased P binding, measured as citrate and HCl extractable P (Ayaga et al. 2006, Gerke 1993). Organic acid anions from the compost may also have reduced potential binding sites on the anion exchange resin during extraction thereby reducing resin P compared to F. These organic compounds likely prevented an increase in citrate P, HCl P and resin P in CM/F compared to CM although more P was added. However, citrate P, HCl P and resin P were higher in CL/F than CL. This suggests that the spatial separation of compost and soil with fertiliser reduced the relative impact of compost compounds on P sorption capacity of soil minerals and the anion exchange resin. Nevertheless, citrate P and HCl P were lower in CL treatments than CM treatments. This is likely because, in the soil adjacent to the compost layer (detritusphere), organic compounds released by the compost are more concentrated than when the compost is mixed into the soil.

The higher MBP in CM treatments than CL treatments can be explained by direct contact of soil microbes and compost as C source in CM whereas soil microbes are spatially separated from compost in CL treatments. The higher MBP in CM treatments is unlikely to influence the other pools because they are an order of magnitude greater than MBP.

Fertiliser addition increased available N in the compost treatments. This is likely due to diffusion of inorganic N from the compost into the surrounding soil. In contrast to P which is poorly mobile in soil nitrate, which was the main form of available N in this experiment, has very low binding affinity to soil particles (Kabala et al. 2017). The high mobility of nitrate can also explain why available N did not differ between CL and CM. Spatial separation of compost and soil in CL would therefore have little effect on N mineralisation. The low N availability in F may be due to denitrification which is likely to be greater with fertiliser than compost because compost N has to be mineralised before it can be lost via denitrification. Ammonium added with the fertiliser, on the other hand, would only require nitrification before it can be denitrified. The fact that ammonium was below detection limit in all treatments indicates that nitrification was rapid.

The second hypothesis (the treatment effect will be greater resin P than in the more stable P pools) can be confirmed. Compared to the control and CL, the relative difference of the other treatments was two- to three-fold greater in resin P than in citrate P and HCl P. Resin P represents immediately available P. Any differences in P release will be more evident in resin P which is a small, readily available P pool compared to citrate P and HCl P which are more stable and larger.

5 Conclusion

The combination of vermicompost with inorganic N and P could be used to minimise loss of N and P after inorganic fertiliser addition and thereby provide a longer-lasting nutrient supply for plants. Vermicompost used as mulch may have little effect on nutrient availability. It may however improve plant growth by reducing water loss and soil erosion.

Acknowledgements Nazia Hassan received a scholarship from the International Research Support Initiative Program of the Higher Education Commission, Pakistan, for her visit to the University of Adelaide.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

Alamgir M, McNeill A, Tang C, Marschner P (2012) Changes in soil P pools during legume residue decomposition. Soil Biol Biochem 49: 70–77

Atiyeh R, Subler S, Edwards C, Bachman G, Metzger J, Shuster W (2000) Effects of vermicomposts and composts on plant growth in horticultural container media and soil. Pedobiologia 44:579–590

Ayaga G, Todd A, Brookes PC (2006) Enhanced biological cycling of phosphorus increases its availability to crops in low-input sub-Saharan farming systems. Soil Biol Biochem 38:81–90

Bremner J, Breitenbeck GA (1983) A simple method for determination of ammonium in semimicro-Kjeldahl analysis of soils and plant materials using a block digester. Commun Soil Sci Plant Anal 14:905–913

DeLuca TH, Glanville HC, Harris M, Emmett BA, Pingree MRA, de Sosa LL, Cerda-Moreno C, Jones DL (2015) A novel biologically-based approach to evaluating soil phosphorus availability across complex landscapes. Soil Biol Biochem 88:110–119

Diacono M, Montemurro F (2019) Olive pomace compost in organic emmer crop: yield, soil properties, and heavy metals' fate in plant and soil. J Soil Sci Plant Nutr 19:63–70

Erinle KO, Li J, Doolette A, Marschner P (2018) Soil phosphorus pools in the detritusphere of plant residues with different C/P ratio—influence of drying and rewetting. Biol Fertil Soils 54:841–852

Frey S, Six J, Elliott E (2003) Reciprocal transfer of carbon and nitrogen by decomposer fungi at the soil–litter interface. Soil Biol Biochem 35:1001–1004

Gee GW, Or D (2002) Particle-size analysis. In: Dane JH, Topp CG (eds) Methods of soil analysis. Part 4 Physical methods. Soil Science Society of America, Madison, pp 255–293

Gerke J (1993) Phosphate adsorption by humic/Fe-oxide mixtures aged at pH 4 and 7 and by poorly ordered Fe-oxide. Geoderma 59:279–288

Gutiérrez-Miceli FA, Santiago-Borraz J, Molina JAM, Nafate CC, Abud-Archila M, Llaven MAO, Rincon-Rosales R, Dendooven L (2007) Vermicompost as a soil supplement to improve growth, yield and fruit quality of tomato (Lycopersicum esculentum). Bioresour Technol 98:2781–2786

Hadas A, Kautsky L, Goek M, Kara EE (2004) Rates of decomposition of plant residues and available nitrogen in soil, related to residue composition through simulation of carbon and nitrogen turnover. Soil Biol Biochem 36:255–266

Hanson WC (1950) The photometric determination of phosphorus in fertilizers using the phosphovanado-molybdate complex. J Sci Food Agric 1:172–173

Kabala C, Karczewska A, Gałka B, Cuske M, Sowiński J, (2017) Seasonal dynamics of nitrate and ammonium ion concentrations in soil solutions collected using MacroRhizon suction cups. Environ Monit Assess 189(7)

Kandeler E, Luxhøi J, Tscherko D, Magid J (1999) Xylanase, invertase and protease at the soil–litter interface of a loamy sand. Soil Biol Biochem 31:1171–1179

Konieczyński P, Wesołowski M (2007) Water extractable forms of nitrogen, phosphorus and iron in fruits and seeds of medicinal plants. Acta Pol Pharm Drug Res 64:385–391

Kouno K, Tuchiya Y, Ando T (1995) Measurement of soil microbial biomass phosphorus by an anion-exchange membrane method. Soil Biol Biochem 27:1353–1357

Lim SL, Wu TY, Lim PN, Shak KPY (2015) The use of vermicompost in organic farming: overview, effects on soil and economics. J Sci Food Agric 95:1143–1156

Liu M, Chen X, Chen S, Li H, Hu F (2011) Resource, biological community and soil functional stability dynamics at the soil–litter interface. Acta Ecol Sin 31:347–352

Marschner P, Marhan S, Kandeler E (2012) Microscale distribution and function of soil microorganisms in the interface between rhizosphere and detritusphere. Soil Biol Biochem 49:174–183

Masunga RH, Uzokwe VN, Mlay PD, Odeh I, Singh A, Buchan D, De Neve S (2016) Nitrogen mineralization dynamics of different valuable organic amendments commonly used in agriculture. Appl Soil Ecol 101:185–193

McLaughlin MJ, Alston AM, Martin JK (1986) Measurement of phosphorus in the soil microbial biomass - a modified procedure for field soils. Soil Biol Biochem 18:437–443

Miranda KM, Espey MG, Wink DA (2001) A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5:62–71

Moritsuka N, Yanai J, Mori K, Kosaki T (2004) Biotic and abiotic processes of nitrogen immobilization in the soil-residue interface. Soil Biol Biochem 36:1141–1148

Ohno T, Zibilske LM (1991) Determination of low concentrations of phosphorus in soil extracts using malachite green. Soil Sci Soc Am J 55:892–895

Poll C, Brune T, Begerow D, Kandeler E (2010) Small-scale diversity and succession of fungi in the detritusphere of rye residues. Microb Ecol 59:130–140

Saleem A, Irshad M, Hassan A, Mahmood Q, Eneji A (2017) Extractability and bioavailability of phosphorus in soils amended with poultry manure co-composted with crop wastes. J Soil Sci Plant Nutr 19:609–623

Sinkevičienė A, Jodaugienė D, Pupalienė R, Urbonienė M (2009) The influence of organic mulches on soil properties and crop yield. Agron Res 7:485–491

Tian G, Kang BT, Brussaard L (1992) Biological effects of plant residues with contrasting chemical compositions under humid tropical conditions—decomposition and nutrient release. Soil Biol Biochem 24:1051–1060

Walkley A, Black IA (1934) An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci 37:29–38

Wilke BM (2005) Determination of chemical and physical soil properties. In: Margesin R, Schinner F (eds) Monitoring and assessing soil bioremediation. Springer, pp 47–95

Willis RB, Montgomery ME, Allen PR (1996) Improved method for manual, colorimetric determination of total Kjeldahl nitrogen using salicylate. J Agric Food Chem 44:1804–1807