# Effect of legume residues on P availability in soil and P uptake by the following wheat

A Thesis submitted to the University of Adelaide

in fulfillment of the requirement for the degree of

Doctor of Philosophy

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**Dedicated to my family**

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## Abstract

Phosphorus (P) deficiency is a common constraint to crop growth in many parts of the world. For optimum plant growth, P is often added to soil as inorganic fertiliser or as crop residues. It has been shown that addition of legume residues can increase P availability by supplying P within the residues but also by mobilising native soil P which could reduce the dependence on inorganic P fertilization for crop growth. In soil, P is found in various organic and inorganic pools which vary in availability. The size of these pools is affected by soil properties such as pH; the flux among these pools determines the relative size of pools and also influences P availability. Less is known about how soil properties such as texture and organic matter content affect the size of the various P pools and if this is modulated by addition of residues or inorganic P fertilisers. The aims of this study were to (i) assess the changes in the P pools over time as affected by residue P concentration and plant part (root or shoot) (ii) to compare the effect of different rates of P added as inorganic P or as residues on soil P pools and growth and P uptake by wheat, (iii) assess short and longer term changes in P pools in soils with different physical and chemical properties amended with residues differing in P concentration.

The research conducted involved laboratory experiments as well as glasshouse experiment. In these experiments three South Australian soils with low P availability and a wide range of legume residues were used. The soils were selected to represent different physical and chemical properties that may affect P availability and were collected from Mount Bold (Mt. Bold) (acidic sandy clay loam), Monarto (neutral loamy sand), and Langhorne Creek (alkaline sandy loam). To have a wide range of P concentrations, the following root and shoot residues from field or glasshouse-grown plants differed in C, N, P content, maturity

were used: mature white lupin (*Lupinus albus* L., low P concentration), mature chick pea (*Cicer arietinum* L., medium P concentration) and young faba bean (*Vicia faba* L., high P concentration).

To investigate the changes in P pools during legume residue decomposition legume shoot or root residues with varying P concentrations of faba bean, chickpea and white lupin (high P, medium P and low P) were added to a loamy sand soil at a rate of 20 g residue $kg^{-1}$ soil and the concentration of various P pools were assessed on day 0 and after 14, 28 and 56 days of incubation. The result of this experiment showed that the size of the P pools changed over time and was affected by both residue P concentration and plant part. The differences in soil P pools among residues were greatest in the first 14 days. Later there was an increase in stable organic and inorganic P in the residue amended soils, indicating net conversion of labile into stable P. Differences in P pools between roots and shoots occurred mainly in the initial phase. The concentration of NaOH-Po increased from d0 to d14 with root and shoot residues, but then decreased from d14 to d28 with addition of shoot residues whereas the concentration of this pool increased when root residues were added. The changes over time were generally more pronounced in low-P than in medium-P residues.

In the second experiment, the short term effects (42 days) of different rates of P added either as inorganic P or as legume residues on soil P pools and wheat growth were compared. In this glass house experiment wheat was grown to the flowering stage (42 days) in a loamy sand soil from Monarto amended with shoot residues of faba bean (high P) chickpea (medium P) and white lupin (low P) at a rate of 5 or 15 g residue $kg^{-1}$ soil. Inorganic P was added at four different rates (3, 10, 30 and 100 mg P $kg^{-1}$) corresponding to the total P added with the different residues at the two residue rates. Soil P pools were

determined at wheat harvest. Compared to inorganic P addition, P added with residues led to a 10-80% greater increase in shoot biomass at the two highest P addition rates. In residue P amended soil, resin P and microbial P were correlated with wheat P uptake whereas in soil amended with inorganic P, resin P and NaOH Pi pools mainly contributed to P uptake.. Over time, the concentration of HCl P decreased in the residue treatments and that of residual P decreased in all treatments suggesting that these so-called non-labile P pools are quite dynamic and could serve as P source for plants.

To assess the impact of soil properties on changes in P pools induced by legume residue addition, three legume different residues differing in P concentration: faba bean (high P) chickpea (medium P) and white lupin (low P), were added at a rate of 20 g $kg^{-1}$ to three soils differing in pH, organic C content and texture from Monarto (pH 7.5), Mount Bold (pH 5.1) and Langhorne Creek (pH 8.1) and incubated for 42 days. In residue-amended soils from day 0 to day 42, the concentration of water soluble and microbial P decreased, whereas the concentrations of $NaHCO_3$ Pi and NaOH Po increased; the magnitude of these changes differed among soils, being greatest in the Mt Bold soil. Residue addition had little or no effect on the concentrations of NaOH Pi, HCl Pi and residual P which also did not change significantly over time. Principal component analysis (PCA) of the data showed that most effects of residue addition to soils on microbial activity and growth and soil P pools can be generalized across the three soil used in this study, but that the size of the P pools is affected by soil properties such as organic carbon content, pH and texture.

To assess longer term temporal changes in P pools in two soils with contrasting physical and chemical properties amended with residues differing in P concentrations, another incubation experiment was carried out with Monarto and Mt Bold soil amended with shoot

residues of faba bean (high P) chickpea (medium P) and white lupin (low P). The concentration of the P pools was measured on days 0, 14, 28, 56, 70 and 98. The PCA plot based on the soil P pools showed a clear separation between the un-amended control soils and those amended with white lupin residues on the one hand and soils amended with faba bean and chickpea residues on the other. The concentrations of most P pools and particularly the labile P pools on days 28 and 56 were higher in soil amended with faba bean and chickpea residues than in the un-amended soil and that with white lupin residues. Despite some differences in temporal changes in P pools between Monarto and Mt. Bold, the PCA showed that the P pool concentrations on day 0 and 98 were quite similar and differed from the P pool concentrations on days 28, 56 and 70 suggesting clear temporal patterns and a limited effect of residue addition on P pool concentrations in the long term. Nevertheless, the temporal changes were more pronounced in the soils amended with faba bean and chickpea residues suggesting that addition of residues with medium or high P concentration has a greater effect on the dynamics among the soil P pools than residues with low P concentration. At the start and the end of the experiment, the concentrations of microbial P and NaOH-Pi were high in both soils, but the concentration of HCl-P was high only in the alkaline Monarto soil whereas the Mt Bold soil was characterized by high resin P concentrations.

## Declaration

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## Structure of this thesis

The thesis is organised into 6 chapters and is presented as a combination of papers that have been published, or have been submitted for publication.

**Chapter 1** provides an overview of the literature review on P dynamics in soil and characterisation of P.

**Chapter 2** comprises a paper published in the *Soil Biology and Biochemistry*. It describes the effect of legume residues with varying P concentration and plant part on changes in soil P pools.

**Chapter 3** comprises a paper submitted for publication in the *Journal of Plant Nutrition and Soil Science*. It describes the comparative effect of inorganic P and P added as residues at different rates on soil P pools and growth and P uptake by wheat at flowering.

**Chapter 4** comprises a manuscript submitted for publication in the *European Journal of Soil Science*. It describes the changes in P pools in three soils with different physical and chemical properties.

**Chapter 5** comprises a manuscript submitted for publication in the *European Journal of Soil Science*. It describes the temporal changes in P pools in two soils induced by residue addition and soil properties.

**Chapter 6** contains general conclusions from all chapters and future research suggestions.

# Chapter 1

# Introduction and review of literature

# 1. Introduction and review of literature

## 1.1 Introduction

Phosphorus (P) is one of the essential nutrients affecting crop production and quality (Khasawneh et al., 1980). For optimum crop production farmers often apply P fertilisers in excess of plant requirements due to low P availability in the soil and low fertiliser use efficiency as a result of P fixation and formation of poorly available P forms. Application of P fertilisers in excess of plant needs has resulted in the accumulation of available P in the surface horizon of agricultural soils in Western and Northern Europe (Barberis et al., 1995; Smet et al., 1995) and a largely unavailable soil P bank in Australian soils (Holford, 1997).

Environmental and economic problems associated with over-use of P fertilisers such as diffuse losses of P from agricultural soils to surface water and water eutrophication (Barberis et al., 1995; Jordan et al., 2000; Sharpley et al., 1994) have led to a renewed interest in alternative management systems, including alternative P sources such as manures, composts and crop residues. Such alternative management systems are also of interest because of the diminishing reserves of P for fertilisers. The main source of all P fertilisers is rock phosphate and more than 75% of the globally commercially exploited phosphate rock is surface mined. Today, the annual global production is 17.5 million tonnes of P, derived from roughly 140 million tons of rock. About 31 million tonnes of P reserves in the earth's crust are consumed annually (Steen, 1998). Therefore, if the current trend continues, world high grade rock phosphate reserve for P fertiliser may run out in 50-100 years (Cordell et al., 2009; Steen, 1998).

Legumes and wheat plays an important role in Australian agriculture and also other parts of the world. It has been shown that crop residues addition improves soil P availability and can be an important source of P for crops but little information exists how incorporation of legume residues influences the P pools in soil and affect growth and P uptake in wheat. Furthermore, there are conflicting results on the interaction of organic amendments and P sorption in soil. To fill this knowledge gap, this study was conducted to assess the effect of legume residues on changes in P pools in soil and growth of wheat.

The aim of this review is to provide an overview of the P dynamics in soil and characterisation of soil P.

### 1.2 Phosphorus in soil

Total soil P ranges from 100 to 300 mg P $kg^{-1}$ soil and is found in various pools that can be categorized into two groups, organic P (Po) and inorganic P (Pi). Within these, pools P is differentiated based on its availability as (i) soluble P in the soil solution which is immediately available to plants, (ii) labile P in the solid phase that is easily exchangeable from the mineral surface or in easily decomposable organic compounds, and (iii) non-labile P that is slowly exchangeable or non-exchangeable from the mineral surface or in recalcitrant organic compounds (Mattingly, 1975) as cited by (Griffin et al., 2003). The dynamics of soil P pools is complex and influenced by biotic and abiotic factors. Different aspects of soil P cycling have been reviewed by several authors e.g. organic P (Stewart and Tiessen, 1987); inorganic P (Syers and Curtin, 1988); cycling processes (Frossard et al., 2000); organic P dynamics (Magid et al., 1996); P mineralisation (Gressel and McColl, 1997) and P dynamics (Shen et al., 2011). The mobility, bioavailability and chemical behavior of different inorganic and organic P fractions vary greatly and are affected by environmental conditions (Sharpley et al., 2000). The P cycle is shown in Figure 1.

### 1.2.1 Inorganic phosphorus

The inorganic fraction of soil P usually accounts for 35% to 70% of total P in soil (Harrison, 1987) and occurs as primary minerals (derived directly from weathered parent material) and secondary minerals (formed by precipitation of P with Al, Ca, and Fe), P dissolved in soil solution, P adsorbed on to the surface of clay minerals, Fe, and Al oxyhydroxides, or Ca carbonates, and P occluded within secondary minerals. Solution P mostly exists as orthophosphate ions, $H_2PO_4^{1-}$ in acidic conditions or $HPO_4^{2-}$ in alkaline conditions. The predominant forms of inorganic P in acid soils are Fe or Al phosphates, while Ca phosphates dominate in alkaline soils. A substantial proportion of inorganic P in the soil is adsorbed (fixed) to clay minerals, Fe/Al oxides, hydroxides, or organic matter complexes (Arai and Sparks, 2007; Hinsinger, 2001). Short-term and long term inorganic P sorption on clay minerals (iron/aluminum oxides and carbonates ) and on soils are well documented (Freese et al., 1995; Parfitt, 1989; Scheinost and Schwertmann, 1995). Transformations among the inorganic forms of soil P are controlled by the processes of precipitation, dissolution and sorption and which also affect organic forms of P (Berg and Joern, 2006). Precipitation refers to reaction of phosphate ions with cations such as Fe, Al, Ca, Mg, forming amorphous precipitate solids. Dissolution reactions involve the solubilisation of the precipitates. Ions and molecules can be sorbed to or desorbed from surfaces of mineral particles.

### 1.2.2 Organic Phosphorus

Organic P may account for up to 80% of total P in soils, mostly in the form of esters (Richardson et al., 2001; Schachtman et al., 1998), phytate (Turner et al., 2002a) and soil microbial biomass (Brookes et al., 1984). As plants take up P as inorganic P, a better understanding of the forms and associated dynamics of organic P in soil is required (Condron and Tiessen, 2005)

Orthosphosphate monoesters e.g. inositol phosphates are the dominant form of organic P and can comprise up to 100% of the total organic P in soils (Turner et al., 2002b). Orthosphosphate diesters such as nucleic acids, phospholipids, teichoic acid account for 0 to 20% of total soil organic P (Magid et al., 1996) and phosphonates for up to 12% of total organic P (Cade-Menun et al., 2000). Phytate (inositol hexakisphosphate) is thought to comprise the majority of soil organic P (Turner et al. 2002b). However recent studies showed that phytate represents just 0.6 to 4.7% of the extractable soil organic P and it has been suggested that phospholipids may constitute a greater proportion of organic P than phytate in a number of soil (Smernik and Dougherty 2007, Doolette, Smernik et al. 2009, Doolette and Smernik 2011). Organic P turnover in soil depends on the rates of mineralisation and immobilisation. Mineralisation is the release of inorganic P from Po and immobilisation is the biological conversion of Pi to Po.

Figure 1. P dynamics in the soil/rhizosphere-plant continuum [adapted from Shen et al. (2011)].

### 1.3 Fate of inorganic P added to soils

Only 10-20% of applied as inorganic P fertilisers is directly used by plants in the year of application, hence the majority of applied P is rapidly fixed, precipitated or immobilised into poorly available forms (Richardson et al., 2001; Vu et al., 2008). The subsequent uptake from the residual fertiliser P rarely exceeds 50% of previous applications (Bolland and Baker, 1998). The amount of fixed or precipitated P in the soil matrix depends on the organic matter and clay concentrations, clay type, soil pH and the concentrations of

exchangeable Al, Fe and Ca (Hansen et al., 2002). In acid soils, P becomes fixed mostly by reactions of $H_2PO_4^-$ with Al, Fe or Mn, either as dissolved ions ($Fe^{2+}$, $Al^{3+}$ and $Mn^{3+}$), as oxides, or as hydrous oxides and with 1:1 type silicate clays. In alkaline soils soluble $H_2PO_4^-$ reacts with Ca to form a sequence of products (Ca-phosphates) of decreasing solubility which may undergo further reactions to form even more insoluble compounds, such as the hydroxy-, oxy-, carbonate-, and fluorapatite compounds.

**1.4 P availability in residue amended soil**

Phosphorus can be added in soil as inorganic fertiliser, manure and crop residues. Residue management plays an important role in soil P cycling. After incorporation of crop residues, P availability is influenced by a number of factors including type and quantity of residues, residue parameters (Palm and Sanchez, 1990; Tian et al., 1992), climatic conditions (Dalal, 1979), potential mineralisation of native organic soil P, P sorption capacity (Baggie, 2002), soil properties (Huffman et al., 1996), cropping systems (Van Den Bossche et al., 2005) and interaction with inorganic fertilisers (Kaur et al., 2005).

Net P mineralisation from crop residues is determined by the rate of residue decomposition and microbial immobilization (Stevenson and Cole, 1999). The water-soluble P in residues is released immediately after residue addition to most soil, later part of organic P in plant residues is mineralised in by phosphatase enzymes which are produced by microorganisms and plant roots (Frossard et al., 2000; Magid et al., 1996; McGill and Cole, 1981; Stewart and Tiessen, 1987). Exudation of low molecular weight organic anions (e.g. citrate, malate, oxalate) by plant roots, mycorrhiza and rhizosphere microbes may also influence the solubility and consequentially the mineralisation of organic P in rhizosphere (Condron et al., 2005). Organic acids released from crop residues can increase P concentration in the soil solution by competing for P sorption sites (Iyamuremye et al., 1996a) and chelation of Al and Fe in the soil solution which in turn reduces the precipitation of P (Fox and

Comerford, 1990). It has been shown that addition of organic residues to soils also affects the relative size of the soil P pools (Iyamuremye et al., 1996b; Nziguheba et al., 1998). There are several methods to characterise the forms of soil P including sequential extraction and spectroscopic methods.

## 1.5 Phosphorus fractionation methods

### 1.5.1 Chemical extraction

Different sequential extraction or chemical fractionation schemes have been developed to quantify the inorganic and organic forms of P in soil and sediments (Bowman and Cole, 1978; Chang and Jackson, 1957; Hedley et al., 1982; Tiessen and Moir, 1993; Turner et al., 2005). Sequential methods rely on differences in solubility of inorganic or organic P in various reagents (Pansu and Gautheyrou, 2006). The early P fractionation scheme proposed by Chang and Jackson (1957) involved determination of Fe, Al and Ca phosphate by sequential extraction with 0.1 N $NH_4F$, 0.1 N NaOH, and 0.5 N $H_2SO_4$ and P in Fe and Al phosphate by extraction with dithionate-citrate and 0.5 N $NH_4F$, respectively. The Chang-Jackson fractionation scheme was subsequently modified by others to apply to a wide variety of soils. Hedley et al. (1982) introduced a sequential extraction procedure to examine P pools that have clearly defined chemical properties. The Hedley fractionation scheme involves a sequence of extractions with water, $NaHCO_3$, NaOH, HCl; acid digestion of the extracts is then used to separate inorganic and organic fractions. The following P pools can be differentiated: labile P (resin-Pi, $NaHCO_3$-Pi and $NaHCO_3$-Po), moderately labile P (NaOH-Pi, NaOH-Po), relatively insoluble apatite-P (HCl-Pi) and resistant P (in primary minerals). Several modifications of Hedley fractionation have been proposed (Perrott, 1992; Potter et al., 1991; Tiessen and Moir, 1993). The modification of Tiessen and Moir (1993) includes another extraction step with concentrated HCl. The P

fractionation scheme of Hedley et al. (1982) as modified by Tiessen and Moir (1993) is shown in Figure 2.



Figure 2. P fractionation scheme of Hedley et al. (1982) as modified by Tiessen and Moir (1993). Note that residue in this scheme refers to soil left after the previous extraction step.

The Hedley fractionation procedure has the advantage over most routine soil tests in its attempts to measure Po and Pi pools. As the same soil sample is sequentially treated with the various reagents it is possible to partition these Po and Pi pools into labile, moderately labile, and recalcitrant P pools based upon to the chemical strength of the P bonds and the plants' ability to access those P pools (Hedley et al., 1982; Tiessen and Moir, 1993). Cross and Schlesinger (1995) provided a review on the Hedley fractionation P data from natural terrestrial ecosystems and Negassa and Leinweber (2009) summarized literature on how

Hedley fractionation P data has been used to assess the impact of land use and management on soil P. .

Some of the limitations of sequential extraction are:

- Inability to isolate discrete mineral phases (Turner and Leytem, 2004)
- The possibility of partial hydrolysis of Po to Pi by some extractants
- Procedure is time and labour intensive
- Inability to discriminate between P fractions that differ in biological importance
- Carry-over of error from step to step e.g. by incomplete removal of P released by the previous step or loss of soil.

Condron and Newman (2011) in their recent review highlighted the fundamental components of Hedley fractionation method that needs to be carefully considered in the assessment of P transformations in various types of soil and sediments. They showed in their review that several aspects of the sequential procedures can significantly influence the apparent nature, distribution and reactivity of the P pools. These include (i) the effect of sample handling and pretreatment on the nature and distribution of P, (ii) accurate determination of respective quantities of inorganic and organic P in different fractions, and most importantly, (iii) fraction validation including the nature and dynamics of sparingly soluble and insoluble organic and inorganic forms of P that are considered to be recalcitrant (residual P). They also identified five major areas of concern relating to the accurate determination of inorganic and organic P in soil extracts, namely humic acid precipitation, acid hydrolysis of organic P, presence of pyrophosphates and polyphosphates, complexation and/or occlusion of inorganic P and incomplete extraction.

Some of the problems associated with chemical extraction of soil P can be overcome by using spectroscopic techniques which do not involve complex chemical extraction procedures.

### 1.5.2 Spectroscopic techniques

The main spectroscopic techniques that have been used for the speciation of organic P in soils are Nuclear Magnetic Resonance (NMR) spectroscopy and X-ray absorption spectroscopy (XAS). These techniques have their advantages and their limitations which need to be understood in order to choose the best technique for any given purpose.

### 1.5.2 .1 $^{31}$P NMR spectroscopy

Characterisation of soil P by $^{31}$P-NMR is a useful alternative to sequential extraction procedures (Taranto et al., 2000). Newman and Tate (1980) pioneered the use of $^{31}$P NMR spectroscopy to quantify organic and inorganic P species in alkali soil extracts. By $^{31}$P NMR it is possible to identify inorganic orthophosphate, pyrophosphate and polyphosphate, and organic orthophosphate monoesters and diesters, and phosphonates (Condron et al., 2005; Condron et al., 1997). $^{31}$P NMR spectroscopy can be based on solution or solid-state.

#### Solution $^{31}$P NMR spectroscopy

Solution $^{31}$P NMR spectroscopy is by far the most widely used spectroscopic technique for the speciation of soil organic P (Doolette and Smernik, 2011). Using solution $^{31}$P NMR spectroscopy, multiple P compounds can be quantified simultaneously with minimal sample preparation and handling (Condron et al., 1997). Solution $^{31}$P NMR is usually carried out on alkaline soil extracts as the solubility of both organic and inorganic P species is maximised at high pH. Previously most NMR studies of soil organic P compounds involved extracting the compounds with 0.5 M NaOH but more recently a mixture of NaOH and EDTA (0.25 M and 0.05 M respectively) is used for extraction (Murphy et al., 2009; Turner et al., 2003). The inclusion of EDTA improves the soil P extraction efficiency and the diversity of P compounds extracted by complexing the paramagnetic cations such as Fe and Mn which interfere with the NMR signal (Bowman and Moir, 1993).

Nuclear magnetic resonance spectroscopy is based on the fact that nuclei of atoms have magnetic properties that can be utilized to identify the chemical forms of that nucleus in a sample. When placed in a magnetic field, NMR active nuclei (such as $^{1}H$, $^{13}C$ or $^{31}P$) absorb electromagnetic radiation and subsequently emit energy which show peaks at different positions in a spectrum, depending on their chemical bonds. The resonant frequency, energy of the absorption, and the intensity of the signal are proportional to the strength of the magnetic field. Phosphate, phosphonates, phosphate monoesters and diesters, pyrophosphate and polyphosphates all exhibit signals at different chemical shifts (resonant frequency of a nucleus relative to a standard), which is the basis for their identification and quantification (McKelvie, 2005). Phosphorus compounds give resonances in characteristic ranges depending primarily on the oxidation state and coordination number of the phosphorus atoms present. Phosphorus nuclei in phosphonates, with a C-P bond, are less shielded and takes less energy for the resonance than P nuclei in polyphosphates, which are phosphate groups linked by energy-rich phosphoanhydride bonds. Chemical shifts also depend on the pH, ionic strength, temperature, solvent effects and the concentration of paramagnetic ions (McDowell and Stewart, 2005; Puppato et al., 2007; Smernik and Dougherty, 2007).

The advantage of NMR spectroscopy is that it is analytically less complex than the partition chromatography techniques previously used to identify specific organic P compounds in soils. Any homogeneous liquid-state sample can usually directly be analysed by NMR. The selective observation of organic P compounds is quite straightforward even in complex samples with $^{31}P$ NMR spectroscopy (Koskela, 2010).

Some limitations of solution $^{31}$P NMR include:

- less than half of the total organic P is extracted and it is questionable whether the extracted portion is representative of the organic P in the soil (Tate and Newman, 1982).
- NMR is expensive to buy and run.
- poor spectral resolution.
- orthophosphate diesters (e.g. RNA and phospholipids) may be hydrolysed.
- the concentrations of orthophosphate diesters may be underestimated whereas the concentrations of orthophosphate monoesters are overestimated.

Compared to solution $^{31}$P NMR minimal sample preparation is required for solid-state $^{31}$P NMR and it overcomes the problem associated with modifying compounds during extraction. However, solid-state $^{31}$P NMR is not sufficiently sensitive for detailed quantitative analysis.

#### 1.5.2.2 X-ray absorption spectroscopy (XAS)

X-ray Absorption Spectroscopy (XAS) is a form of electron spectroscopy where X-rays absorbed by matter excite an absorbing atom's core electron to higher unoccupied states or into a free unbound state. The XAS spectrum can be divided into four sections: (i) Pre-edge (ii) **XANES**: X-ray Absorption Near Edge Structure (iii) **NEXAFS**: Near Edge X-ray Absorption Fine Structure and (iv) **EXAFS**: Extended X-ray Absorption Fine Structure, as illustrated in Figure 3. In practice there is not an obvious division of these regions and the XANES and NEXAFS regions are often modeled together. The combination of XANES and EXAFS is referred to as XAFS.

#### 1.5.2.2.1 X-ray absorption near-edge structure (XANES) spectroscopy

XANES which is synonymous with NEXAFS requires an energy-tunable source of X-rays that is currently only possible with a synchrotron. The fundamental phenomenon underlying XANES is the absorption of an x-ray photon by an atom in a solid and the consequent emission of a photoelectron. XAFS uses differences in an atom's x-ray absorption based on chemical and physical state of the atom to identify P forms. XAFS spectra are especially sensitive to the oxidation state, coordination chemistry, and the distances, coordination number and species of the atoms immediately surrounding the selected element. Because of this dependence, XAFS provides a practical way to determine the chemical state and local atomic structure for a selected atomic species (Matthew, 2004). XANES can be used for speciation of numerous elements in soils and has recently been used to identify P species in soils, manures and marine sediments (Ajiboye et al., 2008; Beauchemin et al., 2003; Brandes et al., 2007; Kruse and Leinweber, 2008; Peak et al., 2002; Sato et al., 2005; Toor et al., 2005). In most of these studies, the focus had been on identifying and quantifying inorganic P species. For XANES analysis soil samples are usually sieved and ground and either placed in a sample holder (Beauchemin et al., 2003) or spread onto adhesive tape (Ajiboye et al., 2007; Ajiboye et al., 2008; Kruse and Leinweber, 2008; Lombi et al., 2006; Sato et al., 2005).

Figure 3. Energy level diagram of X-ray Absorption Spectroscopy (XAS) showing excitation of core electrons (left) and an X-ray absorption spectrum at each of the illustrated transitions (right). (http://www.chemphys.lu.se/research/techniques/xrayxas/)

The advantages of XANES spectroscopy are that minimal sample preparation is required (Beauchemin et al., 2003) and the risks of species transformation by extractants can be eliminated (Toor et al., 2005). XANES analysis can be combined with other analytical techniques including sequential P extraction to characterise the extracted P pools in greater detail (Ajiboye et al., 2007; Beauchemin et al., 2003; Kruse and Leinweber, 2008).

## 1.6 Aim of this study

Previous studies suggested that legume residues upon decomposition can mobilise soil P pools and enhance growth and P uptake of following wheat, (McLaughlin and Alston, 1986; Somado et al., 2007) but the underlying mechanisms are not well understood. Further it is not clear if changes in P pools induced by legume residue addition affect the ability of wheat to access these P pools. Therefore, the objective of this study is to characterise the effect legume residue addition on changes in soil P pools during residue decomposition and during wheat growth.

To fulfil the objective, the study specifically aims to:

- Assess changes in the P pools as affected by residue P concentration and plant part (Chapter 2).
- Compare short-term effects (42 days) of different rates of P added either as inorganic P or as residues on soil P pools and wheat growth (Chapter 3).
- Assess changes in P pools induced by legume residue addition in soils with different physical and chemical properties (Chapter 4).
- Assess longer term (98 days) temporal changes in P pools in two soils amended with residues differing in P concentrations (Chapter 5).

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# Chapter 2

# Changes in soil P pools during legume residue decomposition

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18 Dec 2012

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ABSTRACT

In rotations, cereals after legumes often have higher P uptake than after cereals, and it has been suggested that legume residues may play an important role in this effect by mobilising soil P in the legume phase and by the P added with the residues. However, little is known about the changes in P pools during legume residue decomposition. Residues from faba bean, white lupin or chickpea (shoots or roots) with varying P concentrations were added to a loamy sand soil with a low available P concentration, and the concentration of various soil P pools were assessed by soil P fractionation on days 0, 14, 28 and 56. Residue addition significantly increased cumulative respiration which was positively correlated with amount of C added with residues ($r = 0.54$, $p < 0.05$), and negatively correlated with the C/P ratio ($r = -0.58$, $p < 0.05$). The size of the P pools changed over time and was affected by both residue P concentration and plant part (root or shoot). In the first two weeks, microbial P increased and resin P and $NaHCO_3$–Pi decreased with low-P residues (0.6–1.8 mg $kg^{-1}$) while the reverse was true for high-P residues (6.5–8.3 mg $kg^{-1}$). In medium-P residues (2.9–3.3 mg $kg^{-1}$), there was a balance between mineralisation and immobilisation. Decreases of NaOH–Po occurred earlier with low-P and medium-P residues (d0 to d14) than with high-P residues (d14–d28). The increase in residual P with all residues indicated that part of mineralised P was converted into stable P within 14d; but later (d28–d56), the concentration of residual P strongly decreased. In the period from d28 to d56, there was an increase in NaOH–Po and HCl–P with all residues, indicating net conversion of P into stable organic and inorganic P. Changes in P pools between roots and shoots occurred mainly in the initial phase. The concentration of NaOH–Po increased from d0 to d14, but then decreased from d14 to d28 with addition of shoot residues whereas the reverse was found with roots. These changes were generally more pronounced in low-P than in medium-P residues. This study demonstrates that changes and transformations in soil P pools over time were dependant on residue P concentration and plant part.



## 1. Introduction

Phosphorus (P) is essential for plant growth, crop production and quality. Due to low P availability in soil and low fertiliser use efficiency, farmers often apply P fertilisers in excess of plant requirements. Only 10–20% of the P applied with fertilisers is taken up by plants in the year of application because the majority of applied P is rapidly fixed or precipitated into poorly available forms (Vu et al., 2008), resulting in a largely unavailable soil P bank (Holford, 1997). Environmental and economic problems associated with over-use of P fertilisers such as diffuse losses of P from agricultural soils and surface water eutrophication (Barberis et al., 1995), and the diminishing reserves of P for fertilisers have led to a renewed interest in alternative management systems, including the substitution of chemical fertilisers with manures, composts and crop residues.

Legumes play an important role in agricultural and natural ecosystems. The incorporation of legumes as green manure in cropping systems or as part of the rotation not only positively affects soil properties and increases nitrogen (N) supply, but also increases P supply to the main crop/following crop (Kabir and Koide, 2002). Residue decomposition and nutrient release are affected by residue chemical composition (concentrations of nutrients, and easily available and recalcitrant compounds) as well as by environmental factors such as soil moisture. Much information exists on the effects of residue quality on rates of decomposition and N mineralisation, but fewer studies have evaluated the relationship between residue quality and P release during decomposition. Moreover, soil P may be mobilised during legume residue decomposition. The addition of organic matter to soil can influence

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P availability directly or indirectly through a range of mechanisms (Guppy et al., 2005). Direct effects include (i) P release from the residues, (ii) exchange of sorbed P with organic acid anions produced during residue decomposition (Bolan et al., 1994; Hue et al., 1994), (iii) metal complexation and (iv) dissolution reactions (Bolan et al., 1994). Indirect effects include (i) improvement of water-holding capacity and soil moisture promoting root growth and thereby exploitation of soil P, (ii) greater micro-aggregation causing reduced soil surface area and decreasing number of potential P sorption site (Wang et al., 2001), (iii) microbial immobilisation of inorganic P (Chen et al., 2000), and (iv) short-term increases in soil pH (Mokolobate and Haynes, 2003; Xu et al., 2006). Generally, residues with high P concentrations (>5 mg P $kg^{-1}$) decompose faster and release more P than residues with low P concentrations (Tian et al., 1992) because these residues contain sufficient P (and N) to satisfy the P and N demand of the microbes which have low C/N and C/P ratios. Due to the greater capacity of legumes to utilise soil P, it is expected that legume residues contain more P and have lower C/P ratios than cereal residues (Nuruzzaman et al., 2005), favouring net P mineralisation.

Soil P is found in different pools, including inorganic and organic P. The predominant forms of inorganic P in acid soils are Fe or Al phosphates, while Ca phosphates dominate in alkaline soils. A substantial proportion of inorganic P in the soil is adsorbed (fixed) to clay minerals, Fe/Al oxides, hydroxides, or organic matter. The inorganic and organic P compounds in soils vary in lability/solubility. Organic P is positively correlated with organic C and may account for up to 80% of total P in soils, mostly in the form of esters (Richardson et al., 2001; Schachtman et al., 1998), phytate (Turner et al., 2002) and soil microbial biomass (Brookes et al., 1984). Organic P mineralisation contributes to the available P pool, but the degree of mineralisation is dependent on the stability of organic P fractions and microbial activity (Bowman and Cole, 1978).

Sequential P fractionation can be used to separate the different forms of soil P and is useful to assess the fluxes between P forms in soil (Reddy et al., 2005; Wang et al., 2008). It has also been shown that organic acid anions and phenolics released during residue decomposition mobilise P and decrease P fixation capacity (Ayaga et al., 2006; Schefe et al., 2008), but little is known about the impact of legume residues with varying P concentration on soil P pools over time. Therefore, the aim of this experiment was to determine the impact of residues with different P concentrations and plant parts (shoot or root) on concentrations of soil P pools during residue decomposition over a period of 56 days.

## 2. Materials and methods

### 2.1. Experimental setup

A loamy sand with a low concentration of available P (Table 1) was collected in Monarto, (latitude 35° 05′ S, longitude 139° 04′ E, elevation 212 m), South Australia from the top 10 cm in a natural bushland. The soil was air-dried and passed through a 2 mm sieve. The soil was pre-incubated for 10 days at 70% water-holding capacity, which is the optimum water content for microbial activity in this soil as determined in preliminary experiments.

Ten types of residues from different legume species and growth stages were chosen to represent a wide range of P concentrations and other chemical properties which may affect decomposability and P release. The residues were derived from plants growing in pots with various substrates or from field-grown plants. Total P concentration of the residues ranged from 0.6 to 8.3 g P $kg^{-1}$ with a C/P ratio ranging from 47 to 640 (Table 2). The residues were finely ground and sieved to 0.25–2 mm, added at a rate of 20 g $kg^{-1}$ and mixed thoroughly into the soil. The control soil received no residues but was mixed in the same way as the soils with residues added. There were 4 replicates for each treatment and sampling date. On the basis of P concentration, the residues were grouped into high P: faba bean 1 shoots and roots (6.5–8.3 mg $kg^{-1}$), medium P: faba bean 2 shoots and roots, white lupin 3 shoots (2.9–3.3 mg $kg^{-1}$) and low P: chickpea 1 shoots, faba bean 3 shoots and roots, white lupin 1 shoots, white lupin 2 shoots (0.6–1.8 mg $kg^{-1}$). The average amount of P added was 149, 61 and 26 mg P $kg^{-1}$ with high, medium and low-P residues, respectively.

### 2.2. Analyses

Cores with 20 g soil mixed with residues were placed either inside separate 900 mL glass jars with gas-tight lids equipped with septa to measure respiration (cores for sampling on d14) or in large plastic containers (cores for sampling on d28 and d56) together with containers of water to maintain humidity and minimize drying of the soil during incubation. The cores were incubated in the dark at 22–25 °C for 56 days. Respiration was measured over 14 days only to determine differences in decomposition rates among residues. Respiration rates are highest in the first two weeks after residue addition, therefore differences in decomposition rates are most evident in the first two weeks. Daily, headspace gas was withdrawn and the $CO_2$ concentration measured with an IR $CO_2$ gas analyser (Servomex 1450 Food Package Analyser, Crowborough, UK). After measurement, the jars were opened and flushed with ambient air. After closing, the $CO_2$ concentration at time zero was determined. During incubation, soil moisture was maintained by weight with autoclaved reverse osmosis water. Sampling occurred on days (d) 0 (3 h after residue addition), 14, 28 and 56.

Total C and N of soil and residues were determined using a Leco CNS-2000. Total P was determined by digestion with nitric acid–perchloric acid (Olsen and Sommers, 1982) and then measured colorimetrically by the phosphovanado-molybdate method (Hanson, 1950; Kitson and Mellon, 1944). At each sampling time, the concentration of various P pools was determined by sequential P fractionation based on Hedley et al. (1982), Huang and Zhang (2009), and Tiessen and Moir (1993) with some modifications. One g soil was placed in a 50-ml centrifuge tube and was sequentially extracted (at a 1:30 soil:solution ratio) with anion exchange resin (AER) and 0.5 mL hexanol, 0.5 M $NaHCO_3$, 0.1 M NaOH, 1 M HCl, and again 0.1 M NaOH. Five mL of the extract from the $NaHCO_3$ and NaOH fraction was acidified to pH 1.5 to precipitate organic matter, centrifuged and the supernatant was analysed for inorganic P. Total P in the $NaHCO_3$ and NaOH fractions was analysed by the method as described by Huang and Zhang (2009). Briefly, 5 mL extract was digested with 1 mL 5% potassium persulfate at 90 °C for 16 h and was analysed for total P. Organic P was calculated as the difference between total P and inorganic P. Residual P was determined by digesting the residual soil in $HNO_3$–$HClO_4$ (6:1). For microbial P, another aliquot of soil was extracted with AER with or without hexanol and was calculated from the difference between samples treated with and without hexanol (Kouno et al., 1995). The

**Table 1**
Soil properties.

| Texture | Bulk density | pH ($H_2O$) | Total N % | Total C % | Total P (mg $kg^{-1}$) | Resin P (mg $kg^{-1}$) | Colwell P (mg $kg^{-1}$) | $PBI_{+colP}$ |
|---|---|---|---|---|---|---|---|---|
| loamy sand | 1.63 | 8.82 | 0.09 | 0.73 | 145 | 2.3 | 3.6 | 49.5 |

**Table 2**
Properties of plant residues.

| Treatments | Residues | Plant part | Age | Total P g kg$^{-1}$ | Water soluble P g kg$^{-1}$ | Total C g kg$^{-1}$ | Total N g kg$^{-1}$ | P added with residues mg kg$^{-1}$ | C/N | C/P |
|---|---|---|---|---|---|---|---|---|---|---|
| High P | | | | | | | | | | |
| FB1S | Faba bean 1 | Shoot | Young | 6.6 | 5.9 | 417 | 34.5 | 131.1 | 12 | 63 |
| FB1R | Faba bean 1 | Root | Young | 8.3 | 7.2 | 390 | 30.9 | 166.4 | 13 | 47 |
| Medium P | | | | | | | | | | |
| FB2S | Faba bean 2 | Shoot | Young | 3.0 | 2.4 | 431 | 48.9 | 59.7 | 9 | 144 |
| FB2R | Faba bean 2 | Root | Young | 2.9 | 1.5 | 339 | 31.6 | 58.6 | 11 | 116 |
| WL3 | White Lupin 3 | Shoot | Young | 3.3 | 2.3 | 438 | 34.5 | 65.3 | 13 | 133 |
| Low P | | | | | | | | | | |
| CP1 | Chickpea 1 | Shoot + pods | Mature | 1.8 | 0.6 | 418 | 21.1 | 35.4 | 20 | 232 |
| FB3S | Faba bean 3 | Shoot | Mature | 1.3 | 1.2 | 439 | 37.0 | 25.6 | 12 | 343 |
| FB3R | Faba bean 3 | Root | Mature | 1.7 | 1.0 | 355 | 30.6 | 34.4 | 12 | 206 |
| WL1 | White lupin 1 | Shoot | Mature | 0.6 | 0.2 | 384 | 9.7 | 11.2 | 40 | 640 |
| WL2 | White lupin 2 | Shoot | Young | 1.1 | 1.1 | 442 | 38.3 | 22.5 | 12 | 402 |

P concentration in the fractions was determined colorimetrically (Murphy and Riley, 1962). Total P was obtained by summing sequentially extracted P pools. To check the percentage recovery by fractionation, total P of the un-fractionated soil was also determined after digestion with nitric acid–perchloric acid (6:1) (Kuo, 1996). The P buffering index (PBI) was determined by the method described in Rayment and Lyons (2010). Changes in the size of the P pools between the sampling times were calculated as the difference in the concentration of a given pool: e.g., P concentration in a P pool on d28 − P concentration in a P pool on d14. The changes in the unamended soil were subtracted from those in the residue-amended soils.

### 2.3. Statistical analyses

The concentrations of the different P pools were compared by 2-way ANOVA with residue P concentration and sampling time as factors. Means were compared by using Duncan's Multiple Range Test using PASW Statistics 18, Rel. 18.0.2, (©SPSS Inc., 2010, Chicago) at $\alpha = 0.05$. Correlation analysis was used to measure the strength of relationships between the variables.

## 3. Results

### 3.1. Respiration

Compared to the unamended control, residue addition significantly increased cumulative respiration 9–45 fold after 14 days (Fig. 1). Among the residue-amended soils, cumulative respiration was highest in soil with chickpea shoot residues or white lupin young shoot and lowest in soil amended with white lupin mature shoot. Soil amended with young shoots and roots had higher cumulative respiration than that amended with mature shoots and roots, except for the chickpea residues. Cumulative respiration was lower in the soils amended with low-P residues than in those amended with medium- and high-P residues. Cumulative respiration was positively correlated with the amount of C added with residues ($r = 0.54$, $p < 0.05$), and negatively correlated with the C/P ratio ($r = -0.58$, $p < 0.05$).

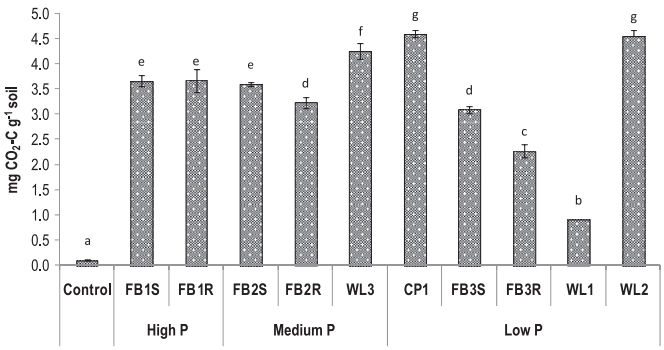


**Fig. 1.** Cumulative respiration (mg $CO_2$–C g$^{-1}$ soil) over 14 days of unamended soil and soil with added legume residues with various P concentrations ($n = 4$). Means with the same letter are not significantly different at $P \leq 0.05$. For abbreviations see Table 1.

### 3.2. General effects on soil P pools

The percentage of total P recovered with the P pools was 94% on d0 and 91% on d56 (91%). On d0 (3 h after residue addition) compared to the unamended control, the concentration of the following P pools was increased with residue addition: resin P (2–26 fold) and microbial P (4–8 fold) and $NaHCO_3$ Pi (2–4 fold). The increases in NaOH–Pi, NaOH–Po and HCl–Pi were smaller (1.0–1.4 fold). On the other hand, the concentration of $NaHCO_3$–Po decreased immediately after residue addition by 36–63%, regardless of the residue P concentration.

Labile P, defined as the sum of resin Pi + microbial P + $NaHCO_3$–Pi + $NaHCO_3$–Po, ranged from 15 to 47% of total P. The size of the labile P pools was positively correlated with total P added with the residues ($r = 0.98$, $p < 0.01$) and with water-extractable P in the residues ($r = 0.98$, $p < 0.01$). The non-labile P (defined as the sum of NaOH–Pi + NaOH–Po + HCl–Pi + residual P) was 53–85% of total soil P. In the residue-amended soils, the percentage of residual P was smaller than in the unamended soil, particularly in soils amended with faba bean 1 shoots and roots. The percentage of organic P was highest in soil with medium-P residues (25% of total P) and lowest in soil with high-P residues (16% of total P). The concentrations of resin P and $NaHCO_3$–Pi were always highest in the soil amended with high-P residues whereas the concentrations of these pools and of NaOH–Pi were lowest in soil with low-P residues.

### 3.3. Effect of P concentration of added residues on changes in soil P pools over time

The concentrations of the P pools changed over time in the unamended soil and the residue-amended soils (Tables 3 and 4), Thus, to assess the effect of residue addition on soil P pools, the changes in the size of the P pools between the sampling times was calculated by subtracting the changes in the unamended control soil (Fig. 2). The changes induced by residue addition varied with residue P concentration.

#### 3.3.1. High-P residues

From d0 to d14, the addition of high-P residues increased the size of all pools except microbial P which decreased (Fig. 2a). The

**Table 3**
Concentrations of different P pools (mg $kg^{-1}$) in soil amended with residues with high P, medium P and low-P concentration and in unamended soil. Data are the means of different residue types with the same P category. Means in columns followed by the same letter are not significantly different at $P \leq 0.05$.

| Day | Resin P | Microbial P | $NaHCO_3$–Pi | $NaHCO_3$–Po | NaOH–Pi | NaOH–Po | HCl–Pi | Residual P |
|---|---|---|---|---|---|---|---|---|
| | mg $kg^{-1}$ | | | | | | | |
| D0 | | | | | | | | |
| High P | 93.5c | 17.4cd | 23.4f | 0.4a | 20.0b | 28.2b | 18.0c | 83.4b |
| Medium P | 14.7b | 20.4d | 12.3d | 0.7a | 19.3b | 27.1b | 19.2cd | 81.7ab |
| Low P | 5.8ab | 10.1b | 8.4bc | 0.5a | 16.8ab | 23.8b | 18.1cd | 81.3ab |
| Unamended soil | 2.3a | 2.5a | 3.6a | 1.1ab | 14.9a | 17.7ab | 14.6b | 87.6bc |
| D14 | | | | | | | | |
| High P | 95.5c | 15.7c | 28.8g | 4.7c | 21.9b | 53.3d | 20.5d | 81.9ab |
| Medium P | 12.9b | 19.6d | 16.3e | 7.8d | 26.1c | 26.0b | 22.7e | 77.7ab |
| Low P | 2.5a | 14.7c | 7.3bc | 5.0c | 19.6b | 29.1a | 19.4cd | 73.7a |
| Unamended soil | 3.2a | 2.6a | 5.7ab | 2.0ab | 16.1ab | 32.7a | 17.2c | 76.2ab |
| D28 | | | | | | | | |
| High P | 101.1c | 15.2c | 36.0h | 2.4b | 34.9d | 14.7b | 21.2de | 104.7d |
| Medium P | 18.5b | 13.8c | 15.3e | 1.5ab | 25.1c | 13.7b | 18.3cd | 105.4d |
| Low P | 5.2ab | 11.0bc | 9.4c | 0.8ab | 20.6b | 10.7a | 17.3c | 100.0cd |
| Unamended soil | 4.4ab | 3.1a | 6.5b | 1.5ab | 19.1b | 10.2a | 15.6b | 98.1cd |
| D56 | | | | | | | | |
| High P | 99.2c | 11.5bc | 26.0f | 1.2ab | 25.6c | 28.9c | 19.9d | 93.9c |
| Medium P | 18.4b | 13.2bc | 12.9de | 0.6a | 18.9b | 30.4c | 16.9bc | 88.1bc |
| Low P | 5.8ab | 12.0bc | 8.7bc | 0.4a | 16.5ab | 26.2b | 17.2c | 89.4bc |
| Unamended soil | 4.6ab | 2.2a | 5.4ab | 1.2ab | 16.8ab | 21.0a | 11.3a | 104.8d |

increase was greatest for NaOH–Po and residual P. The sum of increases was greater than the sum of decreases. From d14 to d28, the concentrations of resin P and inorganic P in the $NaHCO_3$ and NaOH pools as well as HCl–Pi increased, with the greatest increase in NaOH–Pi. In contrast, the concentrations of organic P in the $NaHCO_3$ and particularly in the NaOH pools decreased. In the NaOH pool, the decrease in the organic fraction was greater than the corresponding increase in the inorganic fraction whereas in the $NaHCO_3$ pool, the inorganic fraction increased more than the organic fraction decreased. Microbial P and HCl–Pi changed little from d14 to d28. Overall, the increase in the size of the pools was greater than the decrease. From d28 to d56, the concentration of most P pools decreased with the greatest decreases occurring in the inorganic fractions of the $NaHCO_3$ and NaOH pools and in residual P. Only the concentrations of NaOH–Po and HCl–Pi increased. During this period, the sum of decreases was greater than the sum of increases.

### 3.3.2. Medium-P residues

In soil amended with medium-P residues (Fig. 2b) from d0 to d14, the concentrations of $NaHCO_3$–Po, NaOH–Pi and residual P increased, whereas the concentration of resin P and particularly NaOH–Po decreased. During this period, the sum of increases was greater than the sum of decreases. From d14 to d28, the concentrations of microbial P, $NaHCO_3$–Po, NaOH–Pi and HCl–Pi decreased, whereas those of resin P, NaOH–Po and residual P increased. The sum of decreases was equal to the sum of increases. From d28 to d56, the concentrations of NaOH–Pi and particularly residual P strongly decreased. Only the concentrations of NaOH–Po and HCl–Pi increased. The sum of decreases was greater than the sum of increases.

### 3.3.3. Low-P residues

From d0 to d14, the addition of low-P residues (Fig. 2c) resulted in a decrease in the concentrations of resin P, $NaHCO_3$–Pi, HCl–Pi and particularly NaOH–Po, whereas the concentrations of microbial P, $NaHCO_3$–Po, NaOH–Pi and residual P increased. The sum of decreases was greater than the sum of increases. From d14 to d28, the decrease in microbial P, $NaHCO_3$–Po, NaOH–Po and HCl–Pi closely matched increases in the other pools. The increase was greater for NaOH–Po and residual P than for resin and $NaHCO_3$–Pi. From d28 to d56, the concentration of NaOH–Pi and particularly residual P strongly decreased, whereas that of NaOH–Po and HCl–Pi increased.

## 3.4. Effects of roots and shoots on changes in soil P pools

To assess if root and shoot residues induce differential changes in soil P pools, two pairs of roots and shoots having almost similar P content were compared. The low-P residues were chickpea 1 shoots (1.8 mg P $kg^{-1}$) and faba bean 3 roots (1.7 mg P $kg^{-1}$) (Fig. 3a). The medium-P residues were white lupin 3 shoots (3.3 mg P $kg^{-1}$) and faba bean 2 roots (2.9 mg P $kg^{-1}$) (Fig. 3b). There were no matching roots and shoots with high-P concentration. On d0, in soils amended with medium-P residues the concentrations of resin P and microbial P were higher with shoots than with roots, whereas the reverse was true for $NaHCO_3$–Po, NaOH–Pi and –Po, and HCl–Pi. The concentration of most P pools was higher in the soil amended with low-P roots than in soil with low-P shoots, except for $NaHCO_3$–Po and NaOH–Po pools. The changes in soil P pools over time induced by addition of roots and shoots differed mainly in the initial phase (Fig. 3a, b). Irrespective of residue P concentration, with addition of shoot

**Table 4**
Concentrations of different P pools (mg $kg^{-1}$) on day 0 in soil amended with shoot or root residues with medium and low-P concentration. Means in columns followed by the same letter are not significantly different at $P \leq 0.05$.

| Residue | Resin P | Microbial P | $NaHCO_3$–Pi | $NaHCO_3$–Po | NaOH–Pi | NaOH–Po | HCl–Pi | Residual P |
|---|---|---|---|---|---|---|---|---|
| | mg $kg^{-1}$ | | | | | | | |
| Medium P shoot | 19.9c | 19.1d | 11.9b | 0.3a | 17.8ab | 20.7a | 16.8a | 85.2a |
| Medium P root | 13.1b | 17.2c | 12.6b | 1.4a | 19.4bc | 30.3b | 19.8b | 82.5a |
| Low P shoot | 5.4a | 9.2a | 8.9a | 0.6a | 16.5a | 27.9b | 16.7a | 74.7a |
| Low P root | 6.3a | 12.4b | 10.3ab | 0.1a | 20.7c | 26.3b | 19.6b | 82.1a |

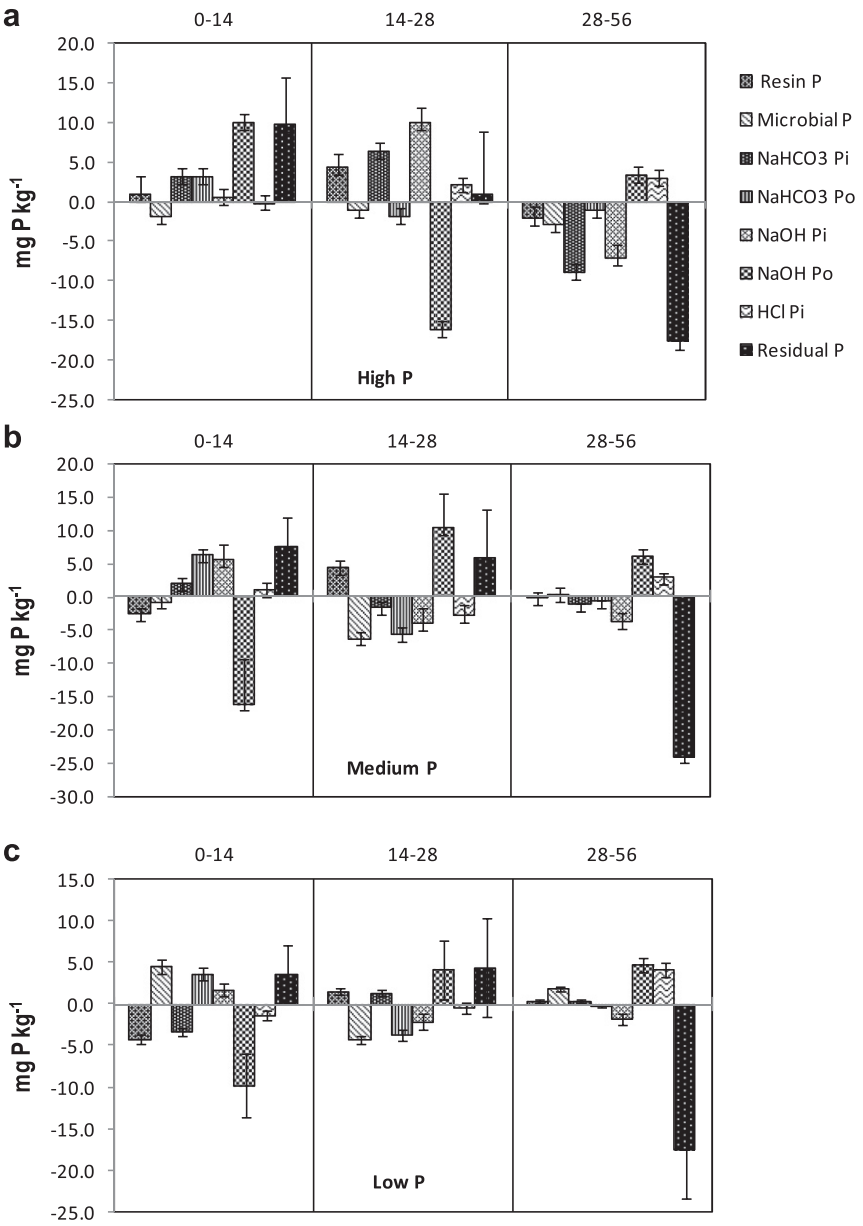


**Fig. 2.** Changes in P pools (mg $kg^{-1}$) in the periods of 0–14, 14–28 and 28–56 days after addition of residues with high (a), medium (b), and low-P concentrations (c). Data are means of different residue types within the same P concentration range (see Table 1). Vertical lines indicate standard deviation.

residues there was an increase in NaOH Po from d0 to d14, but a decrease from d14 to d28, whereas the opposite trend was found with addition of root residues. The concentration of microbial P increased more strongly from d0 to d14 with addition of low-P shoot residue than with low-P root residues. In this phase, the decrease in resin P concentration was greater for shoot than for root residues. From d28 to d56, the concentration of residual P decreased in all amended soils whereas that of NaOH–Po and HCl–Pi increased.

## 4. Discussion

This study showed that residue addition resulted in changes in the concentrations of various soil P pools. These changes occurred immediately after residue addition as well as during residue decomposition. Furthermore, the changes in P pool concentrations were affected by P concentration in residues and plant part.

### 4.1. Respiration

Microbes in soil are usually carbon-limited (Hoyle et al., 2008) and thus the addition of C with the residues increased cumulative respiration. This is confirmed by the positive correlation between cumulative respiration and amount of C added. Although the P concentration of residues was not correlated with cumulative respiration, the negative correlation between cumulative respiration and the C/P ratio indicates that P availability also affected microbial activity.

### 4.2. Comparison of residues with different P concentration

In the first two weeks following addition of low-P residues, the concentration of microbial P increased while resin P and $NaHCO_3$–Pi decreased. The reverse was true for high-P residues. Thus, in agreement with White and Ayoub (1983) who reported that the

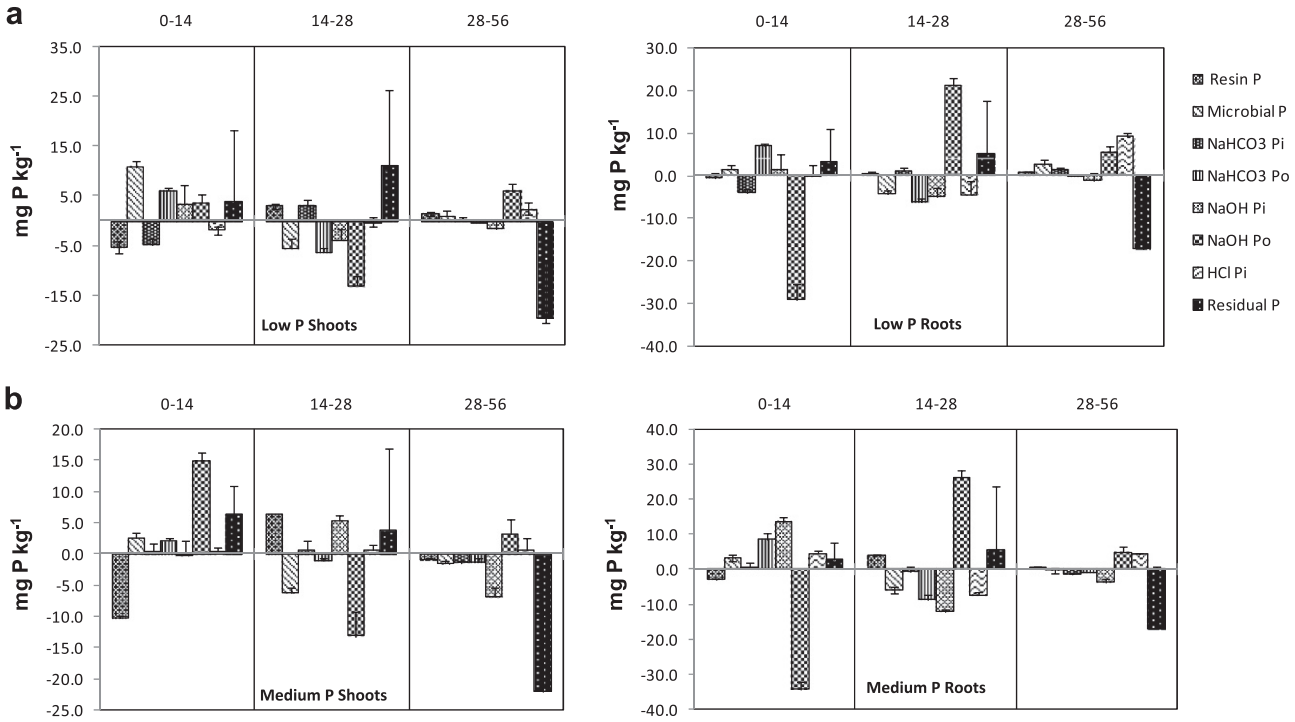


**Fig. 3.** Changes in P pools (mg $kg^{-1}$) in the periods of 0–14, 14–28, and 28–56 days in soil amended with shoots or roots or with low (a) and medium (b) P concentration ($n = 4$). Vertical lines indicate standard deviation.

critical P concentration for immobilisation is 2.4 g $kg^{-1}$, net P immobilisation occurred with the low-P residues (0.6–1.8 mg P $kg^{-1}$), whereas net P mobilisation occurred with high-P residues (6.5–8.3 mg P $kg^{-1}$). According to White and Ayoub (1983), net P mineralisation would also have been expected with medium-P residues (2.9–3.3 mg P $kg^{-1}$), but there was little change in microbial, resin or $NaHCO_3$ Pi in the first 2 weeks with addition of medium-P residues, suggesting mineralisation and immobilisation rates were similar. This may be explained by their C/P ratio which was close to 100 which is the threshold of P immobilisation (Cheshire and Chapman, 1996). The C/P ratio of the low-P residues ranged between 203 and 640 which is well above this threshold and therefore resulted in net P immobilisation. The concentration of NaOH–Po decreased with addition of low- and medium-P residues, suggesting mineralisation of organic P, whereas this pool increased with high-P residues. This indicates mineralisation of organic P (native and residue P) when the amount of P added with the residues is relatively low. With high-P residues on the other hand, there is a net synthesis of organic P because of the abundance of P. The increase in residual P with addition of plant residues indicates that some mineralised P is converted into stable P within the first 14 days.

The decrease in microbial P and $NaHCO_3$–Po concentration between d14 and d28, which was greater with low- and medium-P residues than with high-P residues, suggests that part of the microbial biomass turned over as a result of decreased C availability and also net mineralisation of labile organic P. However with low- and medium-P residues, this decrease did not lead to a corresponding increase in labile inorganic P, as the increase in resin P and $NaHCO_3$–Pi was relatively small. Instead, the concentration of NaOH–Po and residual P increased, suggesting conversion into less labile organic and inorganic P forms. The changes in soil amended with low- and medium-P residues in this period are in contrast to those with high-P residue addition, where there was a decrease in NaOH–Po concentration and strong increases in labile inorganic P (resin and $NaHCO_3$–Pi) as well as non-labile inorganic P (NaOH–Pi), but no change in residual P. The strong decrease in organic P (predominantly NaOH–Po, but also $NaHCO_3$–Po) with high-P residue addition suggests conversion of organic P into inorganic P. The organic P may be derived from the residues as suggested by the strong increase in NaOH–Po concentration in the period of d0 to d14. However, the NaOH–Po concentration decreased from d14 to d28 and this decrease was greater than the increase in the previous period, indicating mineralisation of native organic P. Hence, mineralisation of NaOH–Po occurred earlier with low- and medium-P residues (d0 to d14) than with high-P residues (d14 to d28).

The strongest change in P pools from d28 to d56 was the decrease in residual P concentration in soil amended with all residues regardless of P concentration. In this period the concentrations of NaOH–Po and HCl–P increased in all residue-amended soils, indicating net conversion of P into stable organic and inorganic P. However, the decrease in residual P was greater than the increases in the other P pools. As there were no plants growing and leaching from the soils did not occur, net loss of P from the system can be ruled out. The net decrease suggests that P was converted into P forms that are not accessible with P fractionation. Indeed, Syers et al. (1967) suggested that some soil P forms may remain undetected because they are not dissolved by acids.

### 4.3. Comparison of shoot and root residues

The differences in P pools between soils amended with shoot or root residues were most marked in the first 28 days. Irrespective of the P concentration in the residues, the concentration of NaOH–Po increased from d0 to d14, but then decreased from d14 to d28 with addition of shoot residues whereas the opposite trend was found with roots. These changes were generally more pronounced in the low-P than in the medium-P residues.

The more rapid mineralisation of NaOH–Po in soil following addition of root residues suggests that the organic P in this pool was

more easily decomposable; most likely this was the organic P originating from the root residues. The net increase in NaOH–Po in the initial phase after addition of shoot residues on the other hand indicates synthesis of less labile P, most likely from labile inorganic P (resin P and $NaHCO_3$–Pi) which decreased during this period. The strong decrease in NaOH–Po in the first 14 days with addition of root residues did not correspond with an increase in other P pools, again suggesting conversion into a P pool that is not accessible with P fractionation.

From d14 to d28, the concentration of NaOH–Po in soil increased with addition of root residues, but decreased with shoot residue additions. Hence with root residues the initial mineralisation of organic P from d0 to d14 was followed by a build-up of NaOH–Po in the following 2 weeks, indicating synthesis of organic P. With shoot residues on the other hand, where NaOH–Po was synthesised in the first 2 weeks, there was a net mineralisation of NaOH–Po in period of d14 to d28 which was matched by an increase in inorganic P pools (resin P, NaOH–Pi and NaOH–Pi). This suggests that in soil amended with shoot residues, organic P is mineralised by microbes when the inorganic P sources are depleted. The fact that this difference between soils amended with root and shoot residues occurred in both medium- and low-P residues suggests that there are inherent differences in forms of organic P between plant parts. These differences in P dynamics may also be related to the differential decomposition rate of roots and shoots. Even though the C/N ratio did not differ between shoots and roots and the C/P ratio was greater for the shoots, the decomposition rate of medium and low-P shoots was greater than that of the roots suggesting that roots contain compounds that are more difficult to decompose. Furthermore, root and shoot residues may differ in microbial decomposer community composition and decomposition products (Baumann et al., 2011). Microbial communities may differ in P mobilisation capacity and P uptake and differential decomposition products could indirectly affect soil P dynamics by changing the sorption capacity of the soil. Cobo et al. (2008) also found different P release patterns of leaves, stems, and a leaf/stem mixtures of legume residues.

## 5. Conclusion

This study demonstrated that changes in soil P pools induced by plant residue addition are not only affected by residue P concentration but also by plant part. Furthermore, the concentrations of the various soil P pools changed over time, indicating transformation between P pools. Experiments with longer incubation periods would be required to assess if at some stage in the decomposition process, the size of the P pools stabilizes. Additionally, similar experiments with other soils differing in dominant P pools and P buffering capacity would reveal if the changes observed in the soil used here are generally applicable or if they are soil type specific.

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# Chapter 3

# Short term effects of application of different rates of inorganic P and residue P on soil P pools and wheat growth

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Supervised development of work, data interpretation and manuscript evaluation.

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# Chapter 4

# Changes in P pools in three soils

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**Changes in P pools in three soils**

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**Summary**

Previously we showed that addition of legume residues affected the size of different soil P pools in an alkaline loamy sand soil. Here we tested if the changes in soil P pools induced by residue addition are generally applicable or if they are dependent on certain soil properties. Three legume residues differing in P concentration: faba bean (*Vicia faba*) (high P), chickpea (*Cicer arietinum*) (medium P) and white lupin (*Lupinus albus*) (low P), were added at a rate of 20 g residue $kg^{-1}$ soil to three soils differing in properties but all with low P availability: Mount Bold (sandy clay loam, high organic C content, pH 5.1), Monarto (loamy sand, low organic C content, pH 7.5), and Langhorne Creek (sandy loam, low organic C content, pH 8.1). Soil P pools were assessed by sequential P fractionation on days 0 and 42. In residue-amended soils from day 0 to day 42, the concentration of water soluble and microbial P decreased, whereas the concentrations of $NaHCO_3$ Pi and NaOH Po increased; the magnitude of these changes differed among soils, being greatest in the Mt Bold soil. Residue addition had little or no effect on the concentrations of NaOH Pi, HCl Pi and residual P which also did not change significantly over time. Principal component analysis of the data showed that the size of the P pools was related to soil properties, high concentrations of HCl P were associated with high pH and Ca concentrations, high concentrations of NaOH P and residual P were correlated with high

Al, silt, organic C, total N and P. On day 42, the concentrations of the labile P pools was related to amount of P added with the residues but on day 0 the concentration of $NaHCO_3$-Pi was correlated with the clay content. It can be concluded that most effects of residue addition to soils on microbial activity and growth and soil P pools can be generalized across the three soil used in this study, but that the size of the P pools is affected by soil properties such as organic carbon content, pH and texture.

**Keywords**

C/P ratio; legume residue; microbial biomass carbon; P fractionation; respiration, soil properties

## Introduction

Phosphorus in soil exists in different inorganic and organic P forms and their solubility and availability vary with soil physical and chemical properties (Holford 1997) and management. Two mechanisms control P concentration in the soil solution and the P movement in soils: (1) the solubility of P containing minerals, and (2) the fixation or adsorption of phosphate ions on the surface of soil particles (Brady and Weil 2002). The most important properties that affect solubility and fixation of P in soils are pH; calcium carbonate content; clay content; the nature and surface area of the soil particles; and the concentrations of Fe, Al and Mn hydrous oxides (Brady and Weil 2002; Holford 1997). In acidic soils, the predominant forms of inorganic P are Fe or Al phosphates, P adsorbed by or occluded in Fe/Al oxides and hydroxides, such as gibbsite, hematite, and goethite (Arai and Sparks 2007; Parfitt 1989). In neutral to alkaline soils P occurs as Ca and Mg phosphates and adsorbed on the surfaces of Ca and Mg carbonates. Phosphorus fixing

capacity largely depends on the types and amounts of clay minerals present in soil. Generally soils with high amorphous oxides, allophanes (amorphous aluminium-silicate of variable Al to Si ratios) and kaolinite have high P fixing capacity as these particles have high positive charges to attract phosphate ions.

Organic matter interacts with P in soils through several mechanisms. Humic acids, fulvic acids and aliphatic acids can compete with P ions in solution for soil sorption sites. Furthermore organic compounds can increase solution P concentrations through metal complexation and dissolution reactions (Bolan et al. 1994). Transformation of P in soils involves complex mineralogical, chemical, and biological processes (Zheng et al. 2002) and P added to soil is often transformed into less soluble forms (Zhang et al. 2004).

Soil P pools consist of several fractions of inorganic (Pi) and organic P (Po) compounds that are broadly grouped as labile, moderately labile, relatively insoluble and resistant pools, however the availability of inorganic and organic P in soil is still poorly understood (George et al. 2006; Hinsinger 2001). Various P fractionation schemes have been developed to assess the different forms of soil P (Bowman and Cole 1978; Chang and Jackson 1957; Hedley et al. 1982; Tiessen and Moir 1993; Turner et al. 2005). The sequential P fractionation scheme developed by Hedley et al. (1982) is the most widely used (Negassa and Leinweber 2009) and is useful to assess the fluxes between P forms in soil (Reddy et al. 2005; Wang et al. 2008). The Hedley fractionation scheme involves a sequence of extractions with water, $NaHCO_3$, NaOH, HCl and acid digestion to separate labile (resin-Pi, $NaHCO_3$-Pi and $NaHCO_3$-Po), moderately labile (NaOH-Pi, NaOH-Po), relatively insoluble apatite-P (HCl-Pi) and resistant P (in primary minerals) pools.

In a previous study (Alamgir et al. 2012), we showed that the concentration of various soil P pools changes over time upon addition of legume residue in an alkaline loamy sand. However, given the importance of soil properties on nature and concentration of soil P

pools, it is of interest to assess if the changes in P pools observed in the loamy sand are generally applicable or if they are related to soil properties. Therefore in this study, we added three different legume residues with low, medium and high P concentrations to three soils differing in texture, pH and organic matter content and determined the concentration of soil P pools after 0 and 42 days.

## Materials and Methods

### *Experimental setup*

Three soils collected in South Australia from Monarto 35° 05´ S, 139° 04´E, Mount Bold 35° 04´ S, 138° 42´ E and Langhorne Creek 35° 16´ S, 139° 09´ E were used in this study. The Monarto site was under natural bushland and other sites were under grassland vegetation. Five subsamples (0-10 cm depth) were combined to make a composite sample. After careful mixing, the soils were air-dried and passed through a 2 mm sieve. Soils in Southern Australia remain dry for several months during summer, therefore this treatment is not un-natural. The soils differed in their physical and chemical properties (Table 1). The soils were classified according to USDA soil classification system (USDA 1999). Before onset of the experiment, the soils were pre-incubated for 10 days at the water content optimal for microbial activity. The optimum water content was determined in a preliminary experiment by measuring cumulative respiration for 10 days at different water contents (40-90%). The optimum water contents are 70%, 50% and 60% of water holding capacity for Monarto, Mt. Bold and Langhorne Creek soil, respectively. Three shoot residues differing in total P concentration that induced large, medium and small changes in P pools in a previous experiment (Alamgir et al. 2012) were used: faba bean (*Vicia faba*) (FB, high P), chickpea (*Cicer arietinum*) (CP, medium P) and white lupin (*Lupinus albus*) (WL, low P concentration). The total P concentration of the residues ranged from 0.6 g P $kg^{-1}$ to 6.6 g P $kg^{-1}$ and the C/P ratio from 63 to 640 (Table 2). Water soluble C of the residues varied

from 2 to 16% of total C. The residues were finely ground and sieved to particle size of 0.25 to 2 mm, added at a rate of 20 g residue $kg^{-1}$ soil and mixed thoroughly into the soils. Twenty g soil mixed with residues were placed in 3.7 cm diameter PVC cores fitted with nylon mesh base and the bulk density adjusted to 1.63, 1.43 and 1.46 g $cm^{-3}$ for Monarto, Mt. Bold and Langhorne Creek soil, respectively. This bulk density was calculated using the bulk density calculator based on the U.S. Texture Triangle (http://www.pedosphere.com/resources/bulkdensity/ worktable_us.cfm). The soils were incubated in the dark at 22-25°C for 42 days. During incubation, soil moisture was maintained by weight with autoclaved reverse osmosis water.

***Analyses***

Soil respiration was measured only in the first 14 days after residue addition because after this period, respiration rates were very low and stable and will therefore have little effect on cumulative respiration.

For respiration measurement the cores were placed individually in 1 L glass jars with gas-tight lids equipped with septa that allow sampling of the headspace; containers with water were also placed in the jars to maintain humidity and minimize drying of the soil during incubation. The $CO_2$ concentration was measured daily with an infrared $CO_2$ gas analyser (Servomex 1450 Food Package Analyser, Crowborough, UK). After measurement, the jars were opened and flushed with ambient air. After closing, the $CO_2$ concentration at time zero was determined. On day 14, the cores were removed from the jars and placed together in large plastic containers with loosely fitting lids which were opened regularly for aeration until day 42.

Destructive sampling for the parameters described below occurred on days (d) 0 (3 h after residue addition) and 42. Microbial biomass carbon (MBC) was determined by fumigation

extraction (Guo et al. 2000) at the start (after pre-incubation and before residue addition) and the end of the experiment (day 42). A $k_{EC}$ factor of 2.64 was used to convert the difference in extractable C between the fumigated and non-fumigated samples into microbial biomass C (Tiessen et al. 1992)

Total C and N of the soils and the residues were determined by dry combustion using a Leco CNS-2000. Total Fe, Al, Ca and Mg concentrations were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES) after digesting the soils with nitric acid-perchloric acid (Olsen and Sommers 1982). Total P of the residues was determined after digestion with nitric acid-perchloric acid (Olsen and Sommers 1982); P in the extract was measured colormetrically by the phosphovanado-molybdate method (Hanson 1950; Kitson and Mellon 1944). The concentration of various P pools was determined by sequential P fractionation (Hedley et al. 1982; Huang and Zhang 2009; Tiessen and Moir 1993) with some modifications. One g of soil was sequentially extracted (1:30 soil:solution) with anion exchange resin, 0.5 M $NaHCO_3$, 0.1 M NaOH, 1 M HCl, and again 0.1 M NaOH. Residual P was determined by digesting the soil remaining after the last step of the fractionation in $HNO_3$-$HClO_4$ (6:1). To differentiate between organic and inorganic P, 5 mL of the extract from the $NaHCO_3$ and NaOH fractions was acidified to pH 1.5 to precipitate organic matter, centrifuged and the supernatant was analysed for inorganic P. Total P in the $NaHCO_3$ and NaOH fractions was analysed by digesting 5 mL of the extract with 1 ml 5% potassium persulfate at 90$^{\circ}$C for 16h (Huang and Zhang 2009). Organic P was calculated as the difference between total P and inorganic P. Previous studies showed that only these two fractions contained measurable organic P concentrations. For determination of microbial P, another subsample of the soil was extracted with anion exchange resin with or without hexanol. Microbial P was calculated from the difference between samples treated with hexanol and without hexanol (Kouno et

al. 1995). The P concentration in the extracts was determined colorimetrically (Murphy and Riley 1962). Total P was calculated as the sum of the sequentially extracted P pools.

***Statistical analyses***

Statistical analyses were performed using GenStat for Windows 11th Edition (VSN International, UK, 2008). Cumulative respiration was analysed by two-way ANOVA (fixed factors: residue x soil type). The effects of soils, treatments and sampling dates along with their interactions (fixed factors) on microbial biomass C and P pools was analysed using 3 way ANOVA. Means were compared by Tukey test at $\alpha = 0.05$. Correlation analysis was used to measure the strength of relationships between the variables.

Principal component analysis (PCA) was carried out using the P pools as variables and soil properties as environmental factors for day 0 (unamended soils only) and day 42 (amended and unamended soils) (Primer-E Ltd, Plymouth Marine Laboratory, Plymouth, UK). In PCA plots, samples are shown as symbols, measured parameters as vectors. Values on the x and y axis indicate percentage explanation of the variance of the axes. The distribution of the samples on the plot is based on the measured parameters with vectors pointing towards the samples with the highest concentration of this parameter. The angle between vectors indicates their relationship with a small angle showing a high positive correlation, 90 degree no relationship and 180 degree a negative correlation. Symbols close to each other have similar concentrations of various parameters. Loadings of PCA axis of the different measured parameters indicate how these parameters are correlated with the respective axis. A high loading shows a strong correlation, a negative loading a negative relationship.

## Results

### *Soil properties*

The unamended soils (analysed on day 0 before residue addition) differed in their physical and chemical properties (Table 1). The soil from Mt. Bold was a sandy loam and had the highest total C, N and P concentration and the lowest pH and Ca concentration. The sandy clay loam from Langhorne creek had the highest pH and the lowest total N, P, and Fe concentration. The neutral loamy sand from Monarto was characterised by the lowest total C concentration but highest Fe and Mg concentration.

With faba bean, the largest amount of total C, N and P as well as water-soluble C and P were added and the least with white lupin residues (Table 3).

### *Cumulative respiration*

Cumulative respiration was similar in the unamended soils (Fig. 1). Compared to the unamended control, residue addition increased cumulative respiration 10 to 23 fold (Fig. 1) with the extent of this increase differing among the soils. Chickpea residue addition resulted in the greatest increase in cumulative respiration while the increase was smallest with white lupin. With chickpea and faba bean, cumulative respiration was highest in Monarto soil whereas with white lupin residues, it was highest in Mt Bold soil. Cumulative respiration was positively correlated with the water extractable C of the residues ($r=0.94$, $p<0.01$) and the amount of C added with the residues ($r=0.85$, $p<0.01$), but negatively correlated with residue C/P ratio ($r= -0.86$, $p<0.01$).

### *Microbial biomass carbon*

In the unamended soils on day 0, microbial biomass C was higher in Mt. Bold soil than in the other two soils (Fig. 2). In the amended soils on day 0 (3h after residue addition), microbial biomass C was highest in Mt Bold soil and in all soils highest when amended

with faba bean residues and lowest with white lupin residues. From day 0 to 42, microbial biomass C in the unamended controls remained unchanged in Monarto and Mt Bold soil but increased in Langhorne Creek soil. In all amended soils, the greatest increase in microbial biomass from day 0 to day 42 occurred with chickpea residues. With faba bean residues, microbial biomass remained either unchanged (Monarto and Mt Bold soils) or decreased (Langhorne Creek soil). With addition of white lupin residues, the increase in microbial biomass C from day 0 to day 42 was greatest in Langhorne Creek soil. Microbial biomass C on day 0 was positively correlated with the water extractable C of the residues ($r=0.62$, $p<0.01$), the amount of C added with the residues ($r=0.51$, $p<0.01$) and microbial P ($r=0.56$, $p<0.01$). Microbial biomass carbon was not significantly correlated with residue C/P ratio.

***Soil P pools***

Compared to the other two soils, Mt Bold soil had the highest concentrations of $NaHCO_3$ Po, NaOH Pi and Po and residual P, but the lowest concentrations of HCl Pi (Fig. 3). Langhorne Creek soil had the highest concentrations of resin P, $NaHCO_3$ Pi and HCl Pi, but the lowest residual P concentrations.

In the amended soils, the resin P concentration on day 0 (3 h after residue addition) and day 42 was highest with faba bean residues and lowest with white lupin residues (Fig. 3a). Addition of white lupin residues did not increase resin P concentrations compared to the unamended soils. From day 0 to day 42, the resin P concentration decreased only with faba bean residues in all soils, whereas the resin P concentration remained stable over time in soils amended with chickpea or white lupin residues except for a decrease with chickpea residues in Mt Bold soil.

On day 0 the microbial P concentration was highest in Monarto soil and was higher in soils amended with chickpea and faba bean residues than in the unamended soil or that amended

with white lupin residues. When amended with faba bean and chickpea residues, the microbial P concentration decreased from day 0 to day 42 in Monarto and Langhorne Creek soils. On the other hand in Mt Bold soil, the microbial P concentration did not change over time when amended with faba bean residues and increased with chickpea residues. With white lupin residues, the microbial P concentration increased from day 0 to day 42 in all three soils (Figure 3b).

On day 0 residue addition increased the concentration of $NaHCO_3$ Pi in all soils with the greatest increase with faba bean residues and the smallest with white lupin residues (Figure 3c). In most treatments, the $NaHCO_3$ Pi concentration increased from day 0 to day 42, except for Langhorne Creek soil amended with white lupin residues where it decreased. Compared to the unamended soil, addition of chickpea residues resulted in the greatest increase in $NaHCO_3$ Po concentration in Langhorne Creek and Mt Bold soil on day 0 and in Monarto soil on day 42. Addition of faba bean residues increased the concentration of this pool only in Mt Bold soil whereas white lupin residues had no effect or decreased the $NaHCO_3$ Po concentration. The concentration of $NaHCO_3$ Po changed from day 0 to day 42 only in soils amended with chickpea residues where it decreased in Langhorne Creek and Mt Bold soil but increased in Monarto soil.

Compared to the unamended soil, the concentration of NaOH Pi increased only with addition of chickpea and faba bean residues (Figure 3e). The NaOH Pi concentration changed little over time except for an increase in Mt Bold soil amended with chickpea residues. Compared to the unamended soil, residue addition had no significant effect on the concentration of NaOH Po on day 0 (Figure 3f). However on day 42, the NaOH Po concentration was increased by addition of chickpea and faba bean residues. The concentration of this pool increased from day 0 to day 42 in all treatments, but the increase was greater in residue amended soils, particularly in Mt Bold soil.

On day 0 compared to the unamended soil, the concentration of HCl Pi was increased by addition of white lupin residues in Langhorne Creek and Monarto soils and chickpea residues in Monarto soil (Figure 3g). The concentration of this pool changed little over time except for a decrease in Langhorne Creek amended with white lupin residues and in Monarto soil amended with chickpea or white lupin residues. Residue addition had no effect on the HCl Pi concentration in the Mt Bold soil where it was very low compared to the other two soils. The residual P concentration was not affected by residue addition and did not change significantly over time (Figure 3 h).

The principal component analysis based on the P pools clearly separated the soils on day 0 and day 42, with the Mount Bold soil on the left and Monarto and Langhorne Creek soils on the right (Figure 4). On day 0, Monarto and Langhorne creek soils were separated along PC2 (vertical axis) whereas the samples of these two soils were not clearly separated on day 42. On day 42, the soils with faba bean residues were in the lower part of the plot whereas the control soils and those with white lupin residues were in the upper part. The PCA also showed that the size of the P pools was related to soil properties (Figure 4, Table 4). On both days, high concentrations of HCl P were associated with high pH and Ca concentrations and high concentrations of NaOH Pi, residual and total P with high organic matter content (total C and N), silt, total P and Al concentrations. On day 0 in the unamended soil, high concentrations of $NaHCO_3$-Pi was related to high clay content whereas on day 42 the concentrations of the labile P pools (resin, microbial, $NaHCO_3$-Pi) were correlated with the amount of P added with the residues.

**Discussion**

Our results show that most effects of residues can be generalised across the three soils used in this experiment. From day 0 to day 42, the concentration of water soluble and microbial P decreased in the residue amended soils and there was a corresponding increase in

$NaHCO_3$ Pi and NaOH Po, however the magnitude of these changes differed among soils. The size of the P pools was related to soil properties such as pH, organic matter content and texture.

***Microbial activity and biomass***

The higher organic matter content of the Mt Bold soil can explain its higher microbial biomass on day 0 since organic C and biomass C are correlated (Leirós et al. 2000; Beck et al. 1997). In the unamended soils, the greater microbial biomass in Mt Bold soil did not result in higher cumulative respiration suggesting that a large proportion of the biomass in this soil inactive, probably due to the lack of easily decomposable substrates. Despite the greater microbial biomass on day 0 in Mt Bold soil, cumulative respiration upon residue addition was greater than in the other two soils only with white lupin residues which were the least decomposable residues due to their low concentration of water-soluble C and high C/N and C/P ratio. This suggests that even a small initial microbial biomass in the Langhorne Creek and Monarto soils can rapidly decompose more easily decomposable residues such as faba bean and chickpea residues, but has a limited ability to decompose less decomposable residues. The differential ability to decompose the added residues among the soils may also be due to differences in microbial community composition as soil pH has been shown to have a strong effect on community composition (Fierer et al. 2003; Marschner et al. 2005; Rousk et al. 2010).

The greatest amounts of N, P and water soluble C were added with faba bean residues which can explain the high microbial biomass C and P on day 0 measured after amendment with these residues. It should be noted that chloroform could also release some C and P from the residues, thus microbial biomass on day 0 may be overestimated particularly for faba bean residues with their high concentration of water-soluble C. Cumulative respiration

in the first 14 days after residue addition was greatest in soil amended with chickpea residues although similar amounts of total C and water-soluble C were added than with faba bean residues. On the other hand, microbial P and microbial C on day 42 were similar in soils amended with chickpea and faba bean, except for Langhorne Creek soil where microbial C was higher with chickpea than with faba bean residues. The similar effects of chickpea and faba bean residues on microbial biomass C and P can be explained by the fact that similar amounts of total and water soluble C were added with the two residues. However, the C/N and C/P ratios were lower in chickpea and faba bean residues, which may explain the higher cumulative respiration in soils amended with these residues. The high cumulative respiration would result in greater C loss, which over time will reduce the C/N and C/P ratio of the remaining residues to values closer to those required by the soil microbes to build up biomass.

Cumulative respiration and microbial biomass C and P on day 42 were lowest in soils amended with white lupin residues (except for microbial biomass C in Langhorne Creek soil) which can be explained by the lower amounts of C, N and P added with these residues and their higher C/N and C/P ratio. Thus less substrate was added with the white lupin residues and it was poorly decomposable. The low microbial C on day 42 in the Langhorne Creek soil amended with faba bean may be an analytical or sampling error since microbial P was similar as with chickpea residues.

***Soil P pools***

The acidic Mt. Bold soil with its high organic matter content had the highest concentrations of $NaHCO_3$ Po, NaOH Po and Pi (Fe and Al bound P), whereas the alkaline Langhorne Creek soil had high concentration of HCl Pi (Ca bound P). The differences in concentration of the inorganic P pools among the soils can be explained by the differences in solubility of Fe and Al and Ca phosphates at different pH as with increasing soil pH the solubility of

Fe and Al phosphates increases whereas that of Ca phosphate decreases (Hinsinger 2001). High concentrations of NaOH P, residual and total P were associated with high concentrations of Al, silt and organic matter. Thus, a large proportion of the P in these soils was bound to Al minerals, silt and organic matter. The correlation between $NaHCO_3$-Pi and clay content on day 0 in the unamended soil suggests that P bound to clay minerals is potentially available. However on day 42 the concentration of labile P pools was only associated with the amount of P added, thus P added with the residues over-rode any effects of soil texture on these pools. It has been shown that the relative amounts of Pi and Po are influenced by soil texture within one field site (O'Halloran et al. 1985). Tiessen et al. (1984) reported that the organic C content affects not only the total P concentration but also the size of the major P fractions.

Despite the large differences in initial concentrations of the various P pools, there were few differences among the soils in response of the P pools to residue addition. Addition of faba bean and chickpea residues resulted in strong increases in the labile P pools resin P, microbial P and $NaHCO_3$ Pi on day 0. This can be explained by the high amount of water soluble P added with these residues. Addition of white lupin on the other hand had little effect on the concentration of these labile P pools on day 0 because the amount of water soluble C and P added with this residue was small. Addition of faba bean and chickpea residues generally had little effect on the concentration of the less labile P pools (NaOH P, HCl P, residual P) on day 0 because these pools represent fixed P or P in minerals and transformation of labile P into these forms takes time (Sharpley et al. 1987; Tiessen et al. 1984). From day 0 to day 42 in the residue amended soils, the concentration of resin P decreased in all soils whereas the concentrations of $NaHCO_3$-Pi and NaOH Po increased. Resin and $NaHCO_3$ Pi are both considered to be labile P pools but the extraction method is different. The increase in $NaHCO_3$ Pi indicates that more P was associated with Ca or was

exchangeable by $HCO_3^-$ on day 42 compared to day 0. The increase in NaOH Po concentration suggests conversion of labile P into stable organic P forms. The increase in concentration of NaOH Po was generally greater in the amended than the unamended soil indicating that the formation of NaOH Po was microbially mediated. The decrease in resin P and increase in NaOH Po were greatest in Mt Bold soil which had the highest initial concentration of NaOH Po. This suggests that this soil with its high organic matter content has a greater capacity to convert P into stable organic P forms than the other two soils. The decrease in microbial P from day 0 to day 42 was greatest in Monarto soil which also corresponded to an increase in NaOH Po and $NaHCO_3$ Pi over time. The smallest changes in labile P pools and NaOH Po from day 0 to day 42 occurred in the Langhorne Creek soil; and only in this soil did the concentration of HCl Pi increase over time (except for soil amended with white lupin residues). The alkaline Langhorne Creek soil had also the highest concentrations of HCl Pi; the high pH of this soil may have favoured the formation of HCl Pi from the labile P pools.

**Conclusions**

It can be concluded that the size of P pools is determined by soil properties such as pH, Ca and Al concentration, organic matter content and texture. Most effects of residue addition on microbial activity and growth and soil P pools can be generalised across the three soil used in this study although they differed in soil pH and organic C content. Most of the differences among the residues on the measured parameters can be explained by their differential concentrations of total and water-soluble C and P and C/N and C/P ratios. Similarly, the increase of NaOH Po over time in all soils can, to some extent, be explained by the decrease in resin and microbial P. However, we could only measure net effects and it is not clear how much of the observed changes were due to movement of residue P or P already present in the soil. It would be of interest to determine the fate of residue P in the

different P pools and if this differs among soils with different pH for example by labelling residues with $^{33}P$.

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Table 1 Physical and chemical properties of three soils collected from Monarto, Mount Bold and Langhorne Creek, South Australia (n=3).

| Parameters | Monarto | Mount Bold | Langhorne Creek |
|---|---|---|---|
| Sand g $kg^{-1}$ | 810 | 520 | 720 |
| Silt g $kg^{-1}$ | 100 | 280 | 60 |
| Clay g $kg^{-1}$ | 90 | 200 | 220 |
| Texture | loamy sand | sandy loam | sandy clay loam |
| USDA Soil Classification | Rhodoxeralf | Typic haploxeralf | Calcixerollic Xerochrept–Lithocalcic Calcarosol |
| Bulk density g $cm^{-3}$ | 1.63 | 1.43 | 1.46 |
| Water holding capacity % | 15.8 | 34.5 | 16.4 |
| pH 1:5 ($H_2O$) | 7.5 | 5.1 | 8.1 |
| Total N g $kg^{-1}$ | 0.9 | 4.2 | 0.6 |
| Total C g $kg^{-1}$ | 7.3 | 44.1 | 8.3 |
| Total P mg $kg^{-1}$ | 139 | 246 | 95 |
| Total Fe g $kg^{-1}$ | 17.0 | 16.4 | 4.1 |
| Total Al g $kg^{-1}$ | 16.9 | 28.5 | 7.1 |
| Total Ca g $kg^{-1}$ | 1.5 | 0.6 | 1.8 |
| Total Mg g $kg^{-1}$ | 3.4 | 1.4 | 0.6 |

Table 2 Total and water soluble P and C concentration, total N concentration, C/N, and C/P ratio of legume residues (n=3)

| Residues | Total P | Water soluble P | Total C | Water soluble C | Total N | C/N | C/P |
|---|---|---|---|---|---|---|---|
| | g $kg^{-1}$ | | | | | | |
| White lupin | 0.6 | 0.2 | 384 | 7 | 9.7 | 40 | 640 |
| Chickpea | 1.8 | 0.6 | 418 | 60 | 21.1 | 20 | 232 |
| Faba bean | 6.6 | 5.9 | 417 | 67 | 34.5 | 12 | 63 |

Table 3 Amounts of C, N and P added to soil with the residues added at a rate of 20 g residue $kg^{-1}$ soil.

| Residues | Total C | Total N | Total P | Water soluble P | Water soluble C |
|---|---|---|---|---|---|
| | mg $kg^{-1}$ | | | | |
| White lupin | 7680 | 194 | 12 | 4 | 140 |
| Chickpea | 8360 | 422 | 36 | 12 | 1200 |
| Faba bean | 8340 | 690 | 132 | 118 | 1340 |

The values are based on the total C, N and P concentration shown in Table 2 multiplied by the rates of residue addition.

Table 4 Loadings of PCA axes for P pools and soil properties on days 0 (unamended soil) and 42 (amended and unamended soils). Note that only the most important variables are listed.

| | **P pools** | | | **Soil properties** | |
|---|---|---|---|---|---|
| Variable | PC1 | PC2 | Variable | PC1 | PC2 |
| | **Day 0** | | | | |
| HCl-P | 0.38 | | pH | 0.98 | |
| | | | Ca | 0.97 | |
| NaOH-Pi | -0.36 | | Al | -1.0 | |
| Residual P | -0.37 | | Silt | -0.98 | |
| Total P | -0.38 | | Total N | -0.95 | |
| | | | TOC | -0.91 | |
| | | | Total P | -0.99 | |
| | | | Silt | -0.98 | |
| $NaHCO_3$-Pi | | 0.69 | Clay | | 1.0 |
| | | | K | | -0.93 |
| | | | Mg | | -0.98 |
| | **Day 42** | | | | |
| HCl Pi | 0.38 | | pH | 0.95 | |
| | | | Ca | 0.95 | |
| $NaHCO_3$-Po | -0.39 | | Silt | -0.93 | |
| NaOH-Pi | -0.41 | | Al | -0.95 | |
| NaOH-Po | -0.41 | | Total N | -0.94 | |
| Residual P | -0.37 | | TOC | -0.93 | |
| Total P | -0.40 | | Total P | -0.95 | |
| $NaHCO_3$-Pi | | -0.57 | P added | | -0.90 |
| Microbial P | | -0.42 | | | |
| Resin P | | -0.59 | | | |

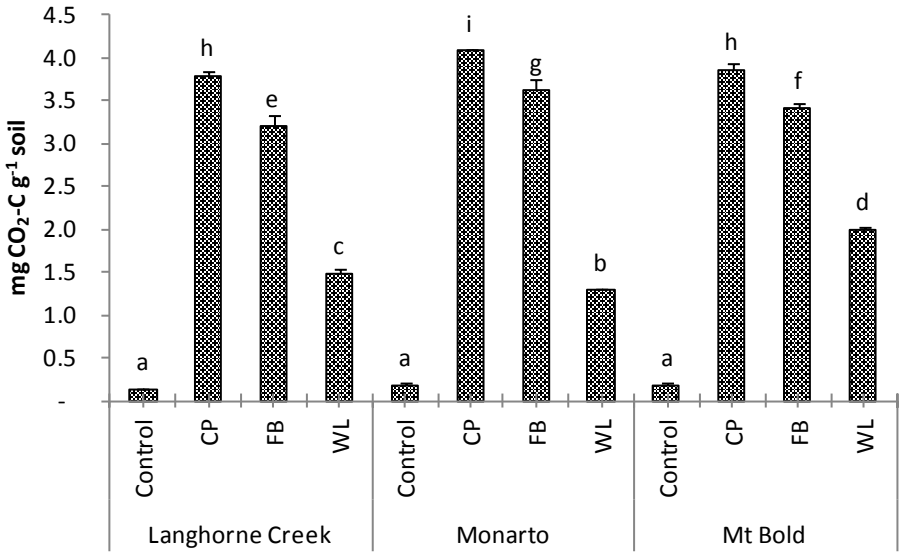


Figure 1 Cumulative respiration (mg $CO_2$-C $g^{-1}$ soil) in Langhorne Creek, Monarto and Mount Bold soils over 14 days in un-amended soil (control) and soil amended with chickpea (CP), faba bean (FB) and white lupin (WL) (n=3). Means with the same letter are not significantly different at $P \leq 0.05$. Vertical lines indicate standard deviation.

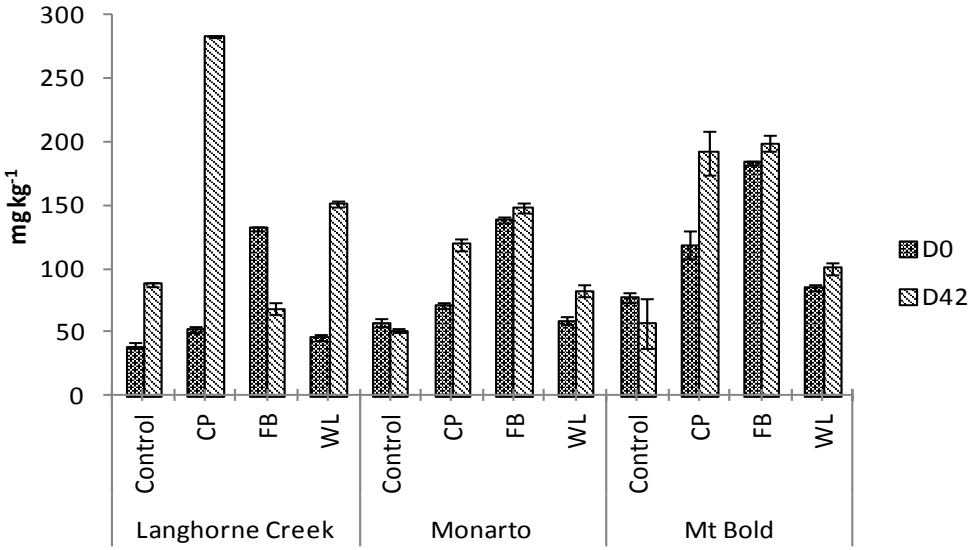


Figure 2 Microbial biomass carbon (mg $kg^{-1}$ soil) in Langhorne Creek, Monarto and Mount Bold soils on day 0 and after 42 days in un-amended soil (control) and soil amended with chickpea (CP), faba bean (FB) and white lupin (WL) residues (n=3). Vertical lines indicate standard deviation.

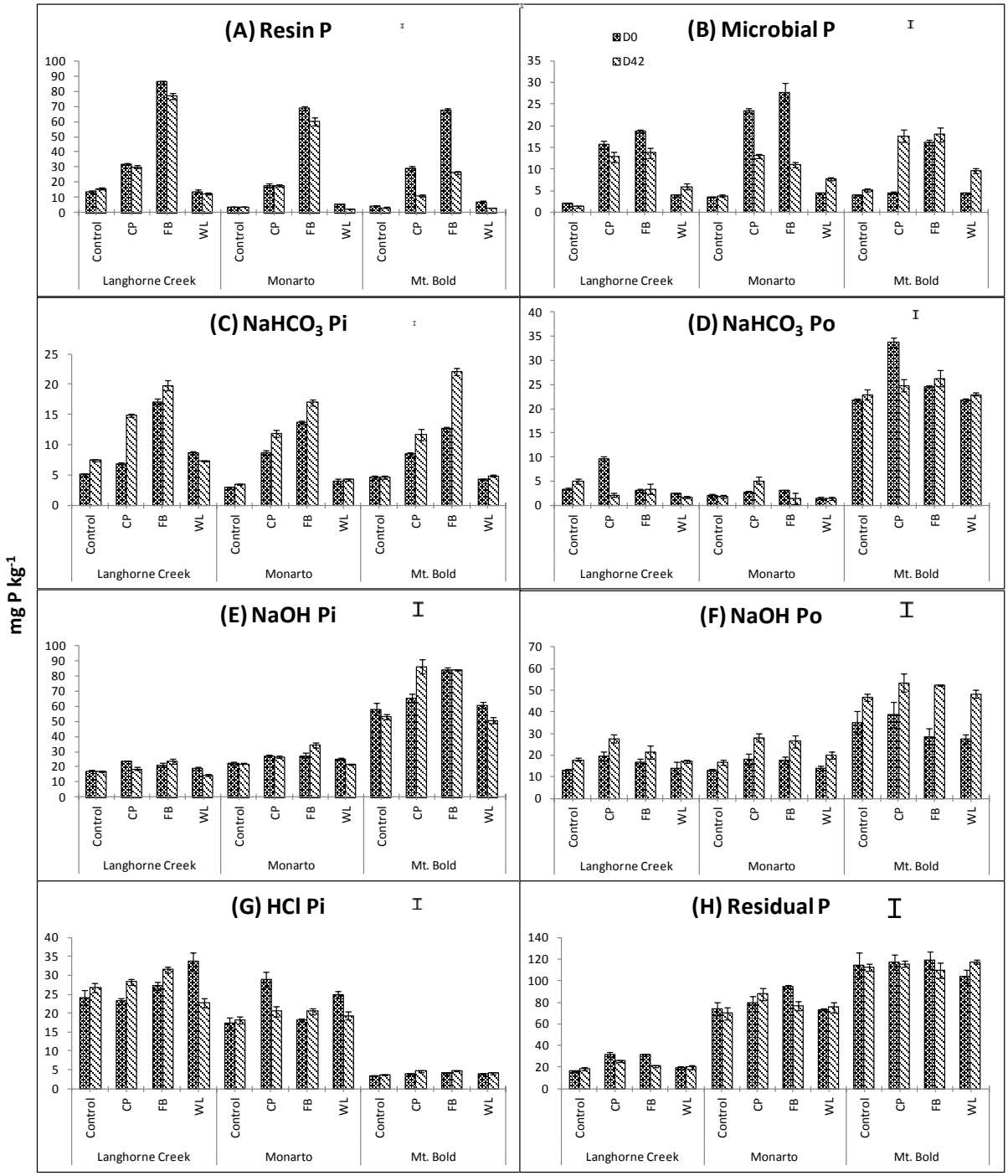


Figure 3 Concentrations of different P pools (mg $kg^{-1}$ soil ) in Langhorne Creek, Monarto and Mount Bold soils: (A) resin P, (B) microbial P, (C) $NaHCO_3$ Pi, (D) $NaHCO_3$ Po, (E) NaOH Pi, (F) NaOH Po, (G) HCl Pi, and (H) residual P on day 0 and after 42 days in unamended soil (control) and soil amended with chickpea (CP), faba bean (FB) and white lupin (WL) residues. Vertical lines indicate standard deviation. The vertical bar in the top right hand corner of each figure indicates LSD (soil x residue) at $P < 0.05$.

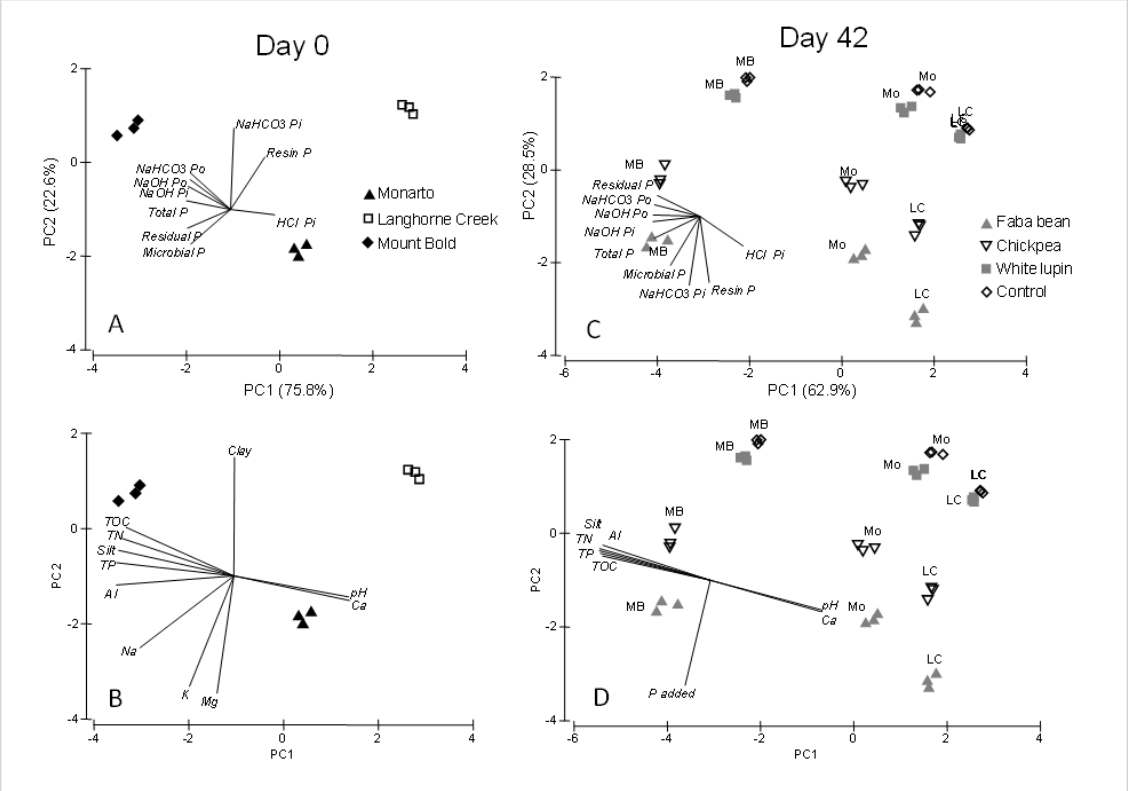


Figure 4 PCA plots of soils based on P pool concentrations for day 0 (unamended soil) and day 42 (amended and unamended soils), with vectors for P pools (A, C) and soil properties (B, D). Mo: Monarto, LC: Langhorne Creek, MB: Mount Bold (n=3).

# Chapter 5

# Changes in P pools over three months in two soils amended with legume residues

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Performed analysis on all samples, interpreted data, wrote manuscript and acted as corresponding author.

I hereby certify that the statement of contribution is accurate.

Signed Date 19 Dec 2012

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Supervised development of work, data interpretation and manuscript evaluation.

I hereby certify that the statement of contribution is accurate and I give permission for the inclusion of the paper in the thesis.

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**Abstract**

To assess the importance of soil properties and residue addition on soil P pool concentrations, we added residues from three different legumes to two soils with contrasting physical and chemical properties and measured the concentration of P pools over three months. Three legume residues differing in total P and N and water-soluble P concentrations were used: faba bean (*Vicia faba*) (high), chickpea (*Cicer arietinum*) (medium) and white lupin (*Lupinus albus*) (low), were added to the soils at a rate of 20 g residue $kg^{-1}$ soil. The size of various P pools was assessed by sequential P fractionation on days 0, 14, 28, 56, 70 and 98.The alkaline Monarto soil which had a low organic matter content, was characterised by high concentrations of HCl-Pi whereas the concentrations of $NaHCO_3$-Po, NaOH-Pi and Po and residual P were high in the acidic Mt. Bold soil with high organic matter content. Addition of faba bean and chickpea residues increased the concentrations of resin P, microbial P, $NaHCO_3$-Pi temporarily whereas amendment with white lupin residues had little effect on P pool concentrations. The decrease in $NaHCO_3$-Po and NaOH-Po towards the end of the experiment coincided with an increase in NaOH-Pi in

Mt. Bold soil and of HCl-Pi in Monarto soil. These temporal changes were more pronounced in soils amended with faba bean and chickpea residues than in the unamended soil or after addition of white lupin residues. The principal component analysis (PCA) plot showed that the P pool concentrations on days 0 and 98 were quite similar and differed from those on days 28, 56 and 70 suggesting clear temporal patterns. The results of this study show that the concentration of various P pools is strongly affected by soil properties such as pH and organic matter content which are further modulated by the properties of the residues. However, residue addition had a limited long-term effect on P pool concentrations.

## Introduction

Appropriate management of crop residues may be an alternative to inorganic fertilisers to increase agricultural sustainability and reduce reliance on mineral fertilisers (Araújo et al. 2012). Incorporation of crop residues may allow maintaining adequate concentrations of available P in soil which is important from both an agronomic and an environmental point of view (Griffin et al. 2003; Sharpley et al. 1989). When crop residues are added, changes in P availability depend on chemical composition of crop residues and on soil properties. Upon decomposition crop residue may increase P availability by (i) $H_2CO_3$ from $CO_2$ released during decomposition which can dissolve Ca phosphates (Tisdale et al. 1985) and (ii) organic acids released during decomposition which can exchange sorbed P and chelate Fe and Al thereby reducing P fixation (Easterwood and Sartain 1990; Sharpley et al. 1989). On the other hand, plant P availability may also decrease following crop residue addition due to microbial immobilisation (McLaughlin and Alston 1986; White and Ayoub 1983). The chemical composition of crop residues varies and the concentrations of C, N, lignin, cellulose, polyphenol and their ratios to P are regarded as predictors of decomposition rate

and P release. Net immobilisation is likely to occur if the total P concentration in the residues is below 2 to 3 g $kg^{-1}$ and a C/P ratio greater than 300:1, while net mineralisation is likely if at C/P < 200:1(Brady and Weil 2002; Singh et al. 1988). Net P release is more likely in the later phases of residue decomposition due to lower microbial demand, but then also more of the P may be fixed because of the lower concentration of organic acid anions.

In soil, P moves between different organic and inorganic pools varying in availability which can be measured by sequential P fractionation. For a better understanding of soil P transformations, the size of these pools over time needs to be determined (Tate et al. 1991). The solubility and fixation of P in soils is highly dependent on the physicochemical properties of soil such as pH (Barrow 1984), organic carbon content (Daly et al. 2001), texture (Toor et al. 1997; Yuan and Lucas 1982), calcium carbonate content (Bertrand et al. 2003), and the concentrations of extractable Fe and Al oxides (Freese et al. 1992). In acidic soils, the predominant forms of inorganic P are Fe or Al phosphates whereas in neutral to alkaline soils P occurs as Ca and Mg phosphates (Hinsinger 2001). In acid soils, P can be adsorbed on the surfaces of clay minerals or occluded in nanopores of Fe or Al oxides (Arai and Sparks 2007). In neutral to alkaline soils it can be precipitated as calcium phosphates with different solubility ranging from dicalcium phosphate to hydroxyapatite (Arai and Sparks 2007). Generally soils with high concentrations of clay, amorphous oxides, allophanes and kaolinite have high P fixing capacity (Brady and Weil 2002; O'Halloran et al. 1985).

In a previous study (Alamgir et al. 2012) we demonstrated that changes in soil P pools induced by plant residue addition are affected by residue P concentration and by plant part (roots or shoots) in a loamy sand soil over 42 days. To better understand the effect of residue addition on soil P pools, longer term studies in soils differing in properties such as

organic matter content, pH and texture are needed. The aim of this study was to examine the changes in P pools over three months after addition of legume residues in two soils with contrasting physical and chemical properties.

## Materials and Methods

### Experimental design

Two soils from South Australia were used in this study. The Rhodoxeralf (USDA 1999) soil was collected from a natural bush land at Monarto (35° 05´ S, 139° 04´E) and the Typic haploxeralf soil from a grassland at Mt. Bold (35° 04´ S, 138° 42´ E). Five sub samples were collected from 0 - 10 cm depth in an area of about 20 $m^2$ and mixed together to form a single composite sample. After collection, the soils were air-dried at room temperature, passed through a 2 mm sieve and analysed for physical and chemical properties (Table 1). Before the experiment, the soils were pre-incubated for 10 days at their optimum water content for microbial activity. The optimum water content was determined in a preliminary experiment (unpublished data) by measuring cumulative respiration for 10 days at different water contents (40-90%). Cumulative respiration was maximal at 70% of water holding capacity for Monarto and 50% for Mt. Bold soil. This pre-incubation was used to activate the microbes and stabilise their activity before the onset of the experiment.

Based on our previous study (Alamgir et al. 2012), we selected three legume shoot residues differing in total P concentration: young faba bean (FB) (*Vicia faba*) (high P), mature chickpea (CP) (*Cicer arietinum*) (medium P) and mature white lupin (WL) (*Lupinus albus*) (low P) which were air-dried, oven-dried at 60°C, finely ground and sieved to particle sizes between 0.25 to 2 mm. The residues also differed in total N, water soluble C and P

concentration which were highest in faba bean residues and lowest in white lupin residues (Table 2). The residues were added at a rate of 20 g residue $kg^{-1}$ soil and mixed thoroughly into the soils. The calculated amounts of total C, N and P and water soluble P added with the residues are given Table 3. Twenty g soil mixed with residues was placed in cores of 3.7 cm diameter fitted with nylon mesh base and the bulk density adjusted to 1.63 and 1.43 g $cm^{-3}$ for Monarto and Mt. Bold soil. The soils were incubated in the dark at 22-25°C for 98 days and sampled on days 0, 14, 28, 56, 70 and 98 with separate samples for each sampling date. During incubation, soil moisture was maintained by weight with autoclaved reverse osmosis water. Thus the experiment consisted of 3 residue types, 2 soils and 6 sampling dates with 3 replicates each.

**Analyses**

Soil pH was determined in a soil:water ratio 1:5. Particle size analysis was carried out by the hydrometer method (Ashworth et al. 2001). The bulk density of the soils was calculated using bulk density calculator based on the U.S. Texture Triangle (http://www.pedosphere.com/resources/bulkdensity/worktable_us.cfm). Total C and N of the soils and the residues were determined by dry combustion using a Leco CNS-2000, carbonates were removed from the soils by acid prior to the analysis. Total Fe, Al, Ca and Mg concentrations of the soils were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES) after digestion with nitric acid-perchloric acid (Olsen and Sommers 1982). Total P of the residues was determined after digestion with nitric acid-perchloric acid (Olsen and Sommers 1982); P in the extract was measured colormetrically by the phosphovanado-molybdate method (Hanson 1950; Kitson and Mellon 1944). The concentration of various P pools was determined by sequential P fractionation (Hedley et al. 1982; Huang and Zhang 2009; Tiessen and Moir 1993). The

sequential extraction was carried out by shaking 1 g of soil (1:30 soil:solution) for 16 h with 0.5 M $NaHCO_3$, 0.1 M NaOH, 1 M HCl, and again 0.1 M NaOH. Residual P was determined by digesting the soil remaining after the last step of the fractionation in $HNO_3$-$HClO_4$ (6:1). Five mL of the extract from the $NaHCO_3$ and NaOH fractions was acidified to pH 1.5 to precipitate organic matter, centrifuged and the supernatant was analysed for inorganic P. Total P in the $NaHCO_3$ and NaOH fractions was analysed separately by digesting 5 mL of the extract with 1 ml 5% potassium persulfate at 90°C for 16h which was then analysed for total P (Huang and Zhang 2009). Organic P was calculated as the difference between total P (after digestion) and inorganic P. For determination of microbial P, another soil subsample was extracted with anion exchange resin with or without hexanol. Microbial P was calculated as the difference between the P concentration with and without hexanol (Kouno et al. 1995). The P concentration in the fractions was determined colorimetrically (Murphy and Riley 1962). Total P was calculated as the sum of the sequentially extracted P pools.

**Statistical analyses**

Three way analysis of variance (ANOVA ) with the main factors residue type, soil type and sampling time (day 14-98) was carried out using GenStat for Windows 14th Edition (VSN International, Hempstead, UK, 2011). Means were compared by Tukey test at 5% significance level. Principal component analysis was carried out using the P pools at the different sampling times as variables (Primer-E Ltd, Plymouth Marine Laboratory, Plymouth, UK).

## Results

### Soil properties

The soils used in this study differed in their physical and chemical properties (Table 1). The acidic Mt. Bold soil had higher clay and silt content and higher total C, N P and Al concentrations compared to the alkaline Monarto soil. Monarto soil had higher sand content and higher concentrations of total Ca and Mg than the Mt. Bold soil.

### Soil P pools

In the unamended soils (control), the percentage as $NaHCO_3$-Po, NaOH-Pi and Po and residual P on day 0 were higher in Mt. Bold soil than in Monarto soil whereas the percentage as HCl-P was lower (Table 4). Residual P was the largest pool in both soils (41.4-42.3% of total P). The percentage of organic P was higher in Mt. Bold soil (22.3% of total P) than in Monarto soil (17.7 % of total P). Residue addition increased the percentage as resin and microbial P with greater changes induced by faba bean and chickpea residue addition than with white lupin residues (Table 4). The percentage of total P in the different P pools did not change from day 0 to day 98 in unamended or amended soils (data not shown).

In both soils the concentration of resin P was significantly higher when amended with high P residue (faba bean), than with medium (chickpea) and low (white lupin) residues (Fig. 1A). During the first two weeks the resin P concentration decreased in both soils amended with residues and the decline was more prominent in Mt. Bold soil. In Mt. Bold soil the decrease in resin P was accompanied by a corresponding increase in microbial P and $NaHCO_3$-Po whereas this was not the case for Monarto soil. After 14 days, the concentration of resin P remained stable in both soils amended with white lupin residues.

When amended with chickpea and faba bean residues, the resin P concentration increased from day 14 to day 98.

Residue addition increased the microbial P concentration, particularly chickpea and faba bean residues with the highest concentrations on day 0 in Monarto soil and on day 98 in Mt. Bold soil (Fig. 1B)

The concentration of $NaHCO_3$-Pi was highest on day 56 in both soils amended with faba bean and chickpea residues whereas the concentration of this pool was little affected by addition of white lupin residues (Fig. 1C).

The $NaHCO_3$-Po concentration was higher in Mt. Bold than in Monarto soil (Fig. 1D); it fluctuated in Mt. Bold soil with the highest concentrations on day 14 and the lowest on day 98. The $NaHCO_3$-Po concentration changed little over time in Monarto soil except for the lowest concentration on day 98 in the residue amended soil.

The concentration of both NaOH-P pools was higher in Mt. Bold than in Monarto soil (Fig. 1E and 1F). In Monarto soil, addition of faba bean residues increased the concentration of NaOH-Pi compared to the unamended soil whereas the other residues had no effect (Fig. 1E). In Mt. Bold soil, the NaOH-Pi concentration was higher on days 0 and 98 than on the other sampling days except for the soil amended with faba bean residues where the NaOH-Pi concentration was also high on day 14.

Addition of chickpea and faba bean residues increased the concentration of NaOH-Po compared to the unamended control in both soils whereas amendment with white lupin residues increased the concentration of this pool only in Mt. Bold soil and only on days 0 and 28 (Fig. 1F). There was little change in the concentration of NaOH-Po over time in the Monarto soil except for a peak on day 56 when amended with faba bean residues. In Mt.

Bold soil, the NaOH-Po concentration increased from day 0 to day 28 or 56 and then decreased to day 98. The decrease in NaOH Po concentration from day 70 to 98 was accompanied by an increase in NaOH Pi concentration in this period.

The HCl-Pi concentration was higher in Monarto than in Mt. Bold soil (Fig. 1G). Residue addition had no effect on the concentration of HCl-Pi in either soil. The size of this pool did not change over time in Mt. Bold soil. In Monarto soil, the HCl-Pi concentration decreased from day 0 to day 14 and increased again from day 70 to day 98.

The residual P concentration was higher in Mt. Bold than in Monarto soil (Fig. 1H) and in both soils the concentration of this pool increased from day 0 to day 28 or 56 and then decreased. Residue addition increased the residual P concentration only temporarily. In Monarto soil, the residual P concentration was higher than in the unamended control on day 28 when amended with chickpea residues and on day 56 in soil amended with white lupin residues. In Mt. Bold soil, addition of faba bean residues increased the residual P concentration on day 28.

The PCA plot based on the soil P pools showed a clear separation between the control soils and those amended with white lupin residues on the left and the soils amended with faba bean and chickpea on the right side in both soils with higher concentrations of all P pools in the latter (Fig. 2). There also is a change in the P pools over time with the samples taken on days 0 and 98 separate from those from day 28, 56 and 70. This temporal change was generally more pronounced in the soils amended with faba bean and chickpea residues than in the unamended control and the soil with white lupin residues. The main P pools on days 0 and 98 were NaOH-Pi and microbial biomass in both soils but there also were some differences among the soils. On days 0 and 98, high concentration of HCl Pi were found in Monarto soil whereas the resin P concentration was high in Mt. Bold soil.

**Discussion**

The results of the study indicate that residue amendment changes the size of the soil P pools and that there is also a distinct temporal pattern in the P pool concentration over the 98 day incubation period which differed between residue and soil type. The higher initial concentrations of $NaHCO_3$-Po, NaOH-Pi and Po (Fe and Al bound P) and total P in the acidic Mt. Bold soil can be explained by its higher organic C and Al concentrations and low pH. The higher concentration of HCl-Pi (Ca bound P) in Monarto soil is probably due to the higher Ca concentration in this soil and its higher pH. Several other studies also reported that size and forms of P in soil depends on the soil properties such as pH, organic matter content, soil texture and even clay mineralogy (Hinsinger 2001; Huffman et al. 1996; Tiessen et al. 1984). However, we show for the first time the temporal changes in P pools and how this modulated by residue addition and soil properties.

The decrease in $NaHCO_3$-Pi and $NaHCO_3$-Po concentration and the increase in NaOH-Pi and HCl-Pi concentration over time suggests the transformation of labile inorganic and organic P into non-labile inorganic P by P sorption or fixation. Mineralisation of $NaHCO_3$-Po can contribute to an increase in resin P (Guo et al. 2000; Zheng et al. 2002) but this was not observed in the present study

The NaOH pool represents organic or inorganic P associated with iron and aluminium (Hedley et al. 1982; Tiessen and Moir 1993). The relative stability of the NaOH-Pi pool in Monarto soil does not mean that little flux occurred in and out of this pool, it could also be that influx was equal to efflux. The concentration of NaOH-Pi in Mt. Bold was high at the start and at the end of the experiment, whereas the concentration was low on days 28 to 70. The increase in NaOH-Pi (representing P associated with Fe and Al) towards the end of the experiment in Mt. Bold soil was accompanied by a decrease in NaOH-Po and $NaHCO_3$-Po suggesting mineralisation of organic P and transformation into this more

stable P pool. In Monarto soil, some the P released from the organic P in the $NaHCO_3$ and NaOH fractions may also have contributed to the increase in HCl-Pi (P associated with Ca) to day 98. Sanyal and De Datta (1991) also reported mobilised P can be adsorbed or precipitated.

Despite the differences in temporal changes in P pools between Monarto and Mt. Bold, the PCA showed that in both soils the P pool concentrations on day 0 and 98 were quite similar and differed from the concentrations on days 28, 56 and 70 suggesting clear temporal patterns and a limited effect of residue addition on these patterns in the long term (Fig. 2). Nevertheless, the temporal changes were more pronounced in the soils amended with faba bean and chickpea residues indicating that addition of residues with medium or high P concentration affects the dynamics among the soil P pools. The decrease in organic P pools over time was followed by an increase in NaOH-Pi but also of microbial biomass P suggesting that some of the P mineralised was taken up by the soil microbes in the later stages of the experiment. Days 0 and 98 were also characterised by high concentrations of HCl-Pi in Monarto soil and resin P in Mt. Bold soil suggesting that P is maintained in plant available form in Mt. Bold soil whereas it is converted into the less labile HCl Pi in Monarto soil. However, HCl-Pi can be mobilised by plant roots and contribute to plant P uptake (Guo et al. 2000; Mat Hassan et al. 2012).

**Conclusions**

This study showed that although the temporal pattern in the P pool concentration differed between residue and soil type, the long term effect of residue addition on P pool concentrations was limited. Studies with $^{32}P$ labelled residues and combining $^{31}P$ Nuclear magnetic resonance spectroscopy or X-ray absorption spectroscopy with chemical fractionation are needed to precisely quantify the fluxes of P in soil.

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Table 1. Properties of Monarto and Mt. Bold soils (n=3).

| | Monarto | Mt. Bold |
|---|---|---|
| Sand (g $kg^{-1}$) | 820 | 530 |
| Silt (g $kg^{-1}$) | 100 | 280 |
| Clay (g $kg^{-1}$) | 80 | 190 |
| Texture | loamy sand | sandy loam |
| USDA soil classification | Rhodoxeralf | Typic haploxeralf |
| Bulk density (g $cm^{-3}$) | 1.63 | 1.43 |
| Water holding capacity (%) | 15.8 | 34.5 |
| pH 1:5 ($H_2O$) | 7.5 | 5.1 |
| Total N (g $kg^{-1}$) | 0.9 | 4.2 |
| Total C (g $kg^{-1}$) | 7.3 | 44.1 |
| Total P (mg $kg^{-1}$) | 139 | 246 |
| Total Fe (g $kg^{-1}$) | 17.0 | 16.4 |
| Total Al (g $kg^{-1}$) | 16.9 | 28.5 |
| Total Ca (g $kg^{-1}$) | 1.5 | 0.6 |
| Total Mg (g $kg^{-1}$) | 3.4 | 1.4 |

Table 2. Total P, C, N, water soluble P concentrations and C/N and C/P ratio of white lupin, chick pea and faba bean residues (n=3).

| Residues | Total P g $kg^{-1}$ | Water soluble P g $kg^{-1}$ | Total C g $kg^{-1}$ | Water soluble C g $kg^{-1}$ | Total N g $kg^{-1}$ | C/N | C/P |
|---|---|---|---|---|---|---|---|
| White lupin | 0.6 | 0.2 | 384 | 7 | 9.7 | 40 | 640 |
| Chickpea | 1.8 | 0.6 | 418 | 60 | 21.1 | 20 | 232 |
| Faba bean | 6.6 | 5.9 | 417 | 67 | 34.5 | 12 | 63 |

Table 3. Amounts of total C, N and P and water-soluble P added with the white lupin, chickpea and faba bean residues at a rate of 20 g $kg^{-1}$.

| Residues | Total C | Total N | Total P | Water soluble P | Water soluble C |
|---|---|---|---|---|---|
| | | | mg $kg^{-1}$ | | |
| White lupin | 7680 | 194 | 12 | 4 | 140 |
| Chickpea | 8360 | 422 | 36 | 12 | 1200 |
| Faba bean | 8340 | 690 | 132 | 118 | 1340 |

Table 4. Percentage of total P in different P pools in Monarto and Mt. Bold soil on day 0 in unamended soil (control) and soil amended with chickpea (CP), faba bean (FB) and white lupin (WL) residues.

| Soil | Treatment | Resin-P | Microbial-P | $NaHCO_3$-Pi | $NaHCO_3$-Po | NaOH-Pi | NaOH-Po | HCl-Pi | Residual-P |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | % of total P | | | | |
| Monarto | Control | 4.5 | 1.6 | 3.0 | 1.1 | 14.2 | 15.1 | 18.3 | 42.3 |
| | CP | 8.1 | 11.9 | 5.1 | 1.6 | 12.5 | 14.0 | 12.2 | 34.6 |
| | FB | 25.7 | 9.0 | 6.2 | 1.6 | 10.7 | 8.8 | 11.1 | 26.9 |
| | WL | 4.6 | 2.5 | 3.4 | 1.4 | 14.7 | 14.0 | 16.6 | 42.8 |
| Mt. Bold | Control | 3.4 | 1.5 | 1.6 | 7.7 | 30.0 | 13.1 | 1.4 | 41.4 |
| | CP | 10.0 | 3.6 | 2.6 | 7.1 | 26.7 | 16.4 | 1.3 | 32.4 |
| | FB | 20.3 | 2.5 | 3.5 | 5.9 | 22.7 | 14.4 | 1.0 | 29.6 |
| | WL | 3.9 | 1.8 | 1.8 | 7.4 | 25.1 | 20.4 | 1.5 | 38.2 |

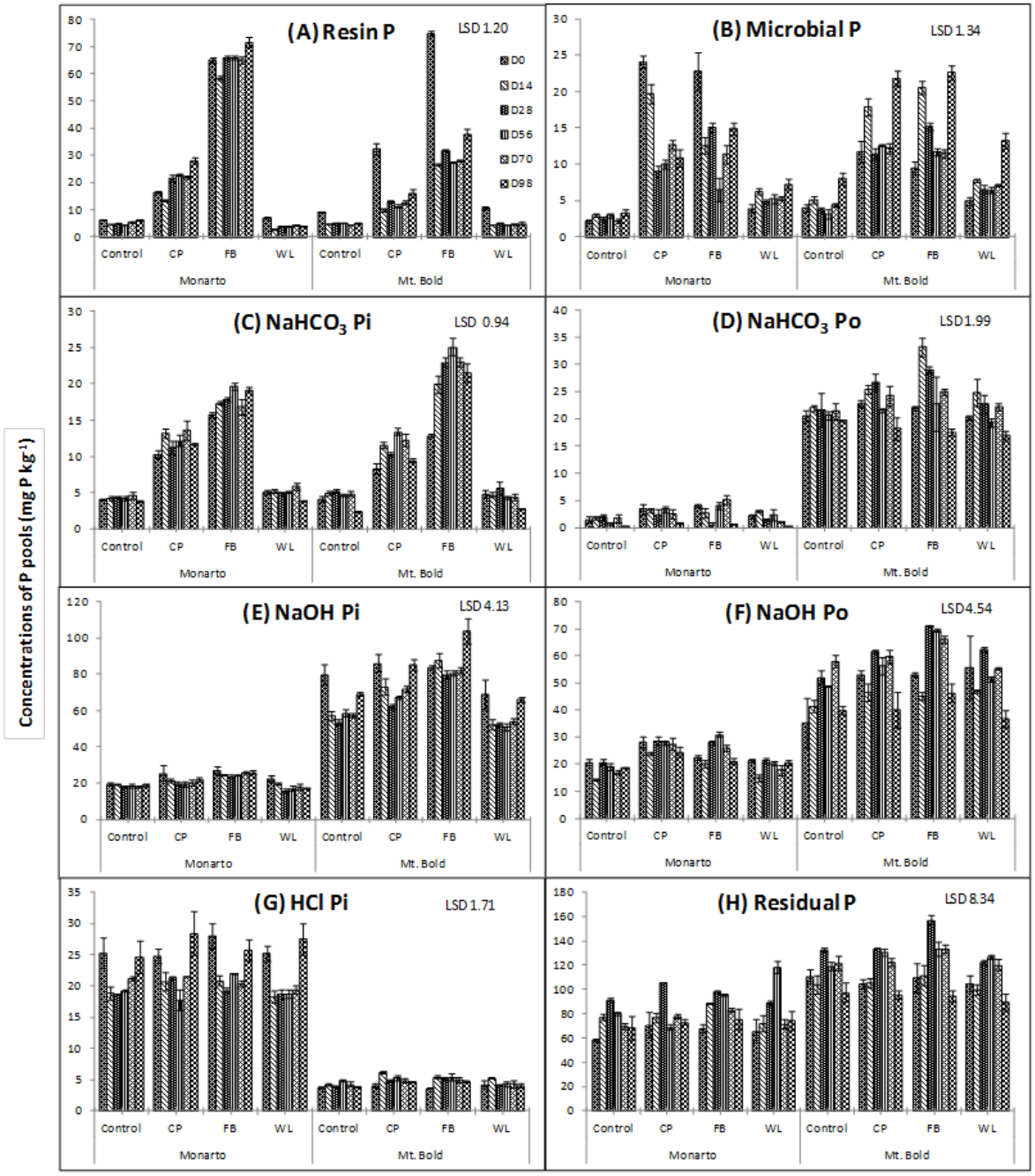


Fig. 1. Concentrations of different P pools in unamended soil (control) and soil amended chickpea (CP), faba bean (FB) and white lupin (WL) residues on days 0, 14, 28, 56, 70 and 98 (n=3). (A) resin P, (B) microbial P, (C) $NaHCO_3$ Pi, (D) $NaHCO_3$ Po, (E) NaOH Pi, (F) NaOH Po, (G) HCl Pi and (H) residual P (n=3), error bar represents standard deviation.

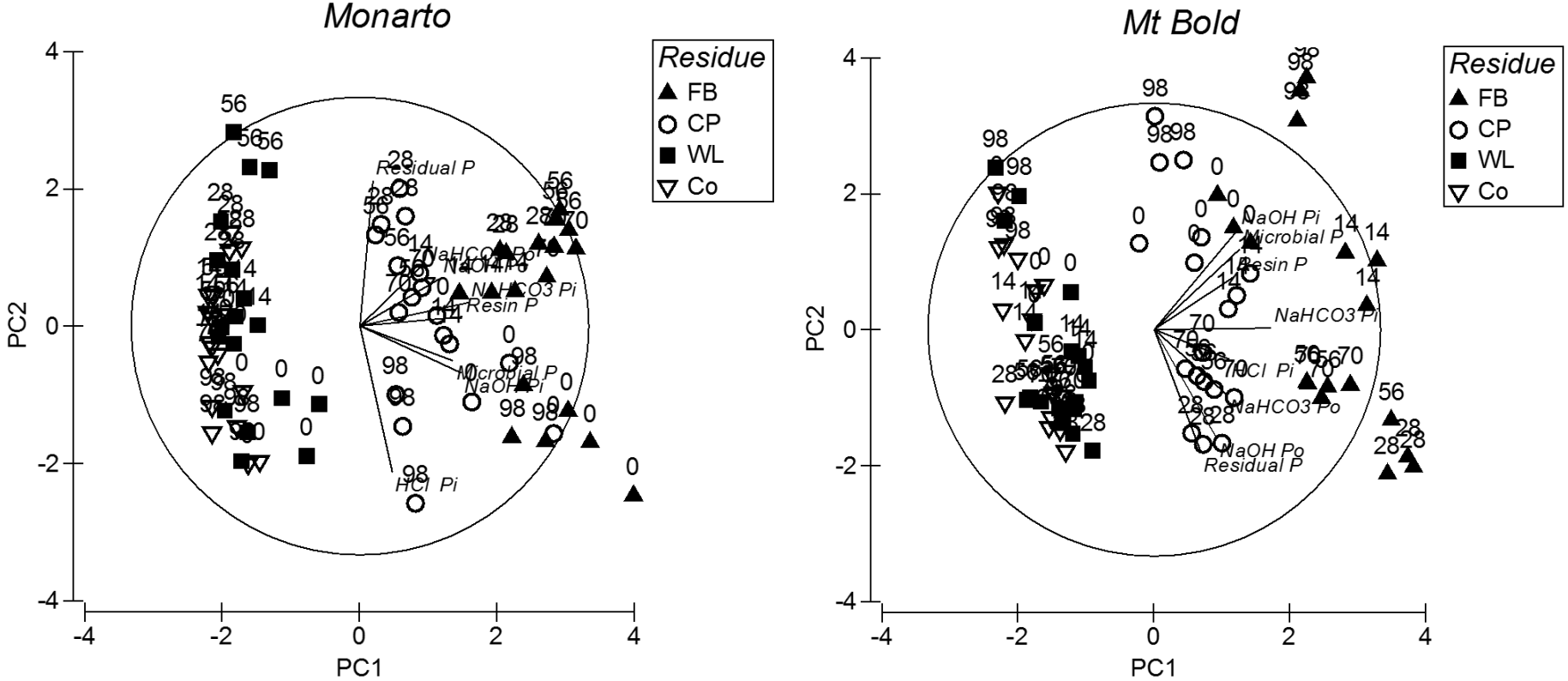


Fig. 2. Principal component analysis based on P pool concentrations on days 0, 14, 28, 56 and 98 in Monarto and Mt. Bold soil amended with faba bean, chickpea and white lupin residues or the unamended control. Values next to the symbols indicate sampling time, vectors the concentration of the P pools.

# Chapter 6

# Conclusions and Future Research

Phosphorus (P) deficiency is one of the major yield-limiting factors worldwide. The majority of the world's agricultural crops today rely on P fertilisers derived from rock phosphate but global rock phosphate reserves may be depleted within this century (Cordell et al. 2009). A better understanding of the P dynamics in soil is necessary to maintain sustainable agriculture, reduce the environmental impacts associated with P fertilisers, increase P availability to crops, and improve farmer's income. In soils, P is present in different pools, including organic and inorganic P and flux among these pools involves complex mineralogical, chemical and biological processes (Zheng et al. 2002). In both acid and alkaline soils, P availability may diminish over time as compounds of decreasing solubility are formed (Brady and Weil 2002). The different forms of soil P and fluxes among them can be assessed by sequential P fractionation which divides the pools into labile P, moderately labile P and stable P (Reddy et al. 2005; Tiessen and Moir 1993).

Wheat is one of the world's most important food crops and legumes are often grown in rotation with wheat. A number of studies in Australia and Africa have shown that some legumes can increase the growth and P uptake of the following wheat which could be due to P mobilised during legume growth and/or during decomposition of legume residues (Carsky et al. 2001; Nuruzzaman et al. 2005a; Nuruzzaman et al. 2005b; Vanlauwe et al. 2000). However, the soil chemical and biological mechanisms behind this have not been studied in detail, particularly changes in various soil P pools and soil biological properties over time during legume residue decomposition and how this is related to plant P uptake. Therefore, the aims of the research described in this thesis were to determine legume

residue effects on the concentration of various soil P pools over time and growth and P uptake of wheat.

Upon addition to soil, residues with different P concentration and roots and shoots of residues may have different effect on P pool concentrations and how they change over time. Therefore, the study described in Chapter 2 was carried out over 56 days after adding residues from faba bean, white lupin or chickpea (shoots or roots) with varying P concentrations to a sandy loam soil with a low available P concentration. These legumes were selected because they are common grain legumes in Australia, and/or have been shown in previous experiments to increase growth of the following cereal (Mat Hassan et al. 2012; Nuruzzaman et al. 2005a; Nuruzzaman et al. 2005b). The results showed that during the first 14 days after residue addition residues with high-P concentration increased the concentrations of labile P fractions (resin and $NaHCO_3$-P) whereas low-P residues resulted immobilization of labile P. When medium-P residues were added the increase in labile P matched the decrease in other pools in this period. From days 28 to 56 there was net conversion of P into stable organic and inorganic P (NaOH-Po and HCl-Pi) with all residues. Differences in P pool concentration between soils amended with root and shoot residues occurred mainly in the first 28 days where the concentration of NaOH Po decreased with shoot residues whereas it decreased with root residues. From this study it can be concluded that short term (56 days) changes and transformations in soil P pools were dependant on residue P concentration and plant part.

Physical and chemical properties of soils e.g. pH, texture, organic carbon content strongly affect P solubility and fixation. Thus changes in P pools induced by legume residues may vary with soil properties. Therefore it is necessary to assess if the changes and

transformation in P pools observed in the alkaline soil (Chapter 2) are generally applicable or if they are soil type-specific. The experiment described in Chapter 4 was carried out in three soils that differed in physical and chemical properties. The concentrations of P pools were measured on day 0 and day 42. The three soils differed in P pool concentrations even after residue addition suggesting that P pool concentrations are mainly affected by soil properties and to a lesser extent by residue properties. Nevertheless, residue addition changed the concentrations of some P pools, namely the labile P pools where the concentrations were highest with the high P residues. Over time in all soils, residue addition induced an increase in the concentration of NaOH Po which can, to some extent, be explained by the decrease in resin and microbial P. Thus, although the soils used in this experiment differed in their organic C content, pH and relative size of P pools the increase in microbial activity and of the concentration of NaOH Po upon residue addition can be generalised across the three soils.

The experiments in Chapters 2 and 4 were carried out for relatively short period of time (56 and 42 days, respectively) The experiment described in Chapter 5 was over 98 days in two soils with different properties to assess if at some stage in the decomposition process, the size of the P pools stabilises. In the residue-amended soils, the P pool concentration on days 0 and 98 differed from those from day 28, 56 and 70 suggesting that residue addition induces transient changes in P pools which were more pronounced in the soils amended with faba bean and chickpea residues than in the soil with white lupin residues. At all sampling times, the concentration of various P pools was strongly affected by soil properties such as pH and organic matter content confirming the P pool concentrations are predominately affected by soil properties and only transiently modulated by residue addition.

The experiments in Chapters 2, 4, and 5 were carried out in absence of plants. However, to assess if residues could be used as alternative to inorganic fertilizers in agriculture, it is important to compare the effect of residues to that of inorganic P not only on P pools but also plant growth and P uptake. Conducting experiments with plants is also important because plants may change the flux among P pools and thus the residue effect on P pool concentrations by taking up P. The experiment described in Chapter 3 was conducted to compare the short term effects of application of different rates of inorganic P and residue P on soil P pools and wheat growth. In contrast to the other experiments which were conducted without plants (Chapters 2, 4, 5), residue addition had no effect on labile organic pool and moderately labile inorganic and organic pool in the study with plants (Chapter 3). This may be, at least in part, due to plant P uptake which may have reduced the movement of P among P pools. At low P addition rates (3 and 10 mg P $kg^{-1}$) wheat growth and P uptake were not increased indicating that not enough P was added to increase P availability. Compared to inorganic fertiliser, P added with residues led to a greater increase in shoot biomass and P uptake at high P addition rates (100 mg P $kg^{-1}$). Furthermore, wheat was able to deplete the less labile pools, (HCl and residual P) which suggests that these pools are quite dynamic and may therefore, through conversion into labile P pools, serve as P sources for plants.

**Suggestions for future studies**

The experiments described in this thesis answered a number of questions with respect to the effect of legume residue addition on changes in soil P pools and growth and P uptake in wheat. However, there are a number of research gaps arising from this study that could be addressed for future studies:

1. The chemical fractionation method used in this study is based on solubility in different extractants and does not provide detailed molecular and structural characterisation of P. A detailed speciation of P in soil and crop residues by $^{31}P$ nuclear magnetic resonance spectroscopy or X-ray absorption spectroscopy would be required to increase the understanding of soil P pools.
2. In all experiments, the relative contribution of P released from legume residues and native soil P to changes in pools and P uptake could not be quantified. To distinguish native soil P and added P, studies with $^{32}P$ labelled residues P would be necessary.

3. In the experiment with plants wheat was grown to flowering whereas in the field cereals are harvested at maturity. The legume residue addition may have different effects on growth and P uptake if wheat is grown to maturity because (i) a larger root system in the older plants may exploit the soil P pools more effectively, and (ii) P taken up by the roots earlier can be released upon root turnover and be taken up by the remaining roots. Therefore, experiments with legume residue addition where the cereal is grown to maturity should be carried out.

4. The experiments were conducted under laboratory and glasshouse conditions at constant soil moisture and temperature, but nutrient release patterns from crop residues and nutrient interactions may differ under different environmental conditions. In Mediterranean climates, soils are frequently exposed to drying and rewetting; fluctuations in soil moisture can affect decomposition rate of residues and P chemistry. In Australia the fallow period between harvest of legumes and sowing of cereals may be up to 6-7 months; during this time drying and rewetting

cycles may occur. Therefore it is necessary to assess the effect of one or several drying and rewetting events on P release from legume residues and P pools in the soil. Further field studies with different legume residues with or without combination of inorganic fertilisers are required to assess under which conditions legume residues could be an alternative to inorganic fertilisers or improve their efficiency.

5. In the experiments described in this thesis, the residues were finely ground and evenly mixed into the soil to maximise their effect and limit the variability among replicates. However in the field, residues particles may be larger and located in patches. This has important implications for P release and accessibility of the P released by the residues top plants. The mineralisation rate of residues may decrease with increasing particle size (Angers and Recous 1997), thus P release from large particles may be slower than from small particles. If the residues are located in patches, root growth towards these patches is critical for uptake of P released from the residues. And this ability to grow towards nutrient-rich patches may differ among crop species. Thus, experiments with plants in soil amended with different residue particle sizes and distribution should be carried out.

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