Incorporation of post–harvest or green manure crop residues influences soil P mobilization and P nutrition of wheat

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DECLARATION

My supervisors Zed Rengel and Bill Bowden contributed advice on my original ideas with the hypothesis and experiments presented in this thesis. The discussions and writing in this thesis are my own with guidance and critical reviews from my supervisor Zed Rengel. This thesis has been completed during the course of enrolment in a Ph. D. degree at the University of Western Australia and has not been previously accepted for a degree at this or any other institution.

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ABSTRACT

Crop residues are valuable sources of organic matter and reservoirs of plant nutrients. Efficient recycling of nutrients from crop residues depends on soil and crop residue management, contributing to sustainability in cropping systems. With the supply of P fertilizer raising questions about a potential future crisis in crop production, recycling P through crop residues is an important component of P nutrient management in agriculture. Crop residues could replace or at least minimize the inorganic P fertilizer requirement in crop production depending on the nature of soil, crop residues, cropping systems and management practices. However, understanding the soil P dynamics as influenced by a crop residue amendment would underpin strategies to improve P recycling by effective crop residue management. Hence, the major focus in this study was to (i) characterize the P pools in soil after incorporation of crop residues varying in P quality and quantity; and (ii) determine the effect of soil incorporation of post-harvest (dry) or green manure crop residues on wheat P nutrition.

Green manuring, a practice of incorporating crop vegetative biomass as fresh, could provide better P nutrition to the subsequently-grown crops because fresh residues contain more readily-available P than dry residues. Also, fresh crop residues are considered of better quality because drying the residues leads to loss of nutrients (e.g. dissolved P) and alters the P fractions in crop residues. A soil incubation study was conducted to (i) compare P release from green manure or dry crop residues (70 DAS) high in P content but varying in quality; and (ii) characterize the temporal variation in soil P pools with decomposing crop residues. Incubation of fresh crop residues resulted in an increase in pH, microbial respiration rate and P availability in soil. Phosphorus was more available from fresh than dry residues, especially early in the incubation period. Soil incorporation of crop residues as fresh (i.e. green manuring) improved biological cycling of soil P in comparison with dry residues. Hence, green manure crops could increase P availability to the subsequently-grown crops, thus enhancing productivity and soil fertility.
Faba bean is capable of mobilizing and acquiring significant amounts of P from native and applied soil P pools, even in P-deficient soils. A glasshouse experiment was conducted in two phases, with faba bean grown for crop residue and wheat grown as subsequent crop. The objectives of the experiment were to (i) determine wheat P uptake after incorporation of shoot residues of faba bean along with whole roots as practiced in the field; and (ii) determine the plant availability of P in soils with freshly applied P fertilizer and residual P fertilizer from a previous crop. Returning faba bean shoots along with roots improved P nutrition in subsequent wheat crop compared with incorporating roots alone. Also, incorporating faba bean residues enhanced mobilization of residual P fertilizer in soil in comparison with freshly applied P fertilizer alone; hence, the efficiency of applied P fertilizer improved with addition of organic matter to soil via faba bean residues, increasing plant-available P in soil.

Another glasshouse experiment was aimed at measuring the capacity of low-quality post-harvest crop residues varying in C:N to provide P nutrition to the subsequent wheat crop in comparison with inorganic P fertilizer at different P and N application rates. The results showed that plant P uptake was related to availability of P added via either inorganic P fertilizers or crop residues. In the absence of residual P fertilizer in unfertilized soil with low native P, incorporation of post-harvest crop residues led to immobilization of added P, reducing plant-available P in soil. Hence, P content of crop residues plays a major role in soil P availability, influencing plant growth especially in P-deficient soils. Also, the dynamics of P pools was affected by the C:N and quantity of added crop residues. Therefore, soil P cycling can be enhanced by integrated nutrient management practices that feature incorporation of post-harvest residues supplemented with inorganic P fertilizer because of increased soil P mobilization resulting in increased availability of added P.

In conclusion, soil P availability improved with addition of high-quality crop residues (green manure) with high P content compared to low-quality post-harvest crop residues. Soil incorporation of fresh crop residues (green manure) rapidly
released P and increased P availability in soil in comparison with post-harvest dry residues. Mobilization of soil and residual fertilizer P was higher with soil incorporation of shoot residues high in P, enhancing P nutrition in the subsequent crop. However, post-harvest residues with low P and high C:N resulted in soil P immobilization. Yet, combined application of low-P post-harvest crop residues along with inorganic P fertilizer at a low rate could compensate for P immobilization, improving P availability. The presented results suggest that green manuring could be an approach to enhance soil P recycling and sustain soil fertility. However, P cycling in soil also depends on the nature of the soil and is influenced by agronomic management practices. Hence, field studies focussing on soil P cycling as influenced by green manuring under integrated nutrient management practices could give a relevant understanding of soil P dynamics to minimize fertilizer usage and sustain P nutrition for the future food production in agriculture.
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CHAPTER 1

General Introduction

Production of food and fibre in agriculture relies on plant nutrition. Phosphorus, a major essential nutrient along with N and K, affects crop growth and productivity. Crop P nutrition is achieved predominantly with inorganic P fertilizers derived from natural rock phosphate reserves with no other substitutes available (Cordell, 2009).

The Green Revolution that started in 1960s increased the use of inorganic fertilizers as a way of boosting crop productivity. However, a decade ago, a high input cost of P fertilizers resulted in limited usage of P fertilizers in developing countries (World Bank, 2004). More recently, the demand for P in crop production has risen more in developing than developed countries (Cordell, 2009; Heffer and Prud’homme, 2013) due to substantial growth in population and an increasing demand for food, which consequently increases the P requirement in crop production.

Increasing the demand and cost of P fertilizers imposes a serious threat to sustaining future food production with depletion of the natural rock phosphate reserves in the world. However, Cordell (2009) stated that applied P fertilizers are never lost from a world eco-system, but are converted to different forms in soil or water or living organisms (plants or animals), making it more or less accessible to plants. Therefore, P cycling plays an important role in linking P availability from various sources in a farming system with the crop P demand.

Agricultural soils of Western Australia are not necessarily low in total P because due to long history of fertilization they now contain relatively large quantities of immobilized P that most crops cannot access (e.g. wheat, Bolland et al., 1999). It has been estimated that immobilized P in Australian agricultural soils is worth AUD$10 billion (Stevens, 1997). Hence, there is a need to develop cropping systems that would increase efficiency of Australian farming and make better use of native and residual soil P, thereby reducing P fertilizer requirement for optimum crop productivity.
Applied P fertilizers become inaccessible with time, placing importance on mobilizing P from inaccessible soil P pools and enhancing P cycling. Growing crops with high P-mobilizing capacity (e.g. legumes) in rotation with less P-efficient crops (e.g. cereals) (Nuruzamman et al., 2005a, b; Rose et al., 2010 b) followed by incorporation of legume residues (Nziguheba et al., 2000; Horst et al., 2001; Kamh et al., 2002) is one of the management practices in the biological farming systems where returning crop residues to soil enhances P availability to the succeeding crops. Effective crop residue management not only improves fertility of Australian soils, but also enhances economic returns to farmers by reducing the input cost of inorganic fertilizers.

Sustainability of most agricultural systems depends on the nutrient recycling in soil. Recently, P cycling from crop residues has been a major focus in agricultural research (Dalal, 1979; McLaughlin et al., 1988a, b, c; Umrit and Friesen, 1994, Rose et al., 2008, 2010a; Alamgir, 2012; Hassan, 2012; Noack, 2014b, c). However, with existing information on P dynamics in soil amended with crop residues being relatively poor and inconsistent, there is a great need to understand P cycling as influenced by inclusion of crop residues, given that P dynamics in soil is governed by a range of soil, crop and crop residue factors.

The studies presented in this thesis were aimed at characterizing soil P dynamics with addition of crop residues differing in quality (green manure or post-harvest) in the presence or absence of subsequent crops and the residue influence on subsequent crop P uptake. An incubation experiment (Chapter 3) was conducted to characterize the soil P dynamics with incorporation of crop residues as fresh or dry, with a hypothesis that P availability will be greater with application of crop residues as fresh (green manure) than dry. The incubation experiment also characterized changes in soil pH and soil P pools with addition of fresh or dry crop residues. The glasshouse experiments (Chapters 4 and 5) included plant P as another P pool, with wheat grown on soils amended with either inorganic P fertilizers or crop residues (Chapter 4) varying in P content and quality (Chapter 5).
Chapter 4 was aimed at determining P availability to wheat grown on soils fertilized with P either just before the wheat phase or before preceding faba bean crop. The treatments with or without crop residues (faba bean) of high P quality were tested. The study presented in Chapter 5 was focused on determining P availability to wheat grown on soils amended with post-harvest crop residues of low P quality differing in C:N ratio combined with N fertilization at low and optimal rates. Also, distribution of P across the various soil P pools was measured. General discussion and conclusions highlight the major results from the studies presented in the thesis.
Phosphorus is a major essential element for all life forms. Naturally, P occurs in rock phosphate, enters the food chain as fertilizer and is returned to soil as plant and animal residues. Even though addition of phosphorus to soil through fertilizers has contributed to a substantial increase in crop production, excess fertilization can have detrimental effects on soils, water bodies and plants. Minimizing fertilizer use not only protects the environment from water pollution caused by leaching of excess phosphorus in soil, but also conserves the global natural resources from being depleted at a fast pace that would impede crop production in the future. This chapter provides a review of literature with a major emphasis on recycling P in soil through crop residues and minimizing fertilizer usage in crop production.

2.1 Soil phosphorus

Soil P varies considerably depending on the nature of parent material, degree of weathering, and various anthropogenic factors, such as land use and management practices. On average, the total P concentration in soil varies widely from 100 - 3000 mg kg$^{-1}$ (Stevenson and Cole, 1999), with low P content found in sandstones (around 100 mg P kg$^{-1}$) and high P content in limestones (around 2000 mg kg$^{-1}$). Soil P exists as inorganic and organic forms.

2.1.1 Inorganic phosphorus in soil

Inorganic forms predominate, except in Histosols, where almost all P occurs in organic forms. More than 90% of soil P occurs in insoluble/fixed forms as primary phosphate minerals, humus P, insoluble Ca, Fe and Al phosphates, and phosphates fixed by colloidal oxides and silicate materials. Only a small fraction of soil P is available to plants, mainly as water-soluble orthophosphate and from mineralization of less labile organic (microbial P) and inorganic (adsorbed P) forms.
2.1.2 Organic phosphorus in soil

Organic P in soil varies from 4% (some podzols) to 90% (alpine soils) of total P. About 1-10% of total soil P is immobilized in soil microbes as organic P (Richardson, 1994), and microbial P constitutes 3-24% of organic P in soil (Brookes et al., 1984). Organic P compounds are added to soil in the form of plant, animal and microbial tissues.

In most agricultural soils, organic P constitutes 30-80% of the total P (McCall et al., 1956; Dalal, 1977; Schachtman et al., 1998; Brady and Weil, 2002) in soil. Organic P occurs in soil as labile polyphosphates, orthophosphate diesters and monoesters (Turner et al., 2002; Condron et al., 2005) and more resistant phytates (Turner et al., 2002; Smernik and Dougherty, 2007). Organic P plays a critical role in soil P cycling, with microbial P fluxes a key intermediary between organic and inorganic P pools (Dalal, 1977; Stewart and Tiessen, 1987).

Previous studies by Dalal (1977), Blair and Bolland (1978), Tate (1985) and Stevenson and Cole (1999) have shown the importance of organic P mineralization in determining availability of P. Also, the amount of P contributed via organic P mineralization is similar to that of applied P fertilizer (Nziguheba et al., 1998). Given that organic P mineralization is considered an important source in increasing P availability in soil, it is necessary to quantify the transformations occurring in soil with addition of crop residues and resulting in enhanced mobilization of labile sources of P in soil.

Organic P stabilized in soils can re-enter the P transformation cycle during biological mineralization of soil organic matter (McGill and Christie, 1983). Transformation of organic phosphorus is correlated with transformation of organic matter (Pierre and Parker, 1927) and associated with decomposition of crop residues (Gressel and McColl, 1997). Organic P accumulates in rotations with high C availability because of plant residues being returned to soil (Bünemann et al., 2006, Iqbal, 2009).
2.2 Soil phosphorus pools

The inorganic (Pi) and organic (Po) P fractions sequentially extracted from soil by the Tiessen and Moir (1993) procedure were grouped into different P pools based on their lability (Hedley et al., 1982, Ivarsson, 1990, Tiessen and Moir, 1993, Schmidt et al., 1996).

2.2.1 Labile P pool

The labile P pool includes water-Pi, resin-Pi (freely exchangeable) and bicarbonate-Pi. Water-Pi and resin-Pi are freely exchangeable in the soil solution and extractable without any modification. In contrast, the bicarbonate-Pi pool is influenced by root respiration because CO₂ produced forms bicarbonates in soil solution, whereas root exudation of protons and organic acid anions may solubilize bicarbonate P in soil (Tiessen and Moir, 1993).

2.2.2 Moderately-labile P pool

The P fractions extracted with alkali (NaOH-Pi and Po) and acid (1 M HCl) are grouped into a moderately-labile soil P pool (Hedley et al., 1982, Schmidt et al., 1996; Ivarsson, 1990). NaOH (Pi + Po) was associated with Fe and Al sesquioxides (Hedley et al., 1982; Tiessen et al., 1984; Wager et al., 1986) and dilute-HCl-Pi was found to be bound to Ca (Kurmies, 1972). In highly weathered soils, NaOH-extracted Pi was in equilibrium with the labile P pool, whereas in slightly weathered soils Pi extracted in dilute HCl acted as a buffer for the labile P pool (Guo et al., 2000).

2.2.3 Stable P pool

The stable P pool extracted with concentrated HCl (11.3 M) contained inorganic and organic residual P fractions that may become available over time (Metha et al., 1954, Tiessen and Moir, 1993).
Figure 2.1: Schematic representation of P fractionation method of Hedley et al. (1982) as modified by Tiessen and Moir (1993). Pi = Inorganic P; Pt = Total P; Organic P (Po) = Pt – Pi
2.2.4 Residual P pool

The residual P pool obtained by concentrated perchloric acid digestion of the soil residue contains highly-recalcitrant P that is unavailable to plants (Tiessen and Moir, 1993). In slightly weathered soils, the residual P decreased over time with plant growth, but in highly weathered soil, the residual P continued to accumulate with plant growth (Guo et al., 2000).

2.3 Soil organic matter in phosphorus cycling

Soil organic matter plays an important role in increasing plant productivity, with organic P a significant part of soil P cycle contributing to P nutrition of plants (Tarafdar and Claassen, 2003; Richardson et al., 2005). Organic matter plays a central role in mineralization and immobilization of N, S and organic P (Williams and Donald, 1957; Williams and Twine, 1967).


Schnitzer and Neely (2000) reported that the oxygen-containing functional groups (-OH, -COOH, -C=O) in soil organic matter can enhance dissolution of soil minerals by complexing and dissolving metals into soil solution and increasing their availability to plants and microorganisms. Singh and Jones (1976) reported that organic material containing 3.1 g kg\(^{-1}\) or more P tends to decrease soil P adsorption in contrast to increasing it when P content is less than 2.2 g kg\(^{-1}\). In many soils, P availability is dependent more on P release from soil organic matter via decomposition than the release of adsorbed phosphate (Yusran, 2005). Also, nutrients applied in the form of fertilizers cannot be efficiently utilized in soils with low organic matter content (Lupwayi et al., 2004).

Besides fertilization and crop species, the cropping sequence in a system
significantly affects the organic matter content in soil. Extensive cultivation and cropping may lead to a substantial loss of soil organic matter. Decreased soil organic matter content and imbalance in nutrients are key factors in declining crop yields (Ladha et al., 2003).

Phosphorus cycling in soil involves the physical (sorption-desorption), chemical (precipitation-dissolution) and biological (immobilization-mineralization) processes. Soil C in organic matter acts as an energy source for microbes affecting biological reactions that contribute to soil P dynamics. Phosphorus release from mineralization of one percent of total organic matter content was estimated to be about 6 kg P ha\textsuperscript{-1} in arable soils and 15 kg P ha\textsuperscript{-1} in grasslands (Gasser, 1962).

Organic residues are variable in composition and differ in the rate of nutrient release, which makes managing soil organic matter and its nutrient cycling complex. Tate (1984, 1985) has discussed P cycle, emphasizing rates and pathways of P through soil organic matter. However, quantifying rates and pathways of C, N, P and S through soil organic matter is challenging (Till et al., 1982). Still, there is a strong need to understand the contribution of soil organic matter in making soil P available to plants (Dalal and Chan, 2001), which is one of the objectives of this research.

2.4 Crop residues as sources of soil organic matter

Crop residues are a primary, convenient and valuable source of organic matter added to soil. Crop residues act as a reservoir of plant nutrients, improving physical and biological properties of soil, and protecting soil from wind and water erosion. Besides providing source of nutrients and inputs to soil organic matter (Allison, 1973), crop residues influence availability of soil nutrients (Wade and Sanchez, 1983). Crop residues represent an important component of stability of agro-ecosystems and are an important factor underpinning sustainable cropping systems.

Burning residues in the field is not only a fire hazard and a source of air pollution, but also leads to loss of some plant nutrients and organic matter (Rasmussen et al., 1980; Biederbeck et al., 1980). However, when left on the soil surface, crop
residues may lead to poor crop establishment (Jessop and Stewart, 1983). Hence, crop residue should be recycled effectively for better soil and crop management.

Crop residues incorporated into soil become soil organic matter that slowly releases nutrients on decomposition. Several studies have reported that decomposition rate of crop residues is affected by their chemical properties such as C:N ratio and lignin and polyphenol content (Wang et al., 2004). Organic residues can modify availability of native P via the products of decomposition (Yadvinder-Singh et al., 1992; Nwuke, 2004; Nuruzamman et al., 2005b, 2006; Rose et al., 2010). Figure 2.2 explains the direct and indirect processes affecting P dynamics in soils with addition of organic matter via crop residues.

Organic P constitutes 30-60% of total P in plants (Harrison, 1987). Adding plant residues to soil may increase soil test P (Black, 1973; Bumaya and Naylor, 1988;

Figure 2.2: Direct and indirect effects of organic matter addition on phosphorus (P) phytoavailability. DOC- Dissolved organic carbon; LOA – low-molecular-weight organic acids; Pi - inorganic P; Po - organic P; MBP - microbial biomass P (Guppy et al., 2005).
McLaughlin et al., 1988c; Li et al., 1990; Vanlauwe et al., 2000), organic P content (Dalal, 1979), and P uptake by a subsequent crop (Till and Blair, 1978; Blair and Boland, 1978; Dalal, 1979, Thibaud et al., 1988; Vanlauwe et al., 2000); in addition, plant residues may reduce adsorption of P in soils (Singh and Jones, 1976; Bumaya and Naylor, 1988).

Recycling nutrients to soil through crop residues helps in maintaining soil organic matter content. Effective utilization of these nutrients occurs only when a nutrient release from organic residue is synchronized with plant demand (Pangga et al., 2000). Hence, crop residues have a major role in improving both P cycling in soil and crop P nutrition, providing they are managed properly in farming systems.

### 2.5 Phosphorus release from crop residues

Phosphorus release from organic matter is dependent on P content in residues and rate of release into soil (Guppy et al., 2005). Blair and Bolland (1978) considered net release of P from added plant material as a sum of inorganic soil P and P accumulated in plants (shoots and roots). Plant P can be in inorganic or organic forms (Bieleski 1973; Mengel and Kirkby, 1982), with 40-80% of total P in plants being inorganic (White and Ayoub, 1983; McLaughlin et al., 1988b; Iqbal 2009). The P forms vary with residues and the state of decomposition (Batten and Wardlaw, 1987). Phosphorus availability from crop residues is dependent on initial P release from residues and soil microbial activity during decomposition (Blair and Bolland, 1978).

Availability of P from crop residues is reduced when residue nutrient content is below the critical range of 2.5-3 g P kg⁻¹ (Power and Legg, 1978). Similar values have been published in other studies as well, eg. net P mineralization of crop residues in soil would occur when the residue P concentration is above 2 g kg⁻¹ (if below, immobilization would occur) (Fuller et al., 1956; Iyamuremye et al., 1996). Phosphorus released via decomposition of low-P residues is insufficient to meet the microbial P demand; hence, P released from crop residues becomes immobilized in microbes and is unavailable for plant uptake.
Curtin et al. (2003) considered C:P ratio as a critical indicator of P mineralization during soil organic matter decomposition. Residues with C:P ratio above 300 are more likely to immobilize P (Brady and Weil, 1996; Iyamuremye et al., 1996). Mineralization of P from residues is positively correlated with the P concentration (Kwabiah et al., 2003b) and negatively correlated with C:P (Lupwayi et al., 2007) and lignin:P (Lupwayi and Haque, 1999; Lupwayi et al., 2007) in crop residues.

Phosphorus requirement by legumes is usually high, resulting in high P content in plants. Return of these high-P plant residues releases 40-60% of P in the available inorganic forms immediately (Tian et al., 1992) after incorporation into soil; later, P is released more slowly by mineralization of organic phosphorus in decomposing legume crop residues, enhancing P availability to a subsequent crop. This is in agreement with previous results of Jones and Bromfield (1969) where 69-78% of total P in Phalaris sp. and clover legumes was water-soluble, with 90% of released P being inorganic P.

Studies by Lupwayi et al. (2007) have shown that amount of P released from legume green manure residues would meet up to 62% of a wheat P requirement, whereas residues of pea, canola and wheat represented only up to 3%, 7% and 4% of the wheat P requirement, respectively. Hence, legume green manure crops are considered best for rotation with cereals due to increased P availability via mobilization and mineralization of P from organic matter.

2.6 Plant-available phosphorus in crop residues

Plant-available P describes a P pool immediately usable by plants. Globally, 5.7 billion hectares of soil contain insufficient available P for sustainable crop production (Gaume, 2000). In the absence of P fertilization, P availability to plants is sustained by recycling P via organic matter decomposition and mineralization of organic P. Organic matter decomposition and turnover of microbial biomass significantly determine availability of P (Ewel et al., 1991; Oberson et al., 2001). Yet, plants have to compete with microbes for available P (He et al., 1997; Kouno et al., 2002).

Phosphorus availability is determined by chemical and biochemical processes
involved in P cycling influenced by soil properties. The P quantity (soil solution P amount) and P intensity (P from other pools that can readily replenish soil solution P) (Holford, 1997) determine P availability for plant growth. The optimum concentration of P in soil solution for plant growth is > 0.2 mg L\(^{-1}\) (Pierzynski et al., 2005).

Incorporation of crop residues into soil increases soil solution P (Birch, 1961; Jones and Bromfield, 1969; Martin and Cunningham, 1973). However, P released to soil solution via decomposition of crop residues may be adsorbed onto soil clay minerals (White and Ayoub, 1983; Friesen and Blair, 1988; Bah et al., 2006) or assimilated by soil microbes, reducing plant availability of added P.

![Figure 2.3: Processes influencing P availability in soil (Haynes and Mokolobate, 2001)](image)

Phosphorus availability from added crop residues is dependent on P sorption capacity of soil (Sharpley, 1996). Adsorption of added P is decreased with addition of organic materials with P content >3.1 g kg\(^{-1}\) (Singh and Jones, 1976), eventually increasing soil solution P. However, addition of organic matter to soil decreases soil P.
sorption capacity in a series of processes (Figure 2.3) explained by Haynes and Mokolobate (2001).

The results from experiments by Bowman and Halvorson (1997) suggested that in the intensive cropping systems return of plant residues to soil enhanced plant available P. The processes mediating mineralization and turnover of organic P are critical in determining available P. It is therefore essential to understand P supply and maximize P availability to plants by measuring fluxes and rates of transformation in P pools during decomposition of crop residues.

2.7 Dynamics of soil P pools with crop residue amendments

Phosphorus released from crop residues enters the soil P pool as soluble P, increasing available P that may become less available (e.g. get converted to the moderately available pool) due to immobilization of available P by microbes or sorption onto soil minerals (Sanyal and De Datta, 1991; Noack, 2014); over time, in the absence of crop removal, P may become chemically occluded in the stable and residual P pools (Tiessen et al., 1984; Sharpley et al., 1987; Alamgir et al., 2012). However, these processes would be influenced by the properties of crop residues (Tian et al., 1992) and soil (Huffman et al., 1996).

Organic residues can modify the soil P pools (Iyamuremye et al., 1996b; Nziguheba et al., 1998); hence, availability of P varies with the added residues. Friesen and Blair (1988) observed an increase in the inorganic P pool in soil 11 days after incorporation of $^{32}$P-labelled oat residues, with 50% of added P being available. In a $^{31}$P NMR spectroscopy study, Noack et al. (2012) observed that 85 % of P in crop residues was in inorganic form as orthophosphates, whereas the organic forms consisted of phospholipids, RNA, pyrophosphate and phytate. Addition of crop residues increased P availability in soil by (i) rapidly releasing water-soluble P to the soil; (ii) mineralization of organic P (Magid et al., 1996; Frossard et al., 2000) via microbial activity, and (iii) releasing P from adsorption sites (Iyamuremye et al., 1996a).

Crop residues can replenish the soil P pools because 10-25% of fertilizer P is
contained in crop residues (Noack et al, 2014b). McLaughlin et al. (1988c) reported that 100% of P added through medic residues got released to soil after 95 days, with 5% of P found in the growing wheat (*Triticum aestivum*) crop (plant pool), 29% was assimilated by soil microbes (microbial pool) and the rest remained in soil as inorganic P (29%) and organic P (37%). This was supported by the recent findings of Noack et al. (2014a) where 80% of crop residue P was found in the plant, microbial and available P pools.

Soil micro-organisms plays an important role in organic P mineralization (McLaughlin and Alston 1986; Richardson, 1994; Magid et al., 1996). Even though an addition of crop residues increased soil C, which in turn increased the microbial activity (Tate, 1985; Bünemann, 2008; Iqbal, 2009), mineralization or immobilization of added P is depended on the P content in crop residues (Iyamuremye et al., 1996a; Nziguheba et al., 1998; Alamgir, 2012; Damon et al., 2014). The labile P pool increased with incorporation of residues high in P, whereas low-P residues led to immobilization of labile P (Bünemann et al., 2012, Alamgir, 2012). Also, transformation of added P was higher when crop residues were ground and incorporated (80% available P) than when left on the surface (25% available P) (Noack et al., 2014a). This implies the importance of conventional tillage where incorporation of crop residues into soil enhances P cycling, whereas leaving crop residues on the surface or burning them leads to slow mobilization and/or losses of P.

### 2.8 Synchronizing P release from soil and P uptake by crops

Synchrony refers to matching soil nutrient availability and crop demand at any given period. A lack of synchrony leads to nutrient deficiency or excess supply, neither of which is beneficial to agricultural crop production. Myers et al. (1994) reviewed the concept of synchrony in agriculture and identified evidence of asynchrony in many crop production systems, but were unable to find improved synchrony through efficient management of agricultural resources.

Research on synchronization of soil nutrient release and crop nutrient uptake is oriented towards maximum nutrient capture in the soil-plant system through
optimization of timing, quantity, quality and location of inorganic and organic nutrient inputs (Myers et al., 1997). Synchronizing P release and uptake can be achieved by promoting processes that maintain P in soil solution for extended periods of time. Manipulating physical compartmentation of organic P enables control of nutrient release from organic residues and enhances synchronization between soil P release and crop P uptake (Schachtman et al., 1998).

Fertilizer application of P leads to asynchrony as the relatively soluble P is highly available in the early stages of crop growth and becomes less available later due to irreversible changes in soil that can be reduced by application of organic materials to soil. Wang et al. (1995) found that adding humic acids to soil with P fertilizer significantly increased soil solution P and increased P uptake and yield of wheat up to 25%, retarding occlusion of P that would decrease its availability. Rebafka et al. (1994) observed an increase in utilization of fertilizer P (about 6-14%) with addition of crop residues (and up to 25% in repeated application of crop residues) that enhanced synchrony.

In practice, promoting synchronization is a challenge. It is therefore necessary to identify proper management practices to enhance synchrony of P supply with P demand that would be acceptable to farmers.

Crop rotation and green manuring are some of the strategies to maintain soil health in biological farming systems. Biological farming systems combine the techniques of organic and conventional farming, with a strong emphasis on improving soil biological properties. Soil biological parameters were found to be higher in biological farming systems than conventional ones (Foissner, 1987; Maire et al., 1990). Oberson et al. (1993) observed higher P fluxes in biologically-managed soils in comparison with conventionally-managed ones because of higher ATP content in the former. In biologically-managed soils, P flux through microbial biomass was more important than P uptake by crops.

In a recent review, Damon et al. (2014) suggested that inclusion of green manure in a cropping system would benefit P nutrition of the subsequent crop.
Especially in low-P soils, rotating legume green manures with cereals is considered very beneficial, whereby incorporation of legume crop residues increases soil P content via mobilization of labile organic P pools and release of nutrients from decomposing crop residues, thus reducing the P fertilizer requirement. Therefore, green manuring and incorporation of crop residues are beneficial and sustainable management practices in restoring soil fertility, minimizing fertilizer usage and sustaining crop productivity.

### 2.9 Integrated use of organic and inorganic sources of P

Crop phosphorus nutrition is not necessarily reliant only on P fertilization because organic materials can replace (at least partly) mineral P fertilizers. However, exclusion of mineral P fertilizers and substitution with crop residues in the long-term may lead to a decrease in P availability (Schjonning et al., 2002; Gosling and Shepherd, 2005) to crops due to exhaustion of soil P (Løes and Øgaard, 1997; Newman, 1997; Askegaard and Eriksen, 2000) and potential asynchrony of P supply from organic residue decomposition with crop demand limiting crop yields (Penfold et al., 1995; Dann et al., 1996; Derrick and Dumaresq, 1999; Ryan and Ash, 1999; Deria et al., 2003). Indeed, crop residues cannot replace P fertilizers completely in soil P cycling because P fertilizers are a source of P to be recycled through crop residues. Integrating the application of P fertilizers with crop residues could minimize fertilizer usage and restore soil P status and fertility in the long-term.

For many decades, superphosphate was a major P fertilizer in many farming systems. More recently, mono- and di-ammonium phosphate as well as triple phosphate and polyphosphates have largely replaced superphosphate as the P fertilizer of choice (Nutrient source specifics, No. 21, IPNI). Continuous P fertilization in cultivated soils has led to build-up of organic P through improved biomass production (Jackman, 1955; Williams and Donald, 1957; Sadler and Stewart, 1975; Dalal, 1977). Application of fertilizer P should be limited to replacing P removed to avoid soil P saturation because it would increase the potential of P loss to the environment.

Efficiency of applied inorganic fertilizers can be improved by combined
application with organic sources, which would avoid not only P overloading via inorganic P fertilizers and possible losses of soluble P, but also would minimize the input cost of fertilizers and preserve the soil fertility. However, limited research (Jenkinson, 1981; Haynes and Swift, 1988; Kwabiah et al., 1999, Iqbal, 2009) has been done on supplementation of organic residue P with inorganic P fertilizer.

Organic acids and anions released during decomposition of crop residues (Figure 2.2) are capable of mobilizing native soil P as well as residual P fertilizer in soil (Nuruzamman et al., 2005a, b; Richardson et al., 2011). Gupta et al. (2007) concluded that integrated use of residues and mineral P fertilizer improved soil P dynamics and maintained a balance in soil P. Similarly, field studies by Sharma and Prasad (2003) and Waigwa et al. (2003) showed that combined application of legume crop residues and rock phosphate significantly increased soil P content, cereal P uptake and cereal yields compared with rock phosphate alone.

Post-harvest residues are generally low in P because about 80-90% of P is removed through grain harvest (Damon et al., 2014). Slow decomposition of post-harvest residues low in P (Iqbal, 2009) and high in C:P and C:N ratios leads to microbial immobilization (Fuller et al., 1956; Iyamuremye et al., 1996a; Nziguheba et al., 1998; Bünemann, 2003) of both added and native soil P due to a high proportion of added C (Marschner et al., 2003) in comparison with added P impeding P availability to plants (Palm, 1995). However, addition of P via crop residues along with P fertilizers could facilitate mineralization of P added in both fertilizers and post-harvest crop residues by the processes outlined in Figure 2.2. Therefore, it is beneficial to use inorganic fertilizer as an additional supplement with organic sources to maximize soil P cycling and improve P availability in agricultural soils (Iqbal, 2009).

Green manure crops harvested in the vegetative stage (Randhawa et al., 2005) are relatively high in P (White and Ayoub, 1983; Friesen and Blair, 1988; Ha et al., 2008). Incorporation of high-P green manure crops not only adds P to soil, but also enhances mobilization of native and residual P in soil due to high content of soluble P and organic C in fresh residues (Duo et al., 2000) facilitating faster decomposition (Hue,
Green manuring is suggested as a good option in nutrient cycling and sustaining soil fertility in the long-term (Randhawa et al., 2005; Iqbal, 2009; Damon et al., 2014). Even though a few studies (Sharma et al., 1987; Haggar et al., 1991; Yashpal et al., 1993; Kamh et al., 1999; Oehl et al., 2001a; Horst et al., 2001; Cavigelli and Thien, 2003; Randhawa et al., 2005) have focused on P cycling via green manures, there is still a great need to understand P transformations in soil with incorporation of green manures (Iqbal, 2009; Damon et al., 2014). Furthermore, future P supply in food production can be sustained by enhancing recycling of P via green manuring (Iqbal, 2009; Damon et al., 2014) or combined application of P fertilizer and post-harvest residues (Iqbal, 2009).

### 2.10 Research gaps

Phosphorus availability is a function of various processes. To predict P uptake in a certain system, it is necessary to characterize these processes. Predictions of P availability and P uptake are extremely difficult because of complexity of P pools, fluxes and processes, and a lack of clearly defined operational pools (Griereson et al., 1999). Hence, there is a need to characterize the transformations of P pools in soils supplemented with P either in organic or inorganic form.

Future crisis in P supply for agricultural production via inorganic P fertilizers from rock phosphate reserves has inevitably created the need to recycle P that is already available in an eco-system. Crop residues are valuable sources of P and are similar to P fertilizers in the capacity to meet the P requirement of growing crops (Nziguheba et al., 1998) depending on the residue quality. As soils are generally low in P, green manure crops with 70% readily-available P (water-soluble) are considered a better option than the post-harvest crop residues containing only about 50% of the total P in a readily-available form (Damon et al., 2014).

The objective of the studies presented in this thesis is (i) to characterize the changes in the soil P pools with crop residue amendments as either green manure or post-harvest residues; and (ii) to determine soil P mobilization and plant P uptake in
Soils amended with crop residues along with residual P fertilizer.

Soil incubation of high quality crop residues (70 DAS) as either fresh or dry was used to characterize the dynamics of P pools in soil supplemented with P via green manure or dry residues. The incubation study in Chapter 3 provided an insight into dynamics of soil P availability with incorporation of fresh or dry residues and characterized their effects on soil pH and microbial activity. Incorporation of crop residues as fresh (green manuring) increased the available P in soil in relatively short-term in comparison with dry residues.

The glasshouse experiment (Chapter 4) was conducted with an objective to measure plant availability of P in soil supplemented with crop residues and residual P fertilizer from a previous crop. The major focus was to determine the effect of returning shoot residues back to soil along with whole roots (as practiced in the field) on subsequent crop P uptake. Also, the study provided information on wheat P uptake from soil supplemented with inorganic P fertilizers as either fresh or residual from previous crop cultivation.

The later glasshouse experiment (Chapter 5) was aimed at measuring the capacity of the low-quality (post-harvest) crop residues to provide P nutrition to the subsequent wheat crop in comparison with inorganic P fertilizer at different P and N fertilization rates. Plant P uptake is related to availability of P added via either inorganic P fertilizers or crop residues. Changes in the soil P pools suggested the importance of crop residue quality and quantity on plant availability of P and soil P recycling.
CHAPTER 3

Phosphorus release from fresh and dry crop residues incubated in soil

3.1 Introduction

Global P resources are declining with increased use of phosphatic fertilizers in agriculture (Cordell et al., 2009). Australian soils are ancient, highly weathered, with poor fertility and low P. Australia, as the fifth largest consumer of phosphate fertilizers in the world, is importing about half of its requirement (Cordell, 2013). Excess usage of phosphatic fertilizers has led to a loss of P to erosion and drainage, causing eutrophication that puts environment at risk (Higgs et al., 2000). The depletion of P reserves in many parts of the world imposes a serious threat to nutrient management in agriculture in the near future. Henceforth, it is important to recycle the nutrients for enhanced sustainability of agriculture; organic amendments act as a major key in recycling nutrients.

Introducing organic amendments of either plant or animal origin in a cropping system produced a significant improvement in crop yields besides reducing the fertilizer requirements (Tisdale et al., 1993; Karlen et al., 1994). Earlier reports found the use of organic amendments in agriculture may improve soil fertility in developed countries such as Australia (Aimers and Rice 2006) and in developing regions such as India and Africa (Jenkinson 2001; Ghosh 2004). However, ‘Green Revolution’ in agriculture increased the use of synthetic fertilizers (Zanden 1991; McGregor and Shepherd 2000) in conventional farming systems, where organic amendments are used little or not at all because the main focus is to increase productivity in the short-term rather than improve soil fertility in the long-term.

Soil fertility can be restored and enhanced with increased organic C content in soil (Ashagrie et al., 2007; Bhogal et al., 2009). Removal of crop residues leads to a significant loss of organic C, imposing a threat to soil health (Skjemstad et al. 2001; Mikha et al. 2006; Lal et al. 2007; Park et al. 2007; Favoino and Hogg 2008). In contrast, organic amendments promote soil health with addition of organic C.

Recently, a wide range of organic amendments have been marketed for
application in broad-acre farming. However, only a few studies (Abbasi et al., 2002; El-
Tarabily et al., 2003; Tenuta and Lazarovits, 2004; Imbue et al., 2005; Mondini et al.,
2008) have researched the possible effects of organic amendments in soil. Inappropriate
use of organic amendments may cause serious threat to the environment, deteriorate soil
health, and decrease productivity. With scientific information on the utility of these
products in broad-acre farming systems being relatively poor, more research is required
to investigate the effects of organic amendments based on the soil, product type and
agronomic management practices in broad-acre farming systems.

Phosphorus inflow for plant nutrition from organic sources depends on the
nature of organic P added, inorganic P in soil solution and phosphatase activity in soil.
Only a few studies have evaluated the effect of plant residues on P cycling in soil
(Dalal, 1979; McLaughlin et al., 1988a, b, c; Umrit and Friesen, 1994), with majority of
the studies on P focusing on its availability. Many studies showed that adding plant
residues to soil increased soil test P (Black, 1973; Bumaya and Naylor, 1988;
McLaughlin et al., 1988c; Li et al., 1990; Vanlauwe et al., 2000), increased organic P
content (Dalal, 1979), enhanced P uptake by subsequent crops (Till and Blair, 1978;
Blair and Boland, 1978; Dalal, 1979, Thibaud et al., 1988; Vanlauwe et al., 2000) and
reduced adsorption of P in soil (Singh and Jones, 1976; Bumaya and Naylor, 1988).

Plant-availability of P in crop-residue-amended soils is a function of organic
matter turnover, concentration of inorganic P in soil solution and P requirement of
microorganisms. Reduction in soil P may be due to a loss of labile P closely associated
with a decrease in soil pH and an increase in sesquioxide content, with increased
occluding and leaching of P (Tiessen and Stewart, 1983). Phosphorus can be leached
from plant residues (Tukey, 1970) due to crop residues being left on the soil surface in
no-till systems. Incorporation of crop residues impedes leaching in dependence on soil
organic matter decomposition (Christensen and Sorensen, 1985).

Phosphorus availability from crop residues is dependent on initial P release
from residues and on microbial activity during decomposition of plant residues (Blair
and Bolland, 1978). Results from the experiments conducted by Bowman and
Halvorson (1997) suggested that under intensive cropping systems returning plant residues to soil enhanced plant-available P and P cycling. Increasing the organic matter content in soil through residue application aids in nutrient cycling in soil and influences nutrient availability in soil both in the short and long term (Bhat et al., 1991; Buchanan and King, 1993; Singh and Singh, 1995).

Phosphorus availability is dependent on P sorption capacity of soil (Sharpley, 1996), whereby addition of organic matter to soil decreases the soil P sorption capacity. Dissolved organic carbon (DOC) released during decomposition of organic amendments influences soil P by interacting with soil adsorption sites and increasing availability of P (Iyamuremye and Dick, 1996). The DOC released from crop residues decreased P sorption (Ohno and Crannell, 1996; Ohno and Erich, 1997), whereas DOC from animal manure (cattle and poultry) had no influence on P sorption. These studies showed that P availability in soil can be improved (eg. reducing soil P sorption) to a greater extent with adding crop residues than manure (Nziguheba et al., 1998).

Application of green rather than dry residues improves the biological P cycling in soil. Green manuring is an agronomic practice of growing a crop and incorporating it into soil at an early stage of development (Randhawa et al., 2005). Decomposing green manures are better sources of readily-available nutrients (Sharma et al., 1987). Bahl and Singh (1997) reported an increase in plant-available P in soils amended with green manures. Hardiputra (2011) showed that in comparison to oven-dried residues, application of fresh faba bean residues had a similar potential to supply P as inorganic P fertilizer. A short-term study by Randhawa et al. (2005) demonstrated a 5-fold increase in organic P mineralization rate in soil amended with green manure in comparison with the unamended soil.

Availability of P in soil was significantly enhanced by inclusion of green manure (Haggar et al., 1991; Yashpal et al., 1993; Oehl et al., 2001a) improving biological P cycling in soil (Kamh et al., 1999; Cavigelli and Thien, 2003). Soil incorporation of green manure improved dissolution of sparingly soluble forms of P, such as rock phosphate (Kamh et al. 1999; Cavigelli and Thien 2003). Growing green
manure legume such as *Crotolaria* released P from soil with a significant decrease in soil pH and a decrease in the P fertilizer requirement of subsequently grown potato (Sharma et al., 1987). Incorporation of green manures increased soil C and N (Randhawa et al., 2005) enhancing soil microbial activity (Horst et al., 2001), a key factor in organic P mineralization. Hardiputra (2011) found P availability with the application of fresh faba bean reside was comparable to the use of P fertilizer, with an added benefit of reduced P sorption minimizing fertilizer cost.

An incubation experiment was conducted (i) to compare P release from fresh and dry crop residues in soil with inorganic P fertilizer application in the controlled environment; and (ii) to evaluate the temporal variation in soil P pools with decomposing fresh and dry crop residues in comparison with the inorganic P fertilizer.

### 3.2 Materials and Methods

#### 3.2.1 Experimental set up

Soil low in total P was collected from the top 10 cm of an unfertilized native vegetation site near the Western Australian town of Bindoon (31° 18’ 58’ S, 116° 07’ 74’ E). Bindoon soil was a moderately P-fixing acid soil, and the basic properties are given in Table 3.1.

Soil texture, Colwell P and pH (1:5 soil:CaCl$_2$) were measured according to Rayment and Higginson (1992). The soil P fractions (Pi – inorganic P; Po – organic P) and total P were determined using a fractionation method of Tiessen and Moir (1993). The soil P fractions were grouped into labile P pool (water-Pi + resin-Pi + bicarbonate-Pi + Po), moderately-labile P pool (NaOH-Pi + NaOH-Po + 1 M HCl-Pi), stable P pool (conc. HCl-Pi + Po) and residual P (persulphate digestion) based on Tiessen and Moir (1993). Total C and N were measured using an elemental analyser (LECO, CHN 1000, MI, USA).

The soil was air dried and sieved through a 2-mm mesh prior to potting. Field capacity moisture content of the soil was determined to be 18 % w/w by saturating 5 kg of soil with water and allowing drainage for 24 h (Rose et al., 2008).
3.2.2 Pre-incubation of soil

About two kilograms of soil were pre-incubated at 80% field capacity in plastic bags in a constant-temperature room at 26°C for 10 days before the start of the actual experiment. The pre-incubation was carried out to trigger and stabilize the microbial activity (Brookes et al., 1982; Oehl et al., 2001b) before incubation of crop residues.

Table 3.1: Basic properties of the experimental soil

<table>
<thead>
<tr>
<th>Properties of Bindoon soil</th>
<th>(Mean ± SE, n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture</td>
<td>clay loam</td>
</tr>
<tr>
<td>pH (1:5 soil:CaCl$_2$)</td>
<td>4.5 ± 0</td>
</tr>
<tr>
<td>Colwell P (mg kg$^{-1}$)</td>
<td>8.2 ± 0.1</td>
</tr>
<tr>
<td>Total P (mg kg$^{-1}$)</td>
<td>111 ± 1.7</td>
</tr>
<tr>
<td>Organic C (g kg$^{-1}$)</td>
<td>28 ± 0.3</td>
</tr>
<tr>
<td>Total N (g kg$^{-1}$)</td>
<td>1.8 ± 0.01</td>
</tr>
<tr>
<td><strong>P pools (mg kg$^{-1}$)</strong></td>
<td></td>
</tr>
<tr>
<td>Labile P</td>
<td>7.4 ± 0.1</td>
</tr>
<tr>
<td>Moderately labile P</td>
<td>35 ± 2.9</td>
</tr>
<tr>
<td>Stable P</td>
<td>32 ± 0.9</td>
</tr>
<tr>
<td>Residual P</td>
<td>39 ± 0.5</td>
</tr>
</tbody>
</table>

3.2.3 Crop residues

Three crops, namely wheat (*Triticum aestivum* L., cv. Crusader), canola (*Brassica napus* L., cv. Boomer) and field peas (*Pisum sativum* L., cv. Kaspa, dun type), were grown for up to 70 days (anthesis or initial flowering stage) to produce crop residues varying in P content (Table 3.2). At harvest, the whole plants with roots were taken out
of soil and washed with deionised water. About 50% of each crop residue was stored fresh in separate plastic bags in the cool room for the fresh residue treatments, and the remaining residues were dried for the dry residue treatments.

Table 3.2: Nutrient content and ratios in crop residues (Mean ± SE, n=3)

<table>
<thead>
<tr>
<th>Residue</th>
<th>Total P (g kg(^{-1}))</th>
<th>Total N (g kg(^{-1}))</th>
<th>Total C (g kg(^{-1}))</th>
<th>C:N</th>
<th>C:P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field pea</td>
<td>5.0 ± 0.1 a</td>
<td>28 ± 0.1 a</td>
<td>371 ± 2.2 a</td>
<td>13.3 ± 0.03 a</td>
<td>74 ± 1.2 a</td>
</tr>
<tr>
<td>Canola</td>
<td>3.8 ± 0.2 b</td>
<td>24 ± 1.2 b</td>
<td>319 ± 22 ab</td>
<td>13.5 ± 0.3 a</td>
<td>84 ± 2.5 b</td>
</tr>
<tr>
<td>Wheat</td>
<td>3.7 ± 0.1 b</td>
<td>18 ± 0.7 c</td>
<td>306 ± 4.7 b</td>
<td>17.3 ± 0.4 b</td>
<td>83 ± 1.5 b</td>
</tr>
</tbody>
</table>

Different letters in a given column denote significant differences among means (Tukey’s HSD\(_{0.05}\)).

The crop residues for dry residue treatments were oven-dried at 70°C until constant weight. A sub-sample of dried residues was ground (<0.5-mm) and digested in 3:1 v/v HNO\(_3\)–HClO\(_4\) mixture (Validazeh et al., 2003) for nutrient analysis. Total P content in the digested samples was measured using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Total C and N in the dried and ground crop residue samples were measured using LECO, CHN 1000, MI, USA.

3.2.4 Soil incubation

The fresh crop residues were chopped and sieved through a 5-mm sieve. Dry residues were ground in a mechanical grinder and sieved to <2-mm size. The residues were added to 1.5 kg of pre-incubated soil as per treatments. Soil with no added P as well as an inorganic P fertilizer treatment (P supplied as KH\(_2\)PO\(_4\)) were included as controls.

The experimental design was complete randomized block with eight soil P treatments: control with nothing added; soil + inorganic fertilizer P (IP); soil + fresh pea (FP); soil + fresh canola (FC); soil + fresh wheat (FW); soil + dry peas (DP); soil + dry canola (DC) and soil + dry wheat (DW). The treatments were replicated thrice.
Basal nutrient solutions except P were applied to all treatments as follows (mg kg\(^{-1}\) soil): 28.5 NH\(_4\)NO\(_3\), 280 K\(_2\)SO\(_4\), 60 MgSO\(_4\)\(\cdot\)7H\(_2\)O, 10 MnSO\(_4\)\(\cdot\)H\(_2\)O, 13.5 ZnSO\(_4\)\(\cdot\)7H\(_2\)O, 3 CuSO\(_4\)\(\cdot\)5H\(_2\)O, 1.05 H\(_2\)BO\(_3\), 0.6 CoSO\(_4\)\(\cdot\)7H\(_2\)O and 0.3 Na\(_2\)MoO\(_4\)\(\cdot\)2H\(_2\)O. The crop residues and inorganic P fertilizer were mixed with soil thoroughly. The soil moisture was maintained at 80% field capacity throughout the incubation period. The soil incubation was conducted in a constant-temperature room at 26 °C in the dark for 126 days.

### 3.2.5 Soil sampling and chemical analysis

The soil samples were collected six times during incubation (0, 21, 42, 63, 84 and 126 days). The samples were air-dried at 40 °C to constant weight in a drying room. The soil samples were sieved through a 2-mm sieve and stored in plastic vials until analysis.

The basic soil properties, namely soil texture, Colwell P and pH (1:5 soil:CaCl\(_2\)) were measured according to Rayment and Higginson (1992). Total elemental C and N were measured on finely ground (<0.5-mm) soil samples using an elementar analyser (LECO, CHN 1000, MI, USA). The soil P fractions and total P were determined using the method of Tiessen and Moir (1993) (Figure 2.1).

The P fractions (Pi – inorganic; Po - organic) sequentially extracted by the above-mentioned procedure were grouped into different P pools mentioned below based on their stability (Hedley et al., 1982, Ivarsson, 1990, Tiessen and Moir, 1993, Schmidt et al., 1996).

i) Labile P pool: water-Pi, resin-Pi and bicarbonate (Pi + Po)

ii) Moderately labile P pool: NaOH-Pi + NaOH-Po (Fe and Al bound) and dilute HCl-Pi (Ca bound)

iii) Stable P pool: concentrated-HCl-extracted Pi + Po

iv) Residual P pool: concentrated-perchloric-acid-extracted Pi

The inorganic P (Pi) in the extracts were determined colorimetrically by Murphy and Riley (1962) procedure. The total P (Pt) in the extracts was determined by digesting
the extracts (25mL) with 0.25 g potassium persulphate (APHA-AWWA-WEF, 1995).

3.2.6 Soil respiration rate measurement

The soil respiration rates were measured in triplicates following the method of Alef (1995) as an amount of CO\textsubscript{2} evolved from soil. The soil treatments were the same as in the soil incubation experiment (Section 3.2.4). The soil amended with crop residues or inorganic P fertilizer was placed in 250-mL vials with 20 mL of 0.5 M NaOH in a separate vial to trap CO\textsubscript{2} evolved. The entire arrangement was placed in 1-L glass jars tightly sealed and incubated in a constant-temperature room at 26\degree C in the dark. To each jar, 5 mL of deionised water was added for humidification. The soil moisture was maintained at 80% field capacity throughout the incubation period of 126 days.

The CO\textsubscript{2} evolved from soil and trapped in 0.5 M NaOH was measured by back-titration of 4 mL of that solution against 0.1 M HCl after addition of 10 mL of 10% w/v barium chloride and 2-3 drops of phenolphthalein as an indicator. The CO\textsubscript{2} measurements were carried out on 10, 20, 35, 50, 70, 90, 113 and 126 days of incubation. The respiration rate was calculated using the formula:

\[
\text{Respiration rate (µg C/g soil per day)} = 12,000 \times (A_1-A_2) \times M \text{ acid} \times V
\]

\[
2 \times W \times v \times \text{time}
\]

where 12,000 is the atomic weight of carbon, A\textsubscript{1} is the volume of acid used in titration against blank, A\textsubscript{2} is the volume of acid used against the sample, M is the molarity of the acid, V is the trap volume of 0.5 M NaOH, 2 is the molar factor, W is soil weight in g, v is titration volume in mL, and time=days of incubation. The cumulative carbon (mg C kg\textsuperscript{-1} soil) was calculated as the total CO\textsubscript{2} evolved from the soil incubation over time.

3.2.7 Statistical analysis

The experimental design was complete randomized block with eight soil P treatments: control with nothing added; soil + inorganic fertilizer P (IP); soil + fresh pea (FP); soil + fresh canola (FC); soil + fresh wheat (FW); soil + dry peas (DP); soil + dry canola (DC) and soil + dry wheat (DW). Soil samples were collected six times during
incubation lasting 126 days.

The statistical analyses were performed using SAS 9.3 statistical software. The ANOVA from repeated measures analysis was not different from the standard 2-way ANOVA. Therefore, 2-way ANOVA with P treatments and sampling time as the main factors was applied to data on soil pH, Colwell P, soil P pools and soil respiration. The 2-way ANOVA with P sources and sampling time as the main factors was used on the log-transformed data of the percent relative P availability. The significant differences in pair-wise comparisons of means were determined using Tukey's Studentized Range (HSD\_0.05) Test. The correlation and regression analyses were performed in MS-Excel.

3.3 Results

Effect of soil incubation with inorganic P fertilizer or crop residues

3.3.1 Soil pH

Supplementing soil with fresh residues tended to elevate soil pH, whereas dry residues or inorganic P fertilizer had little or no significant effect on pH over time (Table 3.3). On day 21, the pH of the soil amended with fresh or dry wheat residues increased by 1.3 or 0.2 units, respectively, and thereafter gradually decreased with time. After 42 days of incubation (DOI), the fresh pea residue amendment resulted in a 0.4-unit increase in the soil pH, whereas the dry pea treatment was on par with the control. The soil incubation with canola residues increased soil pH by up to 0.5 (fresh residues) and 0.3 units (dry residues) at 42 DOI (Table 3.3). The soil amendment with fresh wheat residue had a significant positive effect on the soil pH; this effect was significantly greater than that in other fresh residue treatments during the entire incubation period.
Table 3.3: Soil pH in 0.01 M CaCl$_2$ during incubation of soil with inorganic P fertilizer or crop residues (Mean ± SE, n=3)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Soil pH$_{CaCl_2}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Control</td>
<td>4.4 ± 0.00 a</td>
</tr>
<tr>
<td>Inorganic P</td>
<td>4.4 ± 0.02 a</td>
</tr>
<tr>
<td>Fresh Peas</td>
<td>4.4 ± 0.02 a</td>
</tr>
<tr>
<td>Dry peas</td>
<td>4.4 ± 0.01 a</td>
</tr>
<tr>
<td>Fresh Canola</td>
<td>4.4 ± 0.02 a</td>
</tr>
<tr>
<td>Dry canola</td>
<td>4.4 ± 0.01 a</td>
</tr>
<tr>
<td>Fresh wheat</td>
<td>4.4 ± 0.01 a</td>
</tr>
<tr>
<td>Dry wheat</td>
<td>4.4 ± 0.01 a</td>
</tr>
</tbody>
</table>

Means with different letters in a given column denote significant differences among treatments at a given time (Tukey’s HSD$_{0.05}$, p ≤ 0.05).

### 3.3.2 Plant-available P (Colwell P)

Availability of P in the soil significantly improved with supplementation of an external P source as either inorganic P fertilizer or crop residues (Figure 3.1). Soil amendment with inorganic P fertilizer significantly increased P availability in comparison with the control treatment at 21 DOI, but the increase was insignificant at other sampling times.

The fresh and dry pea residues significantly improved availability of P on day 21. On day 42, the available P in the soil amended with fresh pea residues slightly declined and later stabilized with time and was significantly higher than control, whereas the P release from dry pea residues was insignificant with time after 21 days.

Wheat residues, especially fresh, released significant quantity of P into the soil. Availability of P in the soil amended with fresh wheat residue was significantly higher than in the other treatments at 21 DOI (Figure 3.1). After 21 days, the amount of P
available in soil with fresh wheat residue decreased slightly and stabilized with time. The soil amended with pea and wheat residues showed a greater increase in P availability than the soil amended with canola residues.

Figure 3.1: Availability of soil P (Colwell P) during incubation of inorganic P fertilizer and crop residues in soil (Means, n=3). Vertical bars indicate the minimum significant difference using Tukey's Studentized Range Test (HSD<sub>0.05</sub>) at a given incubation period.

Soil amended with fresh canola residues had a slight (non-significant) increase in P availability at 21 DOI without a significant change thereafter. Phosphorus released from fresh and dry residues of pea and canola was on par with the inorganic P fertilizer treatment. However, P released into soil from fresh wheat residues remained significantly higher than control, whereas there was no significant difference in P availability in the treatments with dry residues (wheat or pea) and inorganic P fertilizer.

3.3.3 Soil P pools

Labile P pool

Phosphorus supplemented into the soil through crop residues or inorganic P fertilizer increased P availability by significantly increasing the labile P fractions. The labile P pool was largest in the wheat-residue-amended soil followed by the soil supplemented
with pea residue, inorganic P fertilizer or canola residue (Figure 3.2). An addition of P in wheat residues contributed to a significant increase in the labile P pool when compared with other soil treatments. However, the labile P pool was more stable with time in the dry wheat than the fresh wheat residue treatment. At the end of incubation (126 days), no significant differences were observed in the labile P pool in the soil amended with fresh or dry residues of each crop.

Table 3.4: Labile organic P, Fe-bound (NaOH-Pi) and Ca-bound (HCl-Pi) inorganic P fractions during incubation of soil with inorganic P fertilizer or crop residues

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Labile Po</th>
<th>Fe-bound Pi</th>
<th>Ca-bound Pi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 DOI</td>
<td>126 DOI</td>
<td>0 DOI</td>
</tr>
<tr>
<td>Control</td>
<td>3.6</td>
<td>5.7</td>
<td>7.4</td>
</tr>
<tr>
<td>Inorganic P</td>
<td>3.6</td>
<td>6.6</td>
<td>7.4</td>
</tr>
<tr>
<td>Fresh peas</td>
<td>3.6</td>
<td>3.5</td>
<td>7.4</td>
</tr>
<tr>
<td>Dry peas</td>
<td>3.6</td>
<td>6.2</td>
<td>7.4</td>
</tr>
<tr>
<td>Fresh canola</td>
<td>3.6</td>
<td>5.3</td>
<td>7.4</td>
</tr>
<tr>
<td>Dry canola</td>
<td>3.6</td>
<td>8.6</td>
<td>7.4</td>
</tr>
<tr>
<td>Fresh wheat</td>
<td>3.6</td>
<td>4.8</td>
<td>7.4</td>
</tr>
<tr>
<td>Dry wheat</td>
<td>3.6</td>
<td>5.6</td>
<td>7.4</td>
</tr>
</tbody>
</table>

Tukey’s test ns ns ns $P < 0.0001$ ns $P < 0.0001$

Means with different letters in a given column denote significant differences among treatments at a given time (Tukey’s HSD$_{0.05}$, $P \leq 0.05$). ns – not significant at $P = 0.05$.

The labile soil P pool contained more organic P in the treatments with dry than fresh crop residue, but the significant difference was noted only in case of canola residues (Table 3.4). Soil supplemented with inorganic P fertilizer had a greater labile organic P pool than the control soil. The labile P pool was highly correlated with the quantity of P added irrespective of the source (Table 3.5).
Figure 3.2: Dynamics of soil P pools during incubation of inorganic P fertilizer or crop residues in soil over time. The data represent means + SE (n=3). Different letters denote significant differences among treatments at a given time (Tukey’s HSD0.05, p ≤ 0.05. The moderately-labile P pool data for the inorganic P treatment at 21 days were omitted because they were found to be outliers.
Moderately-labile P pool

The moderately-labile P pool was higher in soils incubated with fresh than dry residues or inorganic P fertilizer. Soil amendment with fresh wheat residue significantly increased the moderately-labile P pool with time. The moderately-labile P pool size in soil incubated with other crop residues (fresh and dry peas, fresh and dry canola and dry wheat) was similar but significantly lower than in the soil incubated with fresh wheat residues. Soil supplemented with inorganic P fertilizer had a similar moderately-labile P pool as the dry crop residue treatments (Figure 3.2).

Stable P pool

The stable P pool was larger in soil incubated with fresh or dry crop residues or inorganic P fertilizer than in the control. The soil incubated with fresh wheat residues had a significantly higher stable P pool than dry wheat residues and other treatments. The stable P pool in soil amended with fresh wheat residues increased steadily with time (Figure 3.2), whereas that pool in the fresh or dry pea residue-amended soil declined until 42 days and then increased with time. However, at the end of the incubation period on day 126, the stable P pool size was similar in soil supplemented with inorganic P fertilizer or crop residues and the control soil, except for soil incubated with fresh wheat residue that had a significantly larger stable P pool.

Residual P pool

The residual P pool was significantly lowered by soil incubation with fresh or dry crop residues and significantly increased with inorganic P fertilizer (Figure 3.2). Soil incubation with crop residues resulted in a decrease in the residual P pool irrespective of the residue type and was on par with the control soil. In contrast, supplementing P through inorganic P fertilizer significantly increased the residual P pool with time.

3.3.4 Correlation between the crop residue properties, added P and P pools

The quantity of P added in either crop residues or inorganic P fertilizer had a significant positive effect on the labile P pool in soil during the entire incubation period, thereby improving availability of P. The moderately-labile P pool and the stable P pool remained unaffected by the quantity of crop residues and C:N or C:P ratio of crop residues (Table 3.5).
Table 3.5: Correlation between the crop residue properties, added P and the P pools during incubation of soil with inorganic P fertilizer or crop residues

<table>
<thead>
<tr>
<th>Days of incubation</th>
<th>Parameters</th>
<th>Labile P</th>
<th>Moderately-labile P</th>
<th>Stable P</th>
<th>Residual P</th>
<th>Colwell P</th>
</tr>
</thead>
<tbody>
<tr>
<td>21 DOI</td>
<td>Added P</td>
<td>0.95 **</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.88 **</td>
</tr>
<tr>
<td></td>
<td>Residue quantity</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>C:N</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>C:P</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>42 DOI</td>
<td>Added P</td>
<td>0.98 **</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.97 **</td>
</tr>
<tr>
<td></td>
<td>Residue quantity</td>
<td>0.76 *</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.81 *</td>
</tr>
<tr>
<td></td>
<td>C:N</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>C:P</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>63 DOI</td>
<td>Added P</td>
<td>0.82 *</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.96 **</td>
</tr>
<tr>
<td></td>
<td>Residue quantity</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>C:N</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>C:P</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>84 DOI</td>
<td>Added P</td>
<td>0.81 *</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.98 **</td>
</tr>
<tr>
<td></td>
<td>Residue quantity</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-0.80 *</td>
<td>0.78 *</td>
</tr>
<tr>
<td></td>
<td>C:N</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-0.98 **</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>C:P</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-0.97 **</td>
<td>ns</td>
</tr>
<tr>
<td>126 DOI</td>
<td>Added P</td>
<td>0.92 **</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.94 **</td>
</tr>
<tr>
<td></td>
<td>Residue quantity</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>C:N</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-0.91 **</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>C:P</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>-0.97 **</td>
<td>ns</td>
</tr>
</tbody>
</table>

* Significant at P ≤ 0.05; ** significant at P < 0.01; ns – not significant at P = 0.05.

Added P – total P added in either crop residues or inorganic P fertilizer; Residue quantity – quantity of crop residue added; C:N, C:P = ratios in crop residues.
Chapter 3  Phosphorus release from fresh and dry crop residues incubated in soil

The residual P pool in soil was not influenced by the P amendments until 63 DOI; thereafter, a strong negative correlation was observed with C:N or C:P ratio in the P amendments (Table 3.5). An increase in soil C added decreased the residual P pool (Figure 3.2). An addition of inorganic P fertilizer was associated with immobilization of added P with time, increasing the residual P pool (Figure 3.2, Table 3.5). In contrast, P added to soil along with C from decomposing crop residues did not increase the residual P pool.

3.3.5 Correlation between soil pH and P pools

The soil pH had no significant correlation with the labile P and residual P pools in soil during the entire incubation period (Table 3.6). The moderately-labile P pool in soil had a strong positive correlation with the soil pH from 42 DOI onward. The soil pH had a strong positive correlation with the stable P pools from 63 DOI onward. The influence of soil pH on the moderately labile and the stable P pools increased steadily with time (Table 3.6).

Table 3.6: Correlation between soil pH and P pools during incubation of soil with inorganic P fertilizer or crop residues

<table>
<thead>
<tr>
<th>Soil P pools</th>
<th>Soil pH\textsubscript{CaCl\textsubscript{2}}</th>
<th>21 DOI</th>
<th>42 DOI</th>
<th>63 DOI</th>
<th>84 DOI</th>
<th>126 DOI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Labile P</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Moderately-labile P</td>
<td>ns</td>
<td>0.86 *</td>
<td>0.83 *</td>
<td>0.86 *</td>
<td>0.87 *</td>
<td></td>
</tr>
<tr>
<td>Stable P</td>
<td>ns</td>
<td>ns</td>
<td>0.87 *</td>
<td>0.89 *</td>
<td>0.94 *</td>
<td></td>
</tr>
<tr>
<td>Residual P</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Colwell P</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
</tbody>
</table>

* Significant at P ≤ 0.05; ns – not significant at P = 0.05.
3.3.6 P recovery from crop residues and inorganic P fertilizer during soil incubation

The recovery of P with addition of inorganic P fertilizer or crop residues at a given time was calculated as a percent increase in relative availability of P (Colwell P) from initial P availability at week 0.

\[
P \text{ recovery (\%)} = \frac{\text{Available P in treatment at a given time} - \text{available P in treatment at time=0}}{\text{Total P added}} \times 100
\]

Relative availability of P in soil was higher in the treatments with fresh than dry crop residues. However, there were no significant differences among the treatments after 21 DOI, except for fresh peas and dry canola that differed significantly at 63 DOI (Figure 3.3). At 21 DOI, the amount of P released from fresh crop residues (especially pea and wheat, but not canola) was slightly (non-significantly) higher than that from inorganic P fertilizer. About 59 and 61% of added P was made available from fresh pea and wheat residues at 21 DOI; thereafter, P released from the residues decreased steadily to 42% (fresh pea) and 44% (fresh wheat residues) at the end of incubation.

Figure 3.3: Percent P recovered from inorganic P fertilizer or crop residues during soil incubation. Data are means of three replicates (Not significant at P=0.05).
In contrast, fresh and dry canola residue amendment gradually increased P availability in soil with time, reaching up to 42% from fresh canola residue at 126 DOI and 37% from dry canola residue at 84 DOI (Figure 3.3). Soil amended with fresh pea residue had higher P availability than with fresh and dry wheat until 84 DOI, but the difference was not significant. The inorganic-fertilizer-amended soil showed a non-significant trend of higher available P compared with the crop residue treatments and control at the end of incubation.

3.3.7 Soil respiration

After 10 DOI, soil respiration rate was significantly higher in the treatments with crop residue amendments than inorganic P fertilizer and control (Figure 3.4). After 20 DOI, there was no significant treatment difference in respiration rate, except a higher rate for fresh wheat residues. There was no appreciable difference in respiration rate in any treatment from day 50 onwards.

At day 10, soil amended with either fresh or dry wheat residues respired significantly more C than soil in other treatments. Respiration rate was higher in soil incubated with fresh than dry wheat residues until 35 DOI, with no significant differences afterwards (Figure 3.4). A strong decrease (35-65%) in respiration rate was observed between 10 and 20 DOI, except for the soil treatment with fresh wheat residue for which there was no difference between 10 and 20 DOI. At 35 DOI, soil amended with fresh wheat residues respired 63% and in the other treatments 25 to 36% of the rate at 10 DOI.

Soil supplemented with fresh canola residue evolved less CO₂ than the other soil treatments (including control) from day 35 to the end of incubation. From day 50 to 70, soil treated with fresh or dry crop residues respired a similar CO₂ amount as the control and soil fertilised with inorganic P. After 70 DOI, respiration rate was higher in the control soil with no added P than in the treatments with fresh or dry crop residues or inorganic P fertilizer. At the end of incubation, respiration rate was higher in the treatments with no residues added [control (9 mg C kg⁻¹ soil day⁻¹) and inorganic P
treatment (7.2 mg C kg\(^{-1}\) soil day\(^{-1}\)) than the treatments with added residue (4-5 mg C kg\(^{-1}\) soil day\(^{-1}\)).

Figure 3.4: Soil respiration rate measured by CO\(_2\) evolved during soil incubation with inorganic P fertilizer or crop residues. Data are means of three replicates.
The vertical bars indicate the minimum significant difference (Tukey’s HSD$_{0.05}$) among treatments at a given time.

Figure 3.5: Cumulative C released during soil incubation with inorganic P fertilizer or crop residues over time. Data are means of three replicates. The vertical bars indicate the minimum significant difference (Tukey’s HSD$_{0.05}$) among treatments at a given time.

3.3.8 Cumulative C release

Supplementing soil with fresh or dry crop residues resulted in a higher cumulative CO$_2$ release when compared with the control and inorganic P fertilizer treatments until 113
days of incubation (Figure 3.5).

Soil amended with dry pea or canola residues produced non-significantly higher cumulative C than the soil treatments with fresh pea or canola residues throughout the incubation period. Significantly higher quantity of C was released in the treatment with fresh than dry wheat residues during the entire incubation period. At 160 DOI, the highest cumulative C (2.8 g C kg\(^{-1}\) soil) was released from soil supplemented with fresh followed by dry wheat residues (2.1 g C kg\(^{-1}\) soil). Initially, a larger amount of C was accumulated in soil amended with fresh pea or fresh canola residue than the control till 20 DOI, after which time no significant differences were observed. At the end of incubation, there was no significant difference in the cumulative C released regardless of the treatment, except for soil treated with fresh wheat residue that released significantly more C than the other treatments (Figure 3.5).

### 3.4 Discussion

#### 3.4.1 Soil pH

Changes in soil pH with time were highly dependent on the residue type and initial soil pH (Table 3.3, see also Noble et al., 1996; Tang et al., 1999). Several studies showed an increase in soil pH after incubation with plant residues (Hoyt and Turner, 1975; Ritchie and Dolling, 1985; Bessho and Bell, 1992; Pocknee and Sumner, 1997; Tang et al., 1999). The experimental soil tested here was acidic with an initial \(pH_{\text{CaCl}_2}\) of 4.5±0. Soil supplemented with fresh crop residues had slightly increased pH, whereas the soil pH remained unaffected in the treatments with dry crop residues and inorganic P fertilizer (Table 3.3). Similar results were obtained by Hue (2011), with a slight increase in soil pH after organic amendments and a higher increase in soils amended with fresh residues than dry ash from the same residues.

Compared with control, a significant increase in the soil pH was observed initially (21 DOI) only in the treatment with fresh wheat residue (other residue treatments produced non-significant increases in soil pH at that time) (Table 3.3). Wang et al. (2012) and Xiao (2013) observed a significant increase in soil pH during the early
stages of incubation (<21 days) with crop residues high in ash alkalinity and excess cations (Noble and Randall, 1999; Tang et al., 1999) and N content (Wang et al., 2012). Such a relatively rapid increase in soil pH with the residue amendments probably resulted from (i) microbially-mediated decarboxylation of organic acids released with decomposition of crop residues (Yan et al., 1996; Marschner and Noble, 2000; Xu et al., 2006a, Xiao, 2013); (ii) an increase in electrical conductivity with a release of base cations (mainly Ca, Mg, K and Na) from decomposing residues (Wong and Swift, 2003, Li et al., 2008); and (iii) consumption of H⁺ by organic anions released from the fresh residues (Ritchie and Dolling, 1985; Helyar and Porter, 1989; Barekzai and Mengel, 1993; Noble et al., 1996; Chorom and Rengasamy 1997; Tang et al., 1999).

An increase in soil pH was slightly higher in the treatments with fresh than dry residues throughout the incubation period lasting 126 days (Table 3.3). However, no significant differences were observed in the soil pH between crop residue and inorganic P fertilizer amendments (except for the fresh wheat residue treatment). Similar observations on the increase in soil pH with fresh residue amendments were reported by Hue (2011) in Ultisols and Andisols amended with fresh leaf residues of cowpea and pineapple; an increase in soil pH with organic residue amendments was accompanied by a decrease in soil exchangeable Al (Hargrove and Thomas, 1981).

A prominent pH increase in soil amended with fresh wheat residue may be attributed to incorporation of a relatively large quantity of residue. However, the pH in the fresh-wheat-residue-amended soil decreased rapidly after 21 DOI, which might be due to nitrification of NH₄⁺ ions produced during decomposition of fresh wheat residues. Many studies reported nitrification as one of the major processes contributing to a decrease in soil pH with crop residue addition (Yan et al., 1996; Tang et al., 1999; Xu and Coventry, 2003; Xu et al., 2006; Yan et al., 2006; Mao et al., 2010). Except for wheat residues, the soil pH in the present study tended to increase in the residue treatments (pea and canola) until 42 DOI and then decreased. This initial pH increase might have been associated with less microbial activity with addition of a relatively small quantity of C via pea and canola residues in comparison with wheat amendment.
Lower microbial activity might have delayed decomposition of pea and canola residues resulting in a delayed (i) increase in decarboxylation of organic acids (Yan et al., 1996; Marschner and Noble, 2000; Xu et al., 2006a; Xiao, 2013); (ii) release of base cations increasing electrical conductivity (Wong and Swift, 2003; Li et al., 2008); and (iii) increase in consumption of H$^+$ by organic anions (Ritchie and Dolling, 1985; Helyar and Porter, 1989; Barekzai and Mengel, 1993; Noble et al., 1996; Chorom and Rengasamy, 1997; Tang et al., 1999).

### 3.4.2 Soil respiration

Soil respiration rate, as an indicator of microbial activity, is a major factor affecting mobilization of soil P (Oberson et al. 2001; Oehl et al., 2001a; Olander and Vitousek, 2004) and is mainly governed by C availability (Gallardo and Schlesinger, 1990; Wardle, 1992). During early stages of incubation (10 DOI) in the present study, the respiration rates were higher in the residue-amended soils with added C than the inorganic P treatment or the control soil with no added C (Figure 3.4). This might have been mainly due to an increase in microbial activity in response to increased C availability that later declined with decomposition of crop residues. In the fertilizer-amended soil, despite a significant addition of P through inorganic P fertilizer, the soil respiration was low with no added C, indicating less microbial activity, and was on par with the control treatment. This shows that microbial activity was mainly limited by C rather than P. This is in agreement with earlier studies in various soil types by Chauhan et al. (1979, 1981), Ros et al. (2003), Nziguheba et al. (2005) and Iqbal (2009).

Fresh crop residues contain more water-soluble C, N and P, resulting in relatively rapid decomposition (Iqbal, 2009) compared with dry residues. Except for the soil amended with fresh wheat residue, the respiration rate in soil amended with other fresh crop residues declined (albeit not significantly) after 20 DOI. This rapid initial decomposition of fresh crop residues (10-20 DOI) corresponded with an increase in available P at 21 DOI in the residue-amended soils (Figure 3.5). The soil respiration rate with fresh wheat residue decreased gradually after 10 DOI, but was still significantly higher than the other treatments until 35 DOI (Figure 3.4). Previous studies
by Mary et al. (1992), Saggar et al. (1998), Trinsoutrot et al. (2000b), Wang et al. (2004), Abiven et al. (2005), Jensen et al. (2005) and Bertrant et al. (2006) showed that the respiration rate decreased in soils amended with crop residues after 28 days as decomposition of remaining recalcitrant organic material is relatively slow. An addition of large quantities of fresh wheat residues supplied large quantity of water-soluble C, N and P to soil (Trinsoutrot et al., 2000b; Wang et al., 2004, Abiven et al., 2005; Jensen et al., 2005; Bertrant et al., 2006; Raiesi, 2006; Xu et al., 2006; Mathers et al., 2007) when compared to other fresh and dry residue treatments, probably contributing to higher respiration rates in the treatment with fresh wheat residue compared with other residue treatments at 20 and 35 DOI.

High rates of soil respiration in the early stages of crop residue decomposition indicated enhanced microbial activity with added C (Figure 3.4). This increase in microbial activity eventually leads to microbial immobilization of nutrients and microbial accumulation of P (Chauhan et al., 1981; He et al., 1997; Vrede et al., 2004) that can later be mineralized to plant-available form. After 50 DOI, the respiration rates in the soils amended with crop residues declined (albeit not significantly) with time (Figure 3.4). Iqbal (2009) observed a decrease in soil respiration rates with addition of crop residues after 35 DOI, accompanied by a decrease in microbial C, suggesting microbial turnover leading to nutrient mineralization in later stages of decomposition. However, in the present study a decrease in soil respiration after 50 days did not increase the plant-available P in soil, with the amount of P available in soil remaining relatively stable after 42 DOI (Figure 3.1).

Combined addition of P along with C and N in crop residues improved microbial growth (Nordgren, 1992; Giesler et al., 2004; Ilstedt and Singh, 2005) and enhanced availability of added P in soil (Giesler et al., 2002, 2004). Magid et al. (1996) observed enhanced solubilization and mineralization of soil P by microbes, which was influenced mainly by the amount of C added in crop residues and not by the amount of added P (Iqbal, 2009; Bünemann et al., 2004). Furthermore, a large addition of C in wheat residues as either fresh or dry led to prolonged microbial activity in soil,
immobilizing P initially but releasing it later due to microbial turnover (cf. Iqbal, 2009) caused by declining availability of C with decomposition.

### 3.4.3 Plant-available P (Colwell P)

Soil P supplementation with inorganic P fertilizer or crop residues significantly increased P availability in soil (Figure 3.1). Inorganic P fertilizer amendment resulted in a significant increase in P availability at 21 DOI, but was significantly lower than in the crop residue treatments (except for canola residues).

Fresh crop residues increased plant-available P more than dry residues irrespective of the residue type (Figure 3.1). Hardiputra (2011) reported a greater increase in plant-available P with addition of fresh than oven-dried faba bean residues. An increment in P availability with fresh residue amendments might be due to (i) high content of water-soluble C, N and P (Iqbal, 2009); (ii) rapid decomposition (Iqbal, 2009) and high content of dissolved P and volatile organic compounds such as phenolics (Dou et al., 2000; Hardiputra, 2011); and (iii) decreased soil P sorption (Easterwood and Sartain, 1990; Hue et al., 1994; Iyamuremye and Dick, 1996).

Amending soil with crop residues high in P content (Table 3.2) led to increased P availability in soil to a similar or greater extent than in the inorganic P fertilizer treatment (Nziguheba et al., 1998) because of net P mineralization (Fuller et al., 1956; Singh and Jones, 1976; Iyamuremye et al., 1996a; Bumaya and Naylor, 1988). The rate of decomposition of added crop residues and mineralization of organic P added through crop residues is mainly dependent on microbial activity determined by C availability (Bunemann et al., 2004, Iqbal, 2009; Richardson and Simpson, 2011). Hulugalle and Weaver (2005) showed that soil incubation of young wheat residues (collected at anthesis) resulted in greater and faster mineralization compared with mature wheat residues (collected after grain harvest). In the present study, fresh wheat and pea residues resulted in higher P availability in soil than dry residues (Figure 3.1, 3.3), probably because of faster decomposition of fresh than dry residues (cf. Dalton et al., 1952). High soil respiration rates with fresh residue amendments (Figure 3.4) indicated high microbial activity upon addition of P along with C and N (Giesler et al., 2004;
Ilstedt and Singh, 2005; Nordgren 1992), increasing the rate of decomposition of added residues. The available P in the control treatment remained constant with no added C and P.

Plant-available P in soil amended with crop residues slightly decreased after the initial peak at 21 DOI and remained constant until the end of incubation (Figure 3.1). A similar trend in available P was obtained by Scagnozzi et al. (1997) in soils amended with sunflower, rapeseed or soybean residues. In the present study, an initial peak in plant-available P at 21 days in fresh wheat and pea residues might be due to a high amount of water-soluble C, N and P in fresh residues leading to rapid decomposition of the added residues and P release to soil (Tian et al., 1992). However, there was no further increase in P availability with decomposition after 21 days (Figure 3.1).

An application of 30 mg P kg\(^{-1}\) soil as inorganic P fertilizer increased P availability in soil and maintained it during the entire incubation period (Figure 3.1). Soil incubation of fresh wheat (55 mg P kg\(^{-1}\) soil) or pea (38 mg P kg\(^{-1}\) soil) residues increased plant-available P more than the inorganic P fertilizers at 21 days. However, after 21 days, the available P in soil did not increase above 30 mg P kg\(^{-1}\) soil even when applied at a higher rate in wheat and pea residues treatments (Figure 3.1). This might be attributed by the low buffering capacity of the soil (PBI=90.1, Burkitt et al., 2002a, b) which might lead to leaching of P applied in excess.

Colwell P ranging from 29 to 34 mg P kg\(^{-1}\) soil is critical for 95% maximum production of crops in Western Australia (Bolland et al., 2010; Gourley et al., 2010). In recent field trials conducted by Neuhaus and Easton (2014) in Bindoon soil (which was also used in the present study), an application of 36 mg P kg\(^{-1}\) soil was found to be most profitable. This suggests that the response to P fertilization above the Colwell P level of 30 mg P kg\(^{-1}\) (Figure 3.1) soil might be low (Moody, 2007).

Increased P fertilization in excess of critical Colwell P either via inorganic P fertilizers or crop residues can result in P saturation of adsorption sites in soil, leading to P losses due to low soil PBI (Weaver et al., 1988; McDowell and Condron, 2000; Burkitt et al., 2002a, b). However, organic matter (added via crop residues) may interact
with the adsorption sites in soil either decreasing (McDowell and Condron, 2001) or increasing (McLaughlin et al., 1981) sorption of added P.

Given acidic soil pH in the present study ($\text{pH}_{\text{CaCl}_2} = 4.5\pm0$), soil P would be sorbed mainly to Al and Fe hydroxides (Haynes, 1984). Hence, a rapid increase in P availability in soil with application of inorganic P fertilizer followed by levelling off might have been due to low soil P buffering capacity ($\text{PBI} = 90.1$), with a remainder of added P probably transformed into highly recalcitrant pools and retained in soil (cf. Matar et al., 1992). McLaughlin et al. (1988a) observed that addition of crop residues reduced sorption of released P and increased P availability in soil between 18 and 61 days of incubation. In the present study, an increase in soil P availability with addition of inorganic P fertilizer was insignificant after 40 days mainly due to P sorption. Previous study by Hardiputra (2011) found a negative correlation between P availability and P sorption in soils amended with fresh faba bean residues to be greater than in case of dry faba bean residues. Greater enhancement in soil P availability with fresh than dry residues might have been due to differential P desorption, with rapid decomposition of fresh residues releasing products (eg. organic anions) that enhance P desorption (Richardson et al., 2011) via e.g. ligand exchange (Ryan et al., 2001).

Adding fresh crop residues as green manure had a greater positive effect on relative availability of P in soil compared with dry residues (Figure 3.3). In particular, relative availability of P in soil was higher with fresh pea residues compared to other residue or inorganic P fertilizer amendments (Figure 3.3). Pea being a legume crop had higher P, N and C than wheat and canola residues used in the experiment (Table 3.2). The increase in relative P availability in the pea residue treatment was mainly attributed by (i) higher residue P concentration (Fuller et al., 1956; Iyamuremye et al., 1996; Nziguheba et al., 1998; Kwabiah et al., 2003; Guppy et al., 2005) and lower C:P (Hundal et., 1987; Curtin et al., 2003) than wheat and canola residues leading to net P mineralization, and (ii) release of phenolics and organic anions with residue decomposition (Ayaga et al., 2006; Schefe et al., 2008) reducing soil P sorption. However, the relative availability of P was significantly lower in soil amended with dry
than fresh pea residues (Figure 3.3), which might be due to a loss of dissolved P and volatile organic compounds (eg. polyphenols) associated with the process of residue drying (Dou et al., 2000; Hardiputra, 2011).

The relative availability of P in soil with inorganic P fertilizer application was higher than the dry residue treatments and lower than the fresh residue (except canola) treatments (Figure 3.3). Though the quantity of residue and P added in residues was higher in the wheat residue treatments, the relative P availability in soil with wheat residues did not increase (Figure 3.3). Instead, the relative availability of P was mainly dependent of residue quality, and legume residues, being high-quality (Snapp, 1994; Mafongoya et al., 1997; Chikowo et al., 2006), released a relatively large proportion of P into soil.

### 3.4.4 Soil P fractions

#### Labile P pool

Amendment with inorganic P fertilizer or crop residues significantly increased the labile soil P pool (Figure 3.2). At 21 DOI, the labile soil P pool increased more with incorporation of fresh crop residues (except canola) than dry residues or inorganic P fertilizer. Pypers (2005) reported an increase in labile soil P fractions with application of high-quality residues. Crop residues used in the present study can be considered high quality, at least with respect to P content (ranging from 3.7 to 5 g P kg\(^{-1}\) dry matter; Table 3.2), and can be expected to increase the labile P pool as a result of net P mineralization (cf. Bünemann et al., 2008). White and Ayoub (1983) suggested that the addition of crop residues containing less than 2.4 g P kg\(^{-1}\) led to immobilization of added P in soil. Alamgir et al. (2012) observed an increase in labile P pool in soils incubated with medium-P residues (2.9 to 3.3 g kg\(^{-1}\) dry matter) or high-P residues (6.5 to 8.3 g kg\(^{-1}\) dry matter), whereas low-P residues resulted in P immobilization in soil.

An addition of larger quantity of P through wheat than other residues led to a significant increase in the labile soil P pool in the treatments with wheat compared with other crop residues (Figure 3.2). There was a rapid release of P into the labile soil pool.
in the treatments with fresh crop residues (up to 21 days) followed by a gradual decrease until 84 days and then again an increase at the end of incubation. In contrast, the dry residue amendments showed less fluctuation in the labile soil P pool, with a gradual increase until 42 days and then a slow decrease over time (Figure 3.2). The initial P flush in the fresh-residue-incubated soils was mainly attributed to their high content of dissolved P (Dou et al., 2000) enhancing net P mineralization in the short term (Friesen and Blair, 1988), releasing P in excess of microbial requirement (Tian et al., 1992), and contributing to the inorganic fractions of the labile P pool. Tian et al. (1992) found that returning high-P plant residues released 40-60% of P in available inorganic forms immediately. In the later stages of decomposition (21 to 84 days) in the present study, with the amount of P released from fresh residues being low, there was a decline in the labile P pool due to temporary P immobilization with increased microbial activity (Figure 3.2). An increase in the labile P pool at 126 DOI was observed in the fresh residue treatments, probably because of (i) mineralization of initially immobilized P due to microbial turnover (Mary and Recous, 1994) in the later stages of decomposition with a decrease in C supply and/or (ii) desorption of P from the surface of soil particles (Ryan et al., 1985; Iyamuremye and Dick, 1996; Reddy et al., 2005).

Amending soil with fresh residues contributed more to the labile Pi than Po fraction (Table 3.4, Figure 3.2). In contrast, the labile Po fraction in soil increased with dry residue or inorganic P fertilizer amendments (Table 3.4), probably due to initial immobilization with an increase in microbial growth consuming P. Greater labile organic P in soil supplemented with inorganic P fertilizer than the control soil might have resulted from higher microbial activity with P addition leading to build-up of microbial P. Dalal (1977) stated that organic P accumulation in soil mainly results from microbial activity.

The labile P pool was strongly correlated with the amount of P added to soil irrespective of the type of P amendment (Table 3.5). This is consistent with the previous studies by Nziguheba et al. (1998) and Hardiputra (2011) whereby an increase in labile P fractions was observed with addition of P as either inorganic P fertilizers or organic
crop residues.

**Moderately-labile P pool**

An addition of P to soil as either crop residues or inorganic P fertilizer increased the moderately-labile soil P pool during decomposition (Figure 3.2). However, no correlation was observed between the moderately-labile P pool and P addition (Table 3.5), possibly because the moderately-labile P was derived from pre-existing soil P fractions (cf. Reddy et al., 2005). A decrease in labile P with time corresponded with a simultaneous increase in the moderately-labile P and vice-versa (Figure 3.2). This shows that these two P pools are in a dynamic equilibrium (Guo et al., 2000). Adsorption and fixation of P onto clay minerals (Jones, 1998) along with temporary microbial immobilization (Richardson and Simpson, 2011) in soil with an increase in the labile P pool could account for an increase in moderately-labile P, which can become partly available with time in weekly acidic media (Tisdale et al., 1985) created by proton exudation by living roots or soil acidification during residue decomposition. Alamgir et al. (2010) reported an increase in the NaOH-Pi and HCl-Pi fractions 14 days after incubation of faba bean residues in soil mainly due to transformation of P released from decomposing residues.

The moderately-labile P pool in all the soil treatments (including control) comprised mostly NaOH-Pi (up to 72%), associated with Al and Fe (Tiessen and Moir, 1993). The dilute-HCl-Pi fraction (P bound to Ca) (Tiessen and Moir, 1993) constituted only 5-7% of the moderately-labile P pool in the treatments presented here, except for the fresh wheat residue treatment reaching a maximum of 20% at the end of incubation (Table 3.4). As expected, this finding shows higher concentration of Al and Fe than Ca in acidic Bindoon soil used here.

**Stable P pool**

The stable P pool extracted with hot concentrated HCl consists of chemically-occluded P bound inside Fe and Al minerals and does not readily participate in P transformations (Agbenin and Tiessen, 1995). In the present study, the stable P pool was the second largest after the moderately-available pool (Figure 3.2), with relatively high Fe and Al
expected in the acidic soil used here (cf. Tiessen and Moir, 1993). Similar results were obtained by Lilienfein et al. (2000), with the large stable P pool in highly-weathered soils.

Figure 3.2 shows the relatively small stable P pool initially (21 DOI), increasing with time (Figure 3.2). Particularly, fresh wheat residues increased the stable P pool in soil more than the other crop residue or inorganic P fertilizer amendments (Figure 3.2). An increase in the stable P pool with duration of incubation might have been due to re-adsorption of P (Sanyal and De Datta, 1991), released via mineralization in the early stages of decomposition, onto soil minerals and humic substances. Brady and Weil (2002) observed reduction in soil P availability with time due to decreasing P solubility with formation of complexes with clay minerals. Alamgir (2012) reported net conversion to stable P pools of P released from crop residues after 28 days of decomposition. However, growing plants might affect occlusion of mineralized P because plant P uptake reduces the amount of P remaining in soil solution and thus facilitates desorption of adsorbed P (Menzies, 2009) due to the rhizosphere processes (Richardson et al., 2011).

Residual P pool

Incubating soil at constant temperature of 26°C with addition of nutrients and moisture significantly mobilized nearly 40% of the residual P in the soil at 21 DOI (Figure 3.2). Studies by Sparling et al. (1995), Xiao et al. (2007) and Kim et al. (2011) showed that addition of water to soil increased microbial activity. Hence, mobilization of the residual P pool might be explained by intensive microbial activity in the initial stage of incubation (Oehl et al., 2001a; Bünemann et al., 2004; Ehlers et al., 2010; Richardson and Simpson, 2011) whereby a high P requirement for microbial growth led to consumption of the native soil residual P pool (Bünemann et al., 2004; Ehlers et al., 2010), potentially transforming a part of occluded P to microbial P. This initial mobilization of the soil residual P pool was not dependent on P addition in the present study because the control soil with no added P showed a similar decrease in residual pool as the other P-amended treatments.
Supplementing P in soil via inorganic P fertilizer significantly increased the residual P pool over time (Figure 3.2). This is in agreement with the studies by Beck and Sanchez (1994), Friesen et al. (1997) and Lilienfien (2000) on soil fractionation of added P. In contrast, P added in organic form via crop residues was not occluded over time (Figure 3.2). Instead, it was distributed to other P pools that can become available with time (Zhang and MacKenzie, 1997a, b). This might be explained by desorption and decarboxylation processes occurring in soil with a release of organic acid anions inhibiting sorption of P derived from crop residues (Iyamuremye and Dick, 1996; Ohno and Crannell, 1996; Ohno and Erich, 1997).

A gradual increase in the residual P pool after 21 days with inorganic P fertilizer amendment reflected immobilization of fertilizer P by soil aggregates and recalcitrant organic matter (Tiessen and Moir, 1993; Cross and Schlesinger, 1995). Hedley et al. (1982) described residual P as that strongly bound to Al and Fe oxides. In the present study, the acidic nature of Bindoon soil with an expected high amount of Al and Fe would have led to immobilization of inorganic fertilizer P by fixation (Uehara and Gillman, 1981) in the absence of a significant release of organic acids and anions (Hu et al., 2005) from decomposing organic matter.

Statistical analysis showed no significant differences in the residual P pool among the control and crop residue treatments, which implies distribution of P added through crop residues to P pools other than the residual one. A strong negative correlation of residual P pool with C:N and C:P in crop residues suggested that the addition of C via organic matter influenced mobilization of added P by microbial activity (Haynes and Swift, 1988; Richardson, 1994).

### 3.5 Conclusions

Phosphorus supplementation via inorganic P fertilizers improved P availability in the short-term. However, in the long-term, P supplementation in the readily-available forms via inorganic P fertilizers becomes ineffective, mainly due to P sorption or immobilization reducing P availability. The dynamics of soil P pools was greatest in
soil amended with crop residues due to mineralization and immobilization alternating with time in dependence on the quality of added organic matter. Organic matter addition facilitated mobilization of residual P in soil accumulated over time with inorganic P fertilization. Phosphorus added improved P availability more in case of fresh than dry residues early in the incubation period, making it the most suitable soil amendment because plants require large amounts of P early in development. Hence, soil P cycling can be improved through integrated management practices such as green manure crop in rotation with cash crops for increased crop productivity and enhanced soil fertility.
CHAPTER 4

Nutrient uptake by wheat as influenced by soil amendment with legume crop residues

4.1 Introduction

Grain legumes are gaining importance in Australian agriculture as they improve P availability to subsequent crops grown in rotation. Rotating legumes with cereals restored N level and progressively increased soil organic matter content in Australian soils (Greenland, 1971). Wheat-legume rotation is suggested as one of the prominent cropping systems in Australia (Siddique and Skyes, 1997).

Cereals are less efficient in utilizing available P than legumes, and cereal P-use efficiency is enhanced when grown in rotation with legumes (Gardner and Boundy, 1983; Horst and Waschkies, 1987; Hocking and Randall, 2001; Horst et al., 2001). Cereals in rotation with legumes (eg. faba bean) produced more dry matter and higher grain yield than in rotation with grassy pastures (Cresswell and Kirkegaard, 1995, Yunusa and Newton, 2003; Yunussa and Rashid, 2007). Wheat grown after legumes showed an increase in growth and P concentration, indicating enhanced P availability (Nuruzzaman et al., 2005; Hassan et al., 2012).

Enhancement in wheat P uptake following legumes may be due to (i) release of exudates from legume roots mobilizing P in excess of their requirement (Gardner and Boundy, 1983; Horst and Waschkies, 1987; Kamh et al., 1999); (ii) desorption of P from soil mineral surfaces by anion exchange (Parfitt, 1979; Bolan et al., 1994; Jones and Darrah, 1994; Jones, 1998; Hinsinger, 2001) and (iii) mineralization of root-derived organic phosphorus (Nuruzamman et al., 2005a, b). Also, organic residues can modify availability of native P through the products of decomposition (Yadvinder-Singh et al., 1992; Nwuke, 2004) releasing P adsorbed in soil via ligand exchange (Singh and Jones 1976; Bumaya and Naylor, 1988; Hue et al., 1994; Iyamuremye et al. 1996a; Bahl et al. 1998; Nziguheba et al., 1998).

Extensive studies have reported that inclusion of legumes that secrete organic acid anions increased growth and P uptake of subsequent crop (Ae et al. 1990; Kamh et al. 1999; Hocking and Randall 2001; Nuruzzaman et al., 2005a, b; Hassan et al., 2012b). In addition, few studies by Ae et al. (1990), Arihara et al. (1991) and Horst et al. (2001) have proven the rotational P benefit in crops grown after legumes in the field.
The rotational effect is also related to recycling of mobilized P via crop residues. Returning legume residues rich in P has added benefits compared with just growing legumes in crop rotation (Richardson et al., 2011). Retaining legume residues in soil resulted in higher P content in subsequently grown wheat (Whitbread et al., 2000). However, Lupwayi et al. (2007) found that legume green manure residues would meet up to 62% of the wheat P requirement, whereas residues of pea, canola and wheat contributed only 3%, 7% and 4%, respectively, of the requirement. Similarly, Murungu et al. (2010) suggested that incorporation of legume cover crops enhanced growth and nutrient uptake of a subsequent crop.

Phosphorus requirement of legumes is usually high, resulting in high P content in plants. Return of this high-P-content residues released 40-60% of P in available inorganic forms in soil in a short time (Tian et al., 1992). Later, P was released slowly by mineralization of organic P in decomposing legume residues (Dalal 1979), enhancing P availability to a subsequent crop (Black, 1973; Till and Blair, 1978; Blair and Boland, 1978; Dalal, 1979; Bumaya and Naylor, 1988; McLaughlin et al., 1988a; Thibaud et al., 1988; Li et al., 1990; Vanlauwe et al., 2000). Integration of legumes into cropping systems and recycling legume residues effectively contribute to better soil and crop management, enhancing sustainability and productivity of farming systems and minimizing fertilizer costs.

Nutrients applied as inorganic fertilizers cannot be efficiently utilized in soils with low organic matter content (Lupwayi et al., 2004). Efficiency of applied P fertilizers is determined by soil properties, mainly adsorption capacity (Singh and Gilkes, 1991) and crop use efficiency. Incorporation of crop residues enhanced the efficiency of P fertilizers through the products of decomposition releasing adsorbed P from soil (Ohno and Erich, 1997; Ohno and Crannell, 1996; Reddy et al., 2005) and thereby increases P uptake by crops (Till and Blair, 1978; Blair and Boland, 1978; Dalal, 1979, Thibaud et al., 1988; Vanlauwe et al., 2000; Nuruzamman et al., 2005). Richardson et al. (2011) argued for a need to understand the importance of crop residues in P cycling and reducing P fertilizer requirement. Hence, the present study was conducted (i) to determine the effect of incorporation of faba bean shoot residues along with whole roots on the growth and P uptake in subsequent wheat; and (ii) to
characterize nutrient uptake by wheat after addition of faba bean residues in comparison
with application of inorganic P fertilizer.

4.2 Materials and Methods

An experiment was set up on the dynamics of P uptake by wheat grown following a
legume crop in soil supplemented with P as inorganic fertilizer and with legume
residues. The experiment was conducted in a glasshouse at the University of Western
Australia, Perth (31°57’S, 115°51’E) during May to November 2009.

4.2.1 Experimental design

The pot experiment comprised two crop phases: i) legume phase with faba bean (*Vicia
faba* L. cv. Fiesta) (grown until anthesis), and ii) cereal phase with wheat (*Triticum
aestivum* L. cv. Westonia) (grown until anthesis) as the subsequent crop.

Table 4.1: Details of the treatments applied in the legume and cereal phases of the
glasshouse experiment

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Legume phase</th>
<th>Cereal phase</th>
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<tbody>
<tr>
<td>C0W1</td>
<td>Control</td>
<td>Wheat crop with no added inorganic P or faba bean residue</td>
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<tr>
<td></td>
<td>(no added P, no faba bean grown)</td>
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<tr>
<td>C0W3</td>
<td>Control</td>
<td>Wheat crop with freshly added inorganic P</td>
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<tr>
<td></td>
<td>(no added P, no faba bean grown)</td>
<td>(20 mg P kg(^{-1}) soil, no faba bean residue)</td>
</tr>
<tr>
<td>C1W2</td>
<td>Control with added inorganic P</td>
<td>Wheat crop with no freshly added P (no faba bean residues included)</td>
</tr>
<tr>
<td></td>
<td>(50 mg P kg(^{-1}) soil, no faba bean grown)</td>
<td></td>
</tr>
<tr>
<td>R1W2</td>
<td>Faba bean grown with added inorganic P</td>
<td>Wheat crop with no freshly added fertilizer P, but with faba bean residues (whole roots only) added</td>
</tr>
<tr>
<td></td>
<td>(50 mg P kg(^{-1}) soil)</td>
<td></td>
</tr>
<tr>
<td>R2W2</td>
<td>Faba bean grown with added inorganic P</td>
<td>Wheat with previously no freshly added fertilizer P, but with faba bean residues (whole roots + shoots @ 10 t ha(^{-1})) added</td>
</tr>
<tr>
<td></td>
<td>(50 mg P kg(^{-1}) soil)</td>
<td></td>
</tr>
</tbody>
</table>
The experiment was conducted in a generalized randomized block design (Wilk, 1955) with five treatments (Table 4.1) in five blocks for sampling at five plant stages in the cereal phase (14, 28, 42, 56 and 77 DAS). Each treatment was replicated thrice within a block. Pots were re-randomised within blocks weekly.

**4.2.2 Soil**

Soil collected from the top 10 cm of unfertilized native vegetation sites from Bindoon (31° 31’ 70” S, 116° 13’ 79” E) in Western Australia was used in the experiment. The basic properties of the soil were analysed by CSBP, Perth (Table 4.2). Soil texture, Colwell P, Colwell K, pH (1:5 soil: CaCl$_2$), and EC were measured according to Rayment and Higginson (1992). Organic carbon was measured according to Walkley and Black (1934) and nitrate N by Searle (1984). The soil was air-dried for 24 hours and sieved to 4-mm prior to potting.

<table>
<thead>
<tr>
<th>Soil properties</th>
<th>(Mean ± SE, n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Texture</td>
<td>clay loam</td>
</tr>
<tr>
<td>Colwell P (mg kg$^{-1}$)</td>
<td>6.5 ± 2.5</td>
</tr>
<tr>
<td>Colwell K (mg kg$^{-1}$)</td>
<td>240 ± 5.0</td>
</tr>
<tr>
<td>Nitrate N (mg kg$^{-1}$)</td>
<td>6.0 ± 0.0</td>
</tr>
<tr>
<td>Total P (mg kg$^{-1}$)</td>
<td>94.5 ± 0.5</td>
</tr>
<tr>
<td>Organic C (g kg$^{-1}$)</td>
<td>13.7 ± 0.3</td>
</tr>
<tr>
<td>EC (dS m$^{-1}$)</td>
<td>0.064 ± 0.003</td>
</tr>
<tr>
<td>pH (1:5 soil:CaCl$_2$)</td>
<td>6.5 ± 0.2</td>
</tr>
</tbody>
</table>

**4.2.3 Plant material**

Faba bean (*Vicia faba* L. cv. Fiesta) was grown as legume crop followed by wheat (*Triticum aestivum* L. cv. Westonia) in the cereal phase. Cultivar Westonia was found to be efficient in P utilization (Valizadeh et al., 2002).
4.2.4 Plant growth conditions

**Legume phase**

Pots (20 cm diameter and 25 cm deep) were lined with plastic bags and filled with 3 kg of sieved (<4-mm), air-dried soil. The basal nutrients (mg kg\(^{-1}\) soil) consisting of 190 NH\(_4\)NO\(_3\), 280 K\(_2\)SO\(_4\), 60 MgSO\(_4\).7H\(_2\)O, 15 MnSO\(_4\).H\(_2\)O, 13.5 ZnSO\(_4\).7H\(_2\)O, 3 CuSO\(_4\).5H\(_2\)O, 1.05 H\(_3\)BO\(_3\), 0.6 CoSO\(_4\).7H\(_2\)O and 0.3 Na\(_2\)MoO\(_4\).2H\(_2\)O were pipetted on the soil surface in pots a day before sowing and thoroughly mixed. Inorganic P fertilizer (50 mg P kg\(^{-1}\) soil) as KH\(_2\)PO\(_4\) was applied to treatments C1W2, R1W2 and R2W2 (see Table 4.1). Six evenly pre-germinated faba bean seeds were sown per pot 25 mm deep and later thinned to three seedlings per pot at 12 DAS (days after sowing). Pots were watered to 70% field capacity (18 % weight basis) using deionised water every 3 days for the first 21 days and then daily until harvest. The glasshouse temperature during the experimental period (May to November) ranged from a minimum of 8 °C at night to a maximum of 28 °C during the day under natural light conditions.

Nitrogen as NH\(_4\)NO\(_3\) (33 mg N kg\(^{-1}\) soil) was applied every 2 weeks till harvest. The shoots of faba bean were harvested at the initial pod-filling stage (75 DAS) and dried at 70°C for 48 hours. The dry matter content was recorded when the samples reached constant dry weight.

**Cereal phase**

The whole fresh roots remaining in soil after the harvest of faba bean in the treatments R1W2 and R2W2 were sieved and chopped to less than 5 mm size. Chopped roots were evenly mixed with the soil in R1W2 and R2W2 treatments, and pots were re-filled. The dried shoot residues of faba bean were amended in R2W2 treatments at the rate of 10 g per kilogram soil. All the basal nutrients were applied as before the legume phase. Inorganic P fertilizer (20 mg kg\(^{-1}\) soil) as KH\(_2\)PO\(_4\) was applied only to the C0W3 treatment.

Eight uniformly pre-germinated wheat seeds variety Westonia were sown at 20 mm depth in each pot. The seedlings were later thinned to four per pot at 14 DAS. The pots were watered to 70% field capacity (weight basis) once in 2 days throughout the experiment using deionised water. During the growth of wheat, N as NH\(_4\)NO\(_3\) (33 mg N kg\(^{-1}\) soil) was applied every fortnight till harvest.
4.2.5 Harvest and measurements

Soil samples collected at harvest of faba bean were air-dried for 48 hours and stored for analysis. Plant samples were collected at the harvest of wheat crop at 14, 21, 42, 56 and 77 days after sowing (DAS). The shoot and root samples from each harvest were washed with deionised water and oven-dried at 70°C for 72 hours until constant weight. The dry weight of plant samples was recorded, and the samples were stored for further analysis.

4.2.6 Soil analysis

The soil samples collected at the harvest of faba bean were analysed for general soil properties such as soil pH in 0.01 M CaCl\textsubscript{2} (Rayment and Higginson, 1992), cation exchange capacity and exchangeable cations (ICP-OES) (Rayment and Higginson, 1992), total carbon and nitrogen (LECO, CHN 1000, MI, USA) and Colwell P (Rayment and Higginson, 1992).

4.2.7 Plant analysis

Oven-dried shoots and roots were ground finely (<0.5 mm) and digested in 3:1 conc. HNO\textsubscript{3}:conc. HClO\textsubscript{4} mixture (see Validazeh et al., 2003). The nutrient contents in the digested plant samples were measured using ICP-OES. The total nitrogen content in the finely ground (<0.5 mm) plant material was determined using an Elementar analyser (CHN 1000, LECO, MI, USA).

4.2.8 Statistics

The statistical analyses were performed using SAS 9.3 statistical software. Repeated measures ANOVA was applied to determine the interaction effects among the P fertilization treatments over time. The significant differences in pair-wise comparisons of means were determined using Tukey's Studentized Range (HSD\textsubscript{0.05}) Test. The correlation and regression analyses were performed in Excel.

4.3 Results

4.3.1 Soil properties and plant materials
Chapter 4  
Nutrient uptake by wheat as influenced by soil amendment

The experimental soil was near-neutral with a soil pH$_{CaCl_2}$ of 6.5. The available P (Colwell P) in the top 10 cm layer of the soil was 8 mg kg$^{-1}$ soil and hence below the critical level of 10 mg kg$^{-1}$ required for plant growth in clay loam soil (Blaesing, 2006). The soil had low nitrate N (<10 mg kg$^{-1}$) and 14 mg soil organic C per kg (Table 4.1). Colwell K of 240 mg kg$^{-1}$ soil was well above the critical level of K required for plant growth.

The seed P content of faba bean grown in legume phase was 10 times higher than the seed P content of wheat, even though faba bean had almost 20-fold larger seed mass than wheat (Table 4.3).

Table 4.3: Seed P content in faba bean and wheat used in the experiment (Mean ± SE, n=3)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Average seed size (mg seed$^{-1}$)</th>
<th>Seed P content (mg seed$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Vicia faba</em> L. cv. Fiesta</td>
<td>690 ± 5</td>
<td>1.06 ± 0.03</td>
</tr>
<tr>
<td><em>Triticum aestivum</em> L. cv. Westonia</td>
<td>37 ± 1</td>
<td>3.105 ± 0.003</td>
</tr>
</tbody>
</table>

4.3.2  Legume phase

4.3.2.1 Legume crop dry matter production and P uptake

Shoot biomass of faba bean was not significantly higher than root biomass (Table 4.4). However, the root P accumulation in faba bean was higher than shoot P accumulation due to significantly higher concentration of P in roots than in shoots (Table 4.4).

Table 4.4: Plant biomass production and P uptake in faba bean grown in soil fertilized with 50 mg P kg$^{-1}$ for 75 days (Mean ± SE, n=3)

<table>
<thead>
<tr>
<th>Faba bean</th>
<th>Dry weight (g pot$^{-1}$)</th>
<th>P concentration (mg g$^{-1}$ dry matter)</th>
<th>P accumulation (mg pot$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shoots</td>
<td>15.9 ± 0.6 a</td>
<td>2.0 ± 0.1 b</td>
<td>31.3 ± 1.0 b</td>
</tr>
<tr>
<td>Roots</td>
<td>13.7 ± 0.6 a</td>
<td>3.5 ± 0.1 a</td>
<td>49.6 ± 0.3 a</td>
</tr>
</tbody>
</table>

Different letters denote significant differences between the means in a column (Tukey’s HSD$_{0.05}$, P < 0.05).
4.3.2.2 Effect of inorganic P fertilizer amendment and growth of faba bean on soil properties

The initial pH of the native (unplanted) soil significantly decreased from 6.5 to 6.0 a day after soil amendment with inorganic P fertilizer and other nutrients (Table 4.5). In contrast, the pH of the control soil with no P amendment but all other nutrients added (6.2) was not significantly affected. The growth of faba bean crop (at 75 DAS) significantly decreased the soil pH to 5.5, but remained significantly higher than the unplanted controls (5.1) regardless of the P fertilizer addition (Table 4.5).

The plant-available P (Colwell P) increased with addition of inorganic P fertilizer. About 65% of P added as inorganic fertilizer was found in the Colwell-P fraction a day after the addition of inorganic P fertilizer (Table 4.5). The amount of available P in the unplanted control with inorganic P amendment (treatment C1) decreased to 47% of the added P after 75 days. Soil in which faba bean was grown for 75 days showed 31% of P added through inorganic P fertilizers remaining in the Colwell-P fraction (Table 4.5). Hence, growth of faba bean crop significantly decreased availability of fertilizer P (P < 0.0001).

Inorganic P fertilizer amendment significantly increased the cation exchange capacity of soil a day after fertilizer amendment (P < 0.0001, Table 4.5). However, the CEC was found to decrease after 75 days, with no difference among control soils with or without P fertilizer addition and soil in which faba bean was grown for 75 days (Table 4.5). Soil amendment with inorganic P fertilizer had no significant effect on the exchangeable Ca and Mg in soil with time (Table 4.5), whereas growth of faba bean for 75 days significantly decreased exchangeable Ca and Mg in soil (P < 0.01). On the other hand, exchangeable K in soil significantly increased with amendment of inorganic P fertilizer after 75 days, but growing faba bean crop significantly decreased exchangeable K in soil (P < 0.0001, Table 4.5).
Table 4.5: Changes in soil properties with addition of inorganic P fertilizer and growth of faba bean during the legume phase (Means, n=3). No plants were grown in treatments C0 and C1. Faba bean was grown in treatment R.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH&lt;sub&gt;CaCl2&lt;/sub&gt;</th>
<th>Colwell P (mg kg&lt;sup&gt;-1&lt;/sup&gt; soil)</th>
<th>CEC</th>
<th>Exchangeable Ca (cmol(+) kg&lt;sup&gt;-1&lt;/sup&gt; soil)</th>
<th>Exchangeable Mg</th>
<th>Exchangeable K</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A day after fertilizer addition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control C0 (no added P)</td>
<td>6.2 a</td>
<td>9.7 d</td>
<td>9.7 b</td>
<td>6.3 a</td>
<td>3.2 ab</td>
<td>0.7 c</td>
</tr>
<tr>
<td>Control C1 (50 mg P kg&lt;sup&gt;-1&lt;/sup&gt; soil)</td>
<td>6.0 b</td>
<td>42.4 a</td>
<td>10.3 a</td>
<td>6.4 a</td>
<td>3.2 a</td>
<td>0.9 b</td>
</tr>
<tr>
<td><strong>75 days after fertilizer addition (faba bean harvest in treatment R))</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control C0 (no added P)</td>
<td>5.1 d</td>
<td>7.0 d</td>
<td>8.5 c</td>
<td>5.8 ab</td>
<td>2.9 bc</td>
<td>0.9 b</td>
</tr>
<tr>
<td>Control C1 (50 mg P kg&lt;sup&gt;-1&lt;/sup&gt; soil)</td>
<td>5.1 d</td>
<td>33.0 b</td>
<td>8.8 c</td>
<td>6.4 a</td>
<td>3.2 ab</td>
<td>1.3 a</td>
</tr>
<tr>
<td>Control with faba bean crop (R) (50 mg P kg&lt;sup&gt;-1&lt;/sup&gt;soil)</td>
<td>5.5 c</td>
<td>25.4 c</td>
<td>9.0 c</td>
<td>5.7 b</td>
<td>2.8 c</td>
<td>0.6 d</td>
</tr>
</tbody>
</table>

Different letters denote significant differences among treatment means in a column (Tukey’s HSD<sub>0.05</sub>).
4.3.3. Cereal phase

4.3.3.1 Plant shoot and root dry matter production

Wheat shoot biomass was initially (14 DAS) affected more by mixing soil with faba bean shoots (R2W2) than adding inorganic P fertilizer (C1W2, C0W3) or faba bean roots (R1W2), but remained significantly higher (P <0.0001) than the control treatment (C0W2) (Fig 4.1).

![Figure 4.1: Wheat shoot and root dry matter at 14, 28, 42, 56 and 77 days after sowing in soils amended with inorganic P fertilizer (prior to the legume phase - C1W2, or prior to the cereal phase – C0W3), faba bean residues (root only – R1W2, or root+shoot – R2W2) and in the non-amended control (C0W2). Means, n=3. The vertical bars indicate the minimum significant difference among the treatments at a given time (Tukey's HSD_{0.05}).]
After 14 DAS, wheat shoot biomass gradually increased in all treatments, with highest shoot biomass produced in the treatments with inorganic P fertilizer in the legume phase either without residues (C1W2) or root (R2W2) or both root and shoot residues (R1W2) (Figure 4.1); these treatments were significantly higher than the non-residue treatments with freshly added P (C0W3) or without P fertilizer addition (C0W2) prior to the wheat phase (P <0.0001).

Amendment of inorganic P fertilizers prior to either legume (C1W2) or wheat phase (C0W3) significantly increased wheat root biomass compared with the faba bean residue treatments at 14 DAS (Figure 4.1). Soil incorporation of faba bean as either roots+shoots (R2W2) or just roots (R1W2) gradually increased wheat root biomass after 14 DAS to become on par with the treatment with P fertilizers prior to the legume phase (C1W2), but remained significantly higher (P <0.0001) than the treatments with P fertilization prior to the wheat phase (C0W3) or non-fertilised control (C0W2) until the final wheat harvest at 77 DAS.

4.3.3.2 N, P and K concentration in wheat shoots and roots

Nitrogen concentration

Shoot N concentration in wheat remained similar among the treatments until 28 DAS (Figure 4.2); thereafter, shoot N concentration declined in wheat grown in soil amended with faba bean residues (R1W2, R2W2) or residual P fertilizer (C1W2). Application of inorganic P fertilizer prior to the cereal phase (fresh P fertilizer) in the C0W3 treatment was associated with significantly higher shoot N concentration than the C1W2, R2W2 and R1W2 treatments at 42 DAS, and declined subsequently. Shoot N concentration in the control treatment C0W2 did not change over time, but remained significantly higher than the inorganic P fertilizer treatment (C1W2) or faba bean residue amendments in the later stages of wheat growth (56 and 77 DAS).

Root N concentration was significantly higher (P <0.0001) in wheat grown in soil amended with P as either inorganic P fertilizer (C1W2, C0W3) or faba bean residues (R1W2, R2W2), whereas wheat roots in the control with no added P (C0W2) had significantly lower N concentration at 14 DAS (Figure 4.2). Even though N concentration in wheat roots declined over time, no significant differences were observed among the treatments at the end of the wheat-growing period.
Phosphorus concentration

Soil P amendment as either inorganic P fertilizer (C1W2, C0W3) or faba bean residues (R1W2, R2W2) significantly increased shoot P concentration in wheat in comparison with the non-amended control treatment (C0W2) (P <0.0001, Figure 4.2). Wheat shoot P concentration significantly declined over time in the C1W2, R1W2 and R2W2 treatments, whereas the C0W3 treatment showed an increase in P concentration after 42 DAS (Figure 4.2). Wheat had higher shoot P concentration in the R2W2 than R1W2 and C1W2 treatments throughout the growth period. Shoot P concentration was significantly lower (P <0.0001) in the C1W2 than faba bean residue (R2W2, R1W2) treatments at the final harvest (77 DAS). Fresh application of inorganic P fertilizer prior to cereal phase (C0W3) led to significantly lower shoot P concentration in wheat in the initial stages of growth and up to 42 DAS than the inorganic P fertilizer amendment prior to the legume phase (C1W2). Shoot P concentration in C0W3 increased with time and was on par with the faba bean residue-amended treatments at 77 DAS (Figure 4.2). Shoot P concentration in wheat grown on control soil with no P amendment (C0W2) remained unchanged over time and was significantly lower than in the treatments with inorganic P fertilizer or faba bean residues (P <0.0001).

Phosphorus concentration in wheat roots was significantly higher in the R2W2 treatment followed by R1W2 and C1W2 treatments at 14 DAS, and no significant differences were observed among these treatments thereafter (Figure 4.2). Fresh addition of P fertilizer prior to the cereal phase (C0W3) resulted in lower root P concentration in comparison with C1W2, R1W2 or R2W2. However, root P concentration was higher in C0W3 than C1W2 and was on par with R1W2 and R2W2 treatments (Figure 4.2). Root P concentration in the control treatment (C0W2) slightly increased over time and was similar to C1W2 at 77 DAS.

Potassium concentration

Shoot K concentration in wheat significantly increased with P amendments (inorganic P fertilizer or faba bean residues) at 14 DAS and gradually declined over time (P <0.0001, Figure 4.2). At the end of the growth period (77 DAS), the C0W3 treatment had higher shoot K concentration than C1W2, R1W2 and R2W2. Wheat shoot K concentration was significantly lower in the control treatment than other treatments at 14 DAS and was
significantly higher than in the other treatments at 77 DAS (P < 0.0001). Root K concentration was not influenced by soil P amendments or duration of wheat growth (Figure 4.2).

Figure 4.2: N, P and K concentrations in wheat shoots and roots during growth (14, 28, 42, 56 and 77 DAS) in soils amended with inorganic P fertilizer (prior to the legume phase - C1W2, or prior to the cereal phase – C0W3), faba bean residues (root only – R1W2, or root+shoot – R2W2) and in the non-amended control (C0W2). Means, n=3. Different letters denote significant differences among treatments for either shoots or roots at a given time (Tukey’s HSD$_{0.05}$).
4.3.3.3 Total N, P and K uptake in wheat

Total N uptake

Total N uptake was significantly higher in wheat grown in C1W2, R1W2 and R2W2 than the other treatments, despite the same amount of N applied in all the soil treatments (P <0.0001, Figure 4.3). Wheat accumulated more N when grown in the C1W2 than R1W2 and R2W2 treatments (Figure 4.3). Freshly added P in C0W3 did not affect N uptake until 56 DAS and was significantly higher than the control (Figure 4.3). Nitrogen uptake by wheat grown in control soil (C0W2) with no P added was significantly lower than that in the other treatments.

Total P uptake

Wheat grown in soils amended with faba bean residues (R2W2, R1W2) and inorganic residual P fertilizer (C1W2) showed significantly higher total P accumulation (P <0.0001) than the C0W3 and control (C0W2) treatments (Figure 4.3). There was no significant difference in wheat P uptake among R2W2, R1W2 and C1W2 treatments until 56 DAS (Figure 4.3). However, at final harvest (77 DAS), wheat grown with faba bean residues (R1W2, R2W2) accumulated more P from soil, whereas wheat grown in C1W2 showed no further P accumulation after 56 DAS (Figure 4.3). Wheat showed relatively low P accumulation when grown in soil with freshly added inorganic P fertilizer (C0W3), being similar to the control (C0W2) treatment (Figure 4.3).

Total K uptake

Soil amendment with faba bean residues (R2W2, R1W2) or inorganic P fertilizer (C1W2) prior to legume phase resulted in increased K uptake by wheat compared with the C0W3 and control (C0W2) treatments despite the quantity of K added to soil being the same. No significant differences were observed among C1W2, R1W2 and R2W2 in wheat K accumulation. However, returning faba bean shoots (R2W2) to soil increased K accumulation in wheat compared with just whole root incorporation (R1W2) or inorganic P fertilization (C1W2). An addition of fresh P fertilizer (C0W3) had no influence on wheat K uptake in comparison with the control until 56 DAS, with a significant amount of K accumulated between 56 days and 77 DAS (Figure 4.3).
Figure 4.3: Total N, P and K accumulation in wheat crop at 14, 28, 42, 56 and 77 days after sowing in soils amended with inorganic P fertilizer (prior to the legume phase - C1W2, or prior to the cereal phase – C0W3), faba bean residues (root only – R1W2, or root+shoot – R2W2) and in the non-amended control (C0W2). Means, n=3. Different letters denote significant differences among treatments at a given time (Tukey’s HSD$_{0.05}$, p ≤ 0.05).
4.3.3.4 Ca, Mg, S, Zn and Mn concentration in wheat shoots and roots

Wheat grown on soil with no added P (C0W2) tended to have higher Ca and Mg concentration in shoots than on soil with P amendments (Figure 4.4). Lowest Ca concentration in wheat shoots was observed in the R1W2 and R2W2 treatments. Inorganic P fertilization in C1W2 and C0W3 was associated with higher shoot Ca at 14 DAS (P <0.01) that declined with duration of wheat growth and with soil P amendments. Even though Mg concentration in wheat shoots was similar among the treatments until 42 DAS, a significant increase in Mg concentration was observed in the C0W2 treatment between 56 and 77 DAS (Figure 4.4). Root Ca and Mg concentration in wheat was not affected by soil P amendments, and no significant differences were observed among the treatments.

Of the major secondary nutrients, S concentration in wheat shoots was enhanced with soil P amendments (Figure 4.4). Shoot S concentration was significantly higher in the R2W2, R1W2 and C1W2 treatments with higher amounts of added P than in the control treatment (C0W2) with no added P initially at 14 DAS (P <0.05). The highest shoot S concentration was observed in wheat shoots grown in R2W2 at 28 DAS, with differences among the treatments becoming less obvious afterwards (Figure 4.4). However, shoot S concentration was generally lower in C0W3 and C0W2 than the other treatments until 28 DAS. The S concentration in wheat shoots was highly correlated with wheat P uptake (P <0.05). There was no significant difference in root S concentration among the treatments and with duration of wheat growth (Figure 4.4).

Zinc concentration in wheat shoot improved with P amendments in soil at 14 DAS (P <0.01, Figure 4.4). the highest peak in Zn concentration was observed in the C1W2 and R2W2 treatments. There was no significant difference in Zn concentration in wheat shoots between 14 and 56 DAS. A significant decrease in Zn concentration was observed in wheat shoots grown in soils with faba bean residue amendments (R1W2 and R2W2) compared with C0W2 and C0W3 treatments at 77 DAS (Figure 4.4). Root Zn concentration increased with soil P amendments, but no significant difference was observed except for the control (C0W2) that remained significantly lower than the other treatments throughout the wheat-growing period.
Figure 4.4: Ca, Mg, S, Zn and Mn concentration in shoots and roots of wheat at 14, 28, 42, 56 and 77 days after sowing in soils amended with inorganic P fertilizer (prior to the legume phase - C1W2, or prior to the cereal phase – C0W3), faba bean residues (root only – R1W2, or root+shoot – R2W2) and in the non-amended control (C0W2). Means, n=3. The vertical bars denote ±SE. Different letters denote significant differences among treatments at a given time (Tukey’s HSD$_{0.05}$, P ≤ 0.05).
Shoot Mn concentration in wheat significantly increased with crop growth in the control treatment without P added (C0W2) \( (P < 0.05) \). Soil P amendment in the form of inorganic P fertilizer in either the legume (C1W2) or the cereal phase (C0W3) increased Mn concentration in wheat shoots more than incorporation of faba bean residues (R1W2, R2W2) until 28 DAS, but later declined with crop growth (Figure 4.4). Root Mn concentration was significantly higher in the C0W3 treatment throughout the entire growth period \( (P < 0.05) \). Again, Mn concentration in wheat roots was significantly \( (P \leq 0.05) \) lowered by soil incorporation of faba bean residues (Figure 4.4).

### Table 4.6: Correlation between wheat biomass yield and shoot nutrient concentrations

<table>
<thead>
<tr>
<th>Wheat growth stage</th>
<th>Parameters</th>
<th>Shoot nutrient concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N</td>
</tr>
<tr>
<td>14 DAS</td>
<td>Shoot biomass</td>
<td><strong>+</strong></td>
</tr>
<tr>
<td></td>
<td>Root biomass</td>
<td>-*</td>
</tr>
<tr>
<td>28 DAS</td>
<td>Shoot biomass</td>
<td><strong>+</strong></td>
</tr>
<tr>
<td></td>
<td>Root biomass</td>
<td>+*</td>
</tr>
<tr>
<td>42 DAS</td>
<td>Shoot biomass</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Root biomass</td>
<td>ns</td>
</tr>
<tr>
<td>56 DAS</td>
<td>Shoot biomass</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Root biomass</td>
<td>ns</td>
</tr>
<tr>
<td>77 DAS</td>
<td>Shoot biomass</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Root biomass</td>
<td>ns</td>
</tr>
</tbody>
</table>

The symbols + / - denote positive or negative correlation \((r)\); ** significant at \( P \leq 0.0001 \); * significant at \( P \leq 0.001 \); * significant at \( P \leq 0.05 \); and ns not significant at \( P \leq 0.05 \).

### 4.3.3.5 Correlation between accumulations of various nutrients during wheat growth

Shoot biomass had a significant positive influence on nutrient concentration in wheat shoots at 14 DAS. However, shoot nutrient concentration in wheat did not increase with shoot biomass during wheat growth, except for P concentration that increased with an
increase in shoot dry matter (Table 4.6). Root biomass showed a significant positive correlation with shoot P concentration in wheat until 56 DAS.

The initial N concentration in wheat roots at 14 DAS was highly positively correlated with the shoot dry matter yield (P <0.001) and total N, P, K uptake (P <0.05) and was negatively correlated with root dry matter yield (P <0.05) (Table 4.6). The total N, P and K uptake in wheat showed significant positive influence on the root P concentration until 56 DAS (Appendix 4.3). The K concentration in wheat roots was significantly correlated with total N, P and K uptake and dry matter yield (shoot and root) in wheat (P <0.05, Appendix 4.2, 4.3). Root Zn concentration showed significant positive correlation with total N, P and K uptake and dry matter production in wheat until 56 DAS (Table 4.3).

4.4 Discussion

4.4.1 Soil and plants

The native soil used in present study being near neutral (pH = 6.5) was considered favourable for plant growth, but had very low N and P to support plant growth (Table 4.1). The clay loam texture of the soil dominated by clay minerals (Lindsay, 1979; Hinsinger, 2001; Devau et al., 2010) decreased availability of P by the process of adsorption (Vu et al., 2008). However, plants growing in low-P soils showed good mobilization of applied P fertilizers, probably by developing adaptive strategies through modifications of the root system and the rhizosphere (Richardson et al., 2011).

Faba bean as a legume crop with 10-fold higher seed P content than wheat (Table 4.3) is effective in mobilizing and acquiring soil P (Li et al., 2004; Nuruzzaman et al., 2005b; Rose et al., 2010a; Wang et al., 2011; Hassan et al., 2012a, b). Zhu and Smith (2001) showed that plants grown from seeds with high P reserves achieve better root growth and tend to accumulate more P from soil. Previously, Baker (1972) and Lloret et al. (1999) reported that plants from large seeds allocate a large proportion of resources to roots. Several studies have proved that faba bean accumulated more P from applied P fertilizers in comparison with other legumes (Hens and Hocking, 2004; Nuruzzaman et al., 2005b; Li et al., 2007; Rose et al., 2010b) or cereals (Li et al., 2004; Nuruzzaman et al., 2005a). Regardless of the P fertilizer application, availability of soil
Nutrient uptake by wheat as influenced by soil amendment

P improved with growth of faba bean in biologically-managed farming systems (Hardiputra, 2011) with higher organic matter than in conventionally-managed soils (Hassan, 2012). Hence, faba bean is considered a suitable P-mobilizing legume crop in a rotation on soils with low P availability (Nuruzzaman et al., 2005a; Li et al., 2007; Rose et al., 2010b).

4.4.2 Growth and P uptake by faba bean

Soil pH decreased with or without the addition of inorganic P fertilizer and with growth of faba bean (Table 4.5). However, growing faba bean on soil fertilized with inorganic P slightly increased soil pH (Xu et al., 2006a; Rose et al., 2010b; Wang et al., 2012). In the present study, a decrease in soil pH might have been caused by nitrification of NH$_4$ (cf. Neilsen et al., 1994; Bouman et al., 1995) present in NH$_4$NO$_3$ added as basal N fertilizer in all soil treatments.

Even though the soil pH declined at the end of growth period of faba bean (after 75 days) in all soil treatments, the pH in soils was slightly higher in the treatments with the growth of faba bean (75 DAS) in comparison with the unplanted control or inorganic P fertilized treatments (Table 4.5). A similar increase in soil pH with plant growth in Bindoon soil was reported by Rose et al. (2010b), with the highest increase observed in soils with faba bean. Results from studies by Tang et al. (1999), Haynes and Mokolobate (2001), Pearse et al. (2006, 2007) and Rose et al. (2010a) showed that the rhizosphere soil pH may increase, mainly due to plant uptake of NO$_3^-$ as the major source of N. However, soil pH may also increase because of decomposition of old faba bean roots (Tang and Yu, 1999) and release of excess cations followed by decarboxylation of organic anions (Yan et al., 1996; Tang and Yu, 1999) and ammonification (Valles et al., 1993; Hinsinger and Gilkes, 1996; Neumann and Römheld, 2002; Hardiputra, 2011).

Addition of P fertilizer to soil increased P availability initially (Table 4.5), but decreased it later due to (i) decrease in soil pH (Table 4.5) with addition of P fertilizer that might have led to an increase in adsorption of P by Al and Fe hydroxides; and/or (ii) microbial immobilization. About 16% of applied inorganic P fertilizer was taken up by faba bean during 75 days of growth (Table 4.5), with the rest of the added P temporarily immobilized into less available pools in soil (Bolland et al., 1999) due to
sorption or microbial immobilization; with time, these P pools might become available or fixed depending on the soil properties and crop management practices.

Higher P accumulation in faba bean roots than shoots (Table 4.4) was reported before (Nuruzzaman et al., 2005a). These authors also observed shoot P concentration increasing with P fertilization to a greater extent in faba bean than other legumes. Similar results were obtained by Hardiputra (2011), suggesting a high capacity of faba bean to accumulate P in shoots when supplemented with P fertilizers as well as to acquire P from poorly accessible soil P pools without P fertilization. However, this varies with soil type and management practices.

Exchangeable Ca, Mg and K in soil decreased with the growth of faba bean (Table 4.5), which might have been due to uptake by faba bean roots (Marschner 1995; Moritsuka et al., 2000). However, an accumulation of exchangeable cations (Ca, Mg and K) in the rhizosphere of trees has been reported in rhizotron experiments (Bakker et al., 1999; Wang et al., 2001) and in-situ with trees grown in field conditions (Gobran and Clegg 1996; Séguin et al. 2005; Turpault et al. 2005; Calvaruso et al. 2011). Collignon et al. (2011) concluded that exchangeable cations (Ca, Mg and K) in soil decrease during the vegetation period due to tree uptake, but increase with rhizodeposition (Jones et al. 2004; Jones et al. 2009) from roots during winter with no vegetative growth. Hence, returning crop residues back to soil returns nutrients taken up by plants, enhancing nutrient cycling.

4.4.3 Growth and P uptake by wheat following faba bean

Wheat growth following a legume crop with root incorporation was not significantly affected where 40 mg P kg\(^{-1}\) soil was applied to legume (Nuruzzaman et al., 2005b) and even where 100 mg P kg\(^{-1}\) was applied, irrespective of residue incorporation (Hassan, 2012). In contrast, in the present study, returning faba bean residues back to soil as either whole roots or whole roots plus shoots (10 g kg\(^{-1}\) soil) increased the wheat biomass yield more than in the treatments receiving 20 mg P kg\(^{-1}\) soil as fresh P fertilizer (C0W3) or no P fertilizer (C0W2) (Figure 4.1). This is consistent with the results obtained in previous studies by Horst et al. (2001), Kamh et al. (2002), Nuruzzaman et al. (2005a) and Jemo et al. (2006) where wheat growth improved following a legume pre-crop. However, an early decrease in wheat growth in soils
supplemented with faba bean residues might have been due to initial P immobilization in soil via increased microbial activity (McLaughlin and Alston, 1986); later, net P mineralization and a release of P from microbial cells would occur with a decrease in available C with time (Marschner et al., 2011). In the long-term, nutrient release from the faba bean residues would increase availability of the nutrients and thereby would improve soil fertility.

Soil incorporation of faba bean residues improved P tissue concentration in the subsequently grown wheat, particularly regarding P accumulated in shoots. Furthermore, slightly higher shoot P concentration in wheat was found in the treatment with returning shoots of faba bean (R2W2) to soil compared with incorporation of faba bean roots only (R1W2) (Figure 4.2). Nuruzzaman et al. (2005a) reported an increase (albeit not significant) in wheat P concentration and uptake when grown after incorporation of faba bean roots in comparison with root incorporation of other legume crops. Even though growing wheat after legume pre-crops decreased wheat growth irrespective of residue addition, Hassan et al. (2012) observed increased shoot P concentration in wheat grown in rotation with faba bean and with residue incorporation in comparison with other legumes.

Increased P accumulation in wheat shoots with incorporation of faba bean residues may have resulted from (i) higher P availability with P released from decomposition of faba bean residues in soil (Tian et al., 1992; Nziguheba et al., 2000; Ryan et al., 2001); (ii) net mineralization of soil P with addition of high-P faba bean residues (Cheshire and Chapman, 1996; Brady and Weil, 2002); and (iii) desorption of soil-adsorbed P by organic acid anions released from decomposing faba residues (Gahoonia and Nielsen, 1992; Jungk et al., 1993; Iyamureme and Dick, 1996; Barrow et al., 1998; Hu et al., 2005 a, b; Burkitt et al., 2008; Hardiputra, 2011).

Shoot P concentration in wheat grown with faba bean residue amendments (R1W2, R2W2) was adequate (1.6 mg g\(^{-1}\) dry matter at Feekes scale 10.1), whereas the residual P fertilizer resulted in shoot P concentration (<1.5 mg g\(^{-1}\) dry matter at Feekes scale 10.1) below the critical level required for wheat growth (Reuter and Robinson, 1997). In contrast to residual P fertilizer (C1W2), application of fresh inorganic P fertilizer (C0W3) resulted in adequate shoot P concentration at 77 DAS (Reuter and Robinson, 1997), which might be attributed to relatively poor wheat growth in soil.
amended with fresh P fertilizer (Figure 4.2). Results from the incubation study in Chapter 3 showed an increase in residual P pool in soil amended with inorganic P fertilizer due to immobilization and adsorption, whereas incubation of soil with crop residues increased labile soil P. This suggests that the efficiency of inorganic P fertilizer applied at a low rate (C0W3) is low in improving the crop growth, and when applied at a high rate (C1W2) is low in improving P uptake in wheat. However, incorporation of crop residues improves P fertilizer efficiency (Figure 4.3) (Sharma and Prasad, 2003; Waigwa et al., 2003; Nuruzamman et al., 2005a, b; Gupta et al., 2007; Richardson et al., 2011).

Higher P concentration in wheat shoots was observed in the faba bean roots+shoots (R2W2) treatment during the initial stages of crop growth compared with the treatment with only roots returned. This proves the added benefits of returning shoot residues to soil to enhance P nutrition of subsequent wheat crop. Earlier studies by Blair and Bolland (1978) and Dalal (1979) also demonstrated the significance of whole-plant residues in P cycling.

Root P concentration in wheat was initially (14 DAS) higher in the faba residue treatments (particularly in roots+shoots (R2W2), Figure 4.2) due to a lack of dilution in P tissue concentration because of low initial root dry matter production (Figure 4.1). Even though the fresh application of inorganic P fertilizer (20 mg P kg\(^{-1}\) soil) in C0W3 added less P than the residual P fertilizer (50 mg P kg\(^{-1}\) soil), root P concentration at the final harvest (77 DAS) was higher in wheat with freshly applied than residual inorganic P fertilizer (Figure 4.2). Low shoot and root P concentrations in wheat at the later stages of growth in the residual P fertilizer (C1W2) treatment reflected a reduction in P availability in soil due to meeting the P requirement of the growing wheat. In contrast, incorporation of high-P-content faba bean residues (either roots only or roots+shoots) led to net P mineralization (Brady and Weil, 2002) and thus improved soil P availability.

Wheat shoot N concentration improved with increased P availability in early stages of growth irrespective of the treatments (Figure 4.2). A later decline in N concentration was mainly due to a 10-fold increase in shoot biomass compared with root biomass, resulting in dilution of shoot N concentration (Figure 4.1). In wheat grown with no added P, there was little difference in N and P concentration in shoots
during growth, probably because of relatively poor crop growth due to lack of P supplementation despite adequate N nutrition.

Total P uptake in wheat increased in treatments with faba bean residues returned to soil (especially incorporation of shoots and roots). Increased addition of N through faba bean residues might have increased the rate of organic matter decomposition, resulting in net P mineralization (Cheshire and Chapman, 1996; Baggie et al., 2004) of high-P faba bean residues and subsequently increased P uptake by wheat. In contrast, Hassan et al. (2012a, b, c) reported decreased growth and P uptake in subsequently grown wheat with addition of previously grown legume residues. This might be due to differences in management practices in residue incorporation, and properties of crop residues and soils. Hence, nutrient recycling can be enhanced by efficient agronomic management practices.

Shoot Ca concentration in wheat in the early stage was higher in the inorganic P amended treatments than faba bean residue treatments (Fig. 4.4; see also Li et al., 2004). Returning faba bean shoots to soil along with roots initially increased shoot Ca concentration more than root incorporation alone largely due to return of nutrients removed by faba bean shoots (Figure 4.4). Later, Ca concentration in wheat shoots declined in P-amended treatments due to an increase in shoot biomass with wheat growth causing dilution in Ca concentration. A similar effect of P amendments was observed on Mg concentration in wheat shoots (Figure 4.4).

Shoot S concentration in wheat increased with increased soil P availability in the early stages of wheat growth (Figure 4.4). This is in agreement with the findings of Santoso et al. (1995) who reported an increase in S uptake by maize with addition of P fertilizers. Also, S accumulation in wheat shoots significantly improved with increased P application (Mamaril and Millers, 1970; Lefroy et al., 1995). In the present study, growth of faba bean might have enhanced the mobilization of S in the soil rhizosphere by the organic exudates from faba bean roots displacing adsorbed sulphate in soil (Evans and Anderson, 1990). As available P declined with time due to P uptake by growing wheat, S concentration in wheat shoots started to decrease despite adequate S nutrition for wheat growth (shoot S concentration >1.5 mg g\(^{-1}\) dry matter, Reuter and Robinson, 1997) (Figure 4.4).
Zinc concentration in wheat shoots was adequate for plant growth in all treatments irrespective of P fertilization (Reuter and Robinson, 1997). A decrease in soil pH would increase solubility of Zn (Rengel, 2001) and thereby improve Zn uptake. Root Zn concentration increased with low P availability in freshly applied fertilizer P (20 mg P kg\(^{-1}\) soil) compared with residual P fertilizer treatment (50 mg P kg\(^{-1}\) soil). Several studies (Burleson et al., 1961; Robson and Pitman, 1983; Marschner, 1997; Kacar and Katkat, 1998; Zhu et al., 2002; Zhao et al., 2007) showed a decrease in Zn uptake by plant roots with increased P fertilization.

Increased P fertilization increased Mn uptake by wheat (Somers and Shive, 1942). In the present study, the inorganic P fertilizer treatments resulted in increased Mn concentration in shoot (Figure 4.4). Several studies observed a simultaneous increase in P and Mn uptake by plants (Brown and Jones, 1975; Mathan and Amberger, 1977; Singh and Pathak, 1968; Wanasuria and Kuhn, 1977; Kuo and Mikkelsen, 1981). Shoot Mn concentration was higher in the inorganic P fertilizer than faba bean residue treatments (Figure 4.4), with P being released slowly with mineralization of organic P (Li et al., 2004). A decrease in soil pH in inorganic P fertilizer treatments (Table 4.5) may have contributed to increased Mn availability (Rengel, 2008; Khabaz-Saberi et al., 2010) and, thus, improved Mn uptake.

4.5 Conclusions

Enhanced P mobilization by faba bean may be beneficial in rotation with wheat. Wheat growth and P uptake can be improved with incorporation of faba bean roots and shoots due to enhanced soil P cycling. Besides addition of P via decomposition of legume residues, incorporating such residues into soil would mobilize native soil P or residual fertilizer P from a previous crop and thereby can reduce the P fertilizer requirement of subsequent crops, enhancing soil fertility and farm productivity.
CHAPTER 5

Fractionation of soil phosphorus after incorporation of post-harvest crop residues varying in C:N ratio and growing wheat at two N fertilization rates

5.1 Introduction

Phosphorus nutrition in agriculture is reliant on application of mineral fertilizers that can, at least partly, be replaced by organic fertilizers. Nutrient recycling via incorporation of crop residues is one of the measures that can enhance sustainability of agricultural ecosystems. Plant residues improve soil fertility by supplying nutrients and thus minimizing the fertilizer cost in crop production.

Nutrient availability from a crop residue is dependent on a number of factors. Among them, content of C, N, P and S in residues is important in influencing rate of decomposition and nutrient release from crop residues (Schoena and Campbell, 1996).

Several studies have reported that decomposition of crop residues was influenced by properties such as C:N, lignin and polyphenol content (Palm, 1995; Vanlauwe et al., 1996; Lupwayi et al., 2000; Wang et al., 2004; Kriaučiūnienė et al., 2012). The initial mineralization or immobilization of nutrients is mostly influenced by C:N ratio of decomposing residues (Parr and Papendick, 1978; Ghidey and Alberts, 1993). Decomposition of plant residues is governed by the dynamics of N availability (Jenkinson, 1981; Mary et al., 1996) that changes with a release of nutrients from residues over time (Trinsoutrot et al., 2000; Nicolardot et al., 2001). Hence, crop residue decomposition is influenced by the N content and C:N ratio initially, but not later (Gilmour et al., 1998).

An application of N enhanced decomposition of crop residues (Bangar and Patil, 1980; Debnath and Sinha, 1993), influencing availability of P from residues (Fuller et al., 1956; Dalal, 1979). Hence, N mineralization is a crucial process influencing nutrient dynamics in the soil-plant system.

Availability of P from crop residues varies depending on the properties of the soil and added residues (Nwuke et al., 2004). Crop residues could modify availability of native soil P via products of decomposition (Yadvinder-singh et al., 1992; Nwuke, 2004). Concentrations of N and P are usually greater in legume than cereal residues,
which would influence rate of decomposition and determine the amount of these nutrients that could be returned to soil (Strong et al. 1986; Armstrong et al. 1994). Moreover, high total N as well as water-soluble N content of residues had a positive effect on the readily available soil P pool (resin-P fraction), whereas availability of P from decomposing plant residues was inversely proportional to the residue C:N ratio (Iqbal, 2009).

Incorporation of plant residues and farm yard manures increased soil N, P, K, S and Zn contents (Gill and Meelu, 1982; Zia et al., 1992). Agarwal et al. (1997) observed a significant increase in available N and P with incorporation of clusterbean residues and farm yard manure along with fertilizer N under field conditions. In a long-term field experiment conducted to study P transformation in soil supplemented with mineral fertilizers and organic matter via roots and senesced leaf residues, addition of N fertilizer indirectly increased organic P fraction in soil (McKenzie et al., 1992a, b).

With previous research on N and P dynamics in relation to incorporation of crop residues, it is obvious that availability of N has a direct effect on P mineralization. In a recent review, it has been concluded that the interaction between N and P dynamics in soil has received relatively little attention because a majority of research on soil P has been focused on the interaction between P dynamics and soil C (Bünemann et al., 2011). Hence, in the present study, an experiment was conducted to (i) study the effect of different rates of applied N and P amendments (crop residues or inorganic P fertilizer) on growth and P uptake by wheat, and (ii) characterize the soil P pools during crop growth with soil P amendments (inorganic P fertilizer or crop residues varying in C:N ratio) at low and optimal N fertilization.

5.2 Materials and methods

5.2.1 Soil

Soil low in total P was collected from the top 10 cm of unfertilised native vegetation site near Bindoon in Western Australia (31° 18’ 36” S, 116° 07’ 60” E). The general properties of selected soil are listed in Table 5.1. Colwell P and soil pH (1:5 soil: water/CaCl₂) were measured according to Rayment and Higginson (1992). Soil P fractions and total P were determined using the method of Tiessen and Moir (1993)
fractionation. The soil P fractions were grouped into labile Pi (Water-Pi + Resin-Pi + Bicarbonate-Pi), moderately-labile Pi (NaOH-Pi + 1M HCl-Pi), stable Pi (Conc. HCl-Pi) and residual P (persulphate digestion) based on Tiessen and Moir (1993). Total C and N were measured using an Elementar analyser (CHN 1000, LECO, MI, USA).

The water-holding capacity of soil was determined to be 21% w/w by saturating 5 kg soil in a free-draining pot overnight and allowing water to drain for 24 hours (Rose et al., 2008). The soil was air-dried and sieved to 2-mm prior to potting and chemical analyses.

Table 5.1: Basic properties of soil (Mean ± SE, n=3)

<table>
<thead>
<tr>
<th>Soil properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:5 soil:CaCl₂)</td>
<td>4.85 ± 0.03</td>
</tr>
<tr>
<td>pH (1:5 soil:water)</td>
<td>5.99 ± 0.03</td>
</tr>
<tr>
<td>Colwell P (mg kg⁻¹)</td>
<td>9.5 ± 0.5</td>
</tr>
<tr>
<td>Organic C (g kg⁻¹)</td>
<td>24 ± 0.2</td>
</tr>
<tr>
<td>Total C (g kg⁻¹)</td>
<td>26 ± 0.2</td>
</tr>
<tr>
<td>Total N (g kg⁻¹)</td>
<td>2 ± 0.1</td>
</tr>
<tr>
<td>Total P (mg kg⁻¹)</td>
<td>178 ± 0.4</td>
</tr>
</tbody>
</table>

Soil P pools

<table>
<thead>
<tr>
<th>Soil P pools</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Labile Pi (mg kg⁻¹)</td>
<td>9.6 ± 0.6</td>
</tr>
<tr>
<td>Moderately labile Pi (mg kg⁻¹)</td>
<td>30 ± 0.6</td>
</tr>
<tr>
<td>Stable Pi (mg kg⁻¹)</td>
<td>48 ± 1.1</td>
</tr>
<tr>
<td>Residual P (mg kg⁻¹)</td>
<td>39 ± 0.9</td>
</tr>
</tbody>
</table>

5.2.2 Crop residues

Two crop residues (wheat straw and field peas straw) varying in C:N ratio but with similar P content were used. The quality of crop residues used for the experiment is described in Table 5.2.
Table 5.2: Nutrient content of crop residues (Mean ± SE, n=3)

<table>
<thead>
<tr>
<th>Nutrient content</th>
<th>Wheat straw</th>
<th>Pea straw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total P (g kg(^{-1}))</td>
<td>0.5 ± 0.03</td>
<td>0.6 ± 0.01</td>
</tr>
<tr>
<td>Total N (g kg(^{-1}))</td>
<td>5.8 ± 0.09</td>
<td>12.5 ± 0.2</td>
</tr>
<tr>
<td>Total C (g kg(^{-1}))</td>
<td>443 ± 5.3</td>
<td>463 ± 0.6</td>
</tr>
<tr>
<td>C:N</td>
<td>76.6 ± 0.4</td>
<td>37.1 ± 0.5</td>
</tr>
<tr>
<td>C:P</td>
<td>957 ± 14.7</td>
<td>760 ± 12.2</td>
</tr>
</tbody>
</table>

5.2.3 Plant material

Wheat (*Triticum aestivum*) variety Wyalkatchem was used in a bioassay to determine the dynamics of plant-available P during the course of treatments.

5.2.4 Experimental design

The experiment comprised a factorial arrangement of treatments (Table 5.3) with two levels of P (P1 = 10 mg P kg\(^{-1}\) soil, P2 = 40 mg P kg\(^{-1}\) soil) applied as inorganic P fertilizer (KH\(_2\)PO\(_4\)) or crop residues (pea straw or wheat straw) and two levels of N (low N = 75 mg N kg\(^{-1}\) soil, optimal N = 300 mg N kg\(^{-1}\) soil) applied as inorganic fertilizer (NH\(_4\)NO\(_3\)) in five splits (every 2 weeks) along with the controls (soil with no added P and with either low or optimal N fertilization) replicated in four blocks. Each treatment was replicated thrice within each block.

5.2.5 Plant growth conditions

An experiment was conducted in a glasshouse at the University of Western Australia (31.58 S, 115.49 E) during May to August under natural light conditions. Temperatures over the duration of the experiment ranged from minimum of 15 °C at night to maximum of 30 °C during the day.

Three kilograms of sieved soil (<2mm) were weighed into sealed pots (15 cm diameter and 15 cm deep) lined with plastic bags. Basal nutrient solutions except N and P were applied to all pots (mg kg\(^{-1}\) soil): 280 K\(_2\)SO\(_4\), 60 MgSO\(_4\).7H\(_2\)O, 10 MnSO\(_4\).H\(_2\)O, 13.5 ZnSO\(_4\).7H\(_2\)O, 3 CuSO\(_4\).5H\(_2\)O, 1.05 H\(_3\)BO\(_3\), 0.6 CoSO\(_4\).7H\(_2\)O and 0.3
Na$_2$MoO$_4$·2H$_2$O. Nitrogen as NH$_4$NO$_3$ and P as KH$_2$PO$_4$ were applied as nutrient solutions to the corresponding treatments. The soil was air-dried for 24 hours after nutrient application and mixed thoroughly. The dry straw residues were chopped, sieved (<2 mm) and applied to the corresponding treatments in quantities to give the required P levels. The soils with added nutrients and residues were mixed by a mechanical soil mixer to achieve uniform distribution and were re-potted. Soil in the pots was wet to around field capacity with deionized water. The pots were then placed in a glasshouse for 3 weeks to allow time for initial decomposition of crop residues, which would also allow avoiding initial nutrient immobilization phase.

Table 5.3: Details on the treatments applied

<table>
<thead>
<tr>
<th>Treatments</th>
<th>P fertilization, mg P kg$^{-1}$ soil</th>
<th>N fertilization, mg N kg$^{-1}$ soil</th>
<th>Treatment code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control soil with no added P</td>
<td>P0</td>
<td>Low, N75</td>
<td>P0N1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optimal, N300</td>
<td>P0N2</td>
</tr>
<tr>
<td>Soil + added inorganic P fertilizer</td>
<td>P10</td>
<td>Low, N75</td>
<td>P1N1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optimal, N300</td>
<td>P1N2</td>
</tr>
<tr>
<td></td>
<td>P40</td>
<td>Low, N75</td>
<td>P2N1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optimal, N300</td>
<td>P2N2</td>
</tr>
<tr>
<td>Soil + pea straw</td>
<td>P10</td>
<td>Low, N75</td>
<td>R1N1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optimal, N300</td>
<td>R1N2</td>
</tr>
<tr>
<td></td>
<td>P40</td>
<td>Low, N75</td>
<td>R2N1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optimal, N300</td>
<td>R2N2</td>
</tr>
<tr>
<td>Soil + wheat straw</td>
<td>P10</td>
<td>Low, N75</td>
<td>W1N1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optimal, N300</td>
<td>W1N2</td>
</tr>
<tr>
<td></td>
<td>P40</td>
<td>Low, N75</td>
<td>W2N1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Optimal, N300</td>
<td>W2N2</td>
</tr>
</tbody>
</table>
Seeds of wheat variety Wyalkatchem were germinated in Petri dishes on wet filter paper with a film of water for 48 hours at room temperature in darkness. Four pre-germinated wheat seedlings were sown into pots and later thinned to two plants per pot. The pots were watered to field capacity (24 % w/w) with deionized water.

5.2.6 Harvest and measurements

Initial analyses of dry soil were done after sieving (<2 mm) and before nutrient and residue application. After treatment establishment and incubation, soil samples were collected at 0, 3, 6, 9 and 12 weeks after sowing wheat. Plant samples were collected at 3, 6, 9 and 12 weeks of wheat growth. Shoot and root samples were harvested separately at each sampling time.

5.2.7 Soil and plant analyses

Soil samples were air-dried at 40°C for 5 days and mixed to homogenize. The samples were analysed for pH (Rayment and Higginson, 1992), available P (Colwell, 1965) and soil P fractions (Tiessen and Moir, 1993). The soil P fractions were grouped into labile Pi (inorganic P) (water-Pi + resin-Pi + bicarbonate-Pi), moderately-labile Pi (NaOH-Pi + 1 M HCl-Pi), stable Pi (conc. HCl-Pi) and residual P based on Hedley et al. (1982), Ivarsson (1990), Tiessen and Moir (1993) and Schmidt et al. (1996). The Pi content in soil extracts was measured colorimetrically by Murphy and Riley (1962) method.

The shoot and root samples were collected separately in labelled paper bags at each harvest. The number of tillers and dry matter yield were determined at each harvest. The observations on the rate of elongation of the third leaf (Bultynck et al., 2004; Jovanovic et al., 2004) was carried out using a ruler once daily at the same time of the day on one plant in each of three replicates.

The harvested plant samples were washed with deionised water and oven-dried at 70 °C until the samples reached constant weight. The dry matter content in the shoot and root samples was recorded. Oven-dried shoots and roots were ground separately to <0.5-mm and stored in pre-labelled 25-mL high-density polyethylene bottles.

About 0.5 g of finely ground plant samples was digested in a mixture of 3 mL conc. HNO$_3$ and 1 mL conc. HClO$_4$) (Valizadeh et al., 2003). The P concentration in the
digested plant samples was measured using inductively-coupled plasma optical emission spectrometry (ICP-OES).

5.2.8 Statistics

The statistical analyses were performed using SAS 9.3 statistical software. Repeated measures analysis of variance (ANOVA) was performed on the soil and plant data with three factors: P amendments (inorganic P, pea residues, wheat residues), P fertilization (P10, P40) and N fertilization (N75, N300) (Table 5.3) over time (0, 3, 6, 9 and 12 WAS). Statistical analysis for the third leaf emergence and elongation was initially done by 2-way ANOVA (P fertilization and N fertilization as main factors) on the log-transformed data. The significant differences in pair-wise comparisons of means were determined using Tukey's Studentized Range (HSD_{0.05}) Test. The correlation and regression analyses were performed in Excel.

5.3 Results

5.3.1 Growth and P concentration of wheat grown in soils amended with inorganic P fertilizers or crop residues at low or optimal N fertilization

5.3.1.1 Third leaf development in wheat

The growth and development of the third leaf in wheat was significantly influenced by the source of P and rate of N fertilization in soil (Figure 5.1). Crop residue amendments reduced, and the inorganic P treatment significantly enhanced, growth of the third leaf and its development compared with the control.

The third leaf emergence in wheat was significantly influenced by the type of P amendment (P <0.0001) but not the amount of P added to soil. The emergence of third leaf in wheat grown in soils amended with inorganic P fertilizers (12 DAS) was 5 to 8 days earlier than that of wheat grown in soils amended with crop residues (17 to 20 DAS) and P-zero control (Figure 5.1). In inorganic P fertilizer treatments, the third leaf emergence was not affected by the rates of P and N added via fertilizers. In contrast, in the crop residue treatments, the third leaf emerged a day or two earlier in wheat crops grown with optimal N (N2) compared with low N fertilization (N1).
Chapter 5 Fractionation of soil phosphorus after incorporation of post-harvest residues

Figure 5.1: Third leaf development in wheat grown in soils amended with inorganic P fertilizer or crop residues at low or optimal N fertilization. n=3. Vertical bars indicate ±SE. □ - maximum leaf length (cm), □ – leaf emergence (DAS) and ▲ - duration of leaf expansion (days). Significant differences were observed in the log-transformed means of leaf length (P < 0.0001), leaf emergence (P < 0.0001) and duration of leaf expansion (P < 0.05) among the soil P amendments (Tukey’s HSD 0.05). For treatment details, see Table 5.3.

Third leaf emergence in P-zero control soil was influenced by N fertilization (Figure 5.1). Optimal N fertilization (N2) stimulated earlier emergence of third leaf (12 DAS) and was on par with the inorganic P fertilizer treatments, but it was delayed by 4 days in wheat receiving low N fertilization (N1).

The third leaf was significantly longer (P < 0.0001) in wheat grown in soil amended with inorganic P fertilizer than crop residues. The length of the third leaf in wheat decreased with an increase in the quantity of residue added to soil. Increased leaf length was achieved by adding less crop residue (R1, W1) and by N fertilization (Figure 5.1). No significant differences were observed in the length of third leaf of wheat grown in soils amended with crop residues, except for R1N2 and W2N2 that differed significantly.
Figure 5.2: Leaf elongation rate of third leaf in wheat grown in soils amended with inorganic P fertilizer or crop residues at low or optimal N fertilization. n=3. LER - leaf elongation rate (mm day$^{-1}$) representing a slope of the linear part of the relationship (P < 0.0001 among the treatments at P level). Significant differences was observed in the log-transformed means of LER among the soil P amendments (Tukey’s HSD$_{0.05}$). For treatment details, see Table 5.3.

The duration of expansion of the third leaf was not significantly different among the treatments (Figure 5.1). However, the leaf expansion rate (LER) was significantly higher in the inorganic P fertilized treatments (P2 > P1) than the control and crop residue amended treatments (Figure 5.2).
5.3.1.2 Tiller formation

Tiller formation was earlier (data not presented) and the number of tillers and productive tillers at harvest (12 WAS) (Table 5.4) were significantly higher in wheat grown in soils amended with inorganic P fertilizers than crop residues. Soil P fertilization combined with N fertilization enhanced the number of tillers and productive tillers at harvest (12 WAS), especially in inorganic P fertilizer treatments.

Table 5.4: Tiller formation in wheat (12 WAS) grown in soils amended with inorganic P fertilizer or crop residues at low or optimal N fertilization.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total number of tillers at 12 WAS</th>
<th>Number of productive tillers with visible heads at 12 WAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0N1</td>
<td>4 cd</td>
<td>3 c</td>
</tr>
<tr>
<td>P0N2</td>
<td>6 bc</td>
<td>4 b</td>
</tr>
<tr>
<td>P1N1</td>
<td>7 b</td>
<td>5 b</td>
</tr>
<tr>
<td>P1N2</td>
<td>8 ab</td>
<td>5 ab</td>
</tr>
<tr>
<td>P2N1</td>
<td>7 b</td>
<td>5 ab</td>
</tr>
<tr>
<td>P2N2</td>
<td>9 a</td>
<td>6 a</td>
</tr>
<tr>
<td>R1N1</td>
<td>3 d</td>
<td>1 d</td>
</tr>
<tr>
<td>R1N2</td>
<td>3 d</td>
<td>1 d</td>
</tr>
<tr>
<td>R2N1</td>
<td>0 e</td>
<td>0 d</td>
</tr>
<tr>
<td>R2N2</td>
<td>0 e</td>
<td>0 d</td>
</tr>
<tr>
<td>W1N1</td>
<td>0 e</td>
<td>0 d</td>
</tr>
<tr>
<td>W1N2</td>
<td>0 e</td>
<td>0 d</td>
</tr>
<tr>
<td>W2N1</td>
<td>0 e</td>
<td>0 d</td>
</tr>
<tr>
<td>W2N2</td>
<td>0 e</td>
<td>0 d</td>
</tr>
</tbody>
</table>

Means with different letters in a given column denote significant differences (Tukey’s HSD) among the treatments (P < 0.0001, n=3). For treatment details, see Table 5.3.
Wheat grown in soils with high inorganic P and optimum N amendment (P2N2) had higher number of productive tillers than low P and N amendment (P1N1) and control (P0N1, P0N2) (Table 5.4). No tiller formation was observed in wheat grown in soil amended with crop residues, except for R1N1 and R1N2 treatments that produced three tillers per plant and one productive tiller; these numbers were significantly higher than in other crop residue treatments (Table 5.4).

Table 5.5: Shoot biomass production at different stages of wheat growth (Mean ± SE, n=3)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Shoot dry weight (g pot⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3 WAS</td>
</tr>
<tr>
<td>P0N1</td>
<td>0.05 ± 0.00 abcd</td>
</tr>
<tr>
<td>P0N2</td>
<td>0.05 ± 0.00 abcd</td>
</tr>
<tr>
<td>P1N1</td>
<td>0.07 ± 0.01 abc</td>
</tr>
<tr>
<td>P1N2</td>
<td>0.08 ± 0.01 a</td>
</tr>
<tr>
<td>P2N1</td>
<td>0.07 ± 0.01 abcd</td>
</tr>
<tr>
<td>P2N2</td>
<td>0.07 ± 0.01 ab</td>
</tr>
<tr>
<td>R1N1</td>
<td>0.04 ± 0.00 abcd</td>
</tr>
<tr>
<td>R1N2</td>
<td>0.03 ± 0.00 bcd</td>
</tr>
<tr>
<td>R2N1</td>
<td>0.04 ± 0.00 bcd</td>
</tr>
<tr>
<td>R2N2</td>
<td>0.06 ± 0.02 abcd</td>
</tr>
<tr>
<td>W1N1</td>
<td>0.03 ± 0.00 cd</td>
</tr>
<tr>
<td>W1N2</td>
<td>0.04 ± 0.00 abcd</td>
</tr>
<tr>
<td>W2N1</td>
<td>0.04 ± 0.00 bcd</td>
</tr>
<tr>
<td>W2N2</td>
<td>0.03 ± 0.00 d</td>
</tr>
</tbody>
</table>

Means with different letters in a given column denote significant differences among the treatments at a given time (Tukey’s HSDₘ₀.₀₅, p ≤ 0.05). For treatment details, see Table 5.3.
5.3.1.3 Dry matter production

Shoot and root biomass production was significantly affected by the soil P amendments (Appendix 5.1). Adding inorganic P fertilizer increased shoot and root biomass with time more than in the P-zero control and crop residue amended treatments (Tables 5.5, 5.6). Amending soil with crop residues resulted in significantly lower plant biomass than in the P-zero control.

Table 5.6: Root biomass production at different stages of wheat growth (Mean ± SE, n=3)

<table>
<thead>
<tr>
<th>Treatments</th>
<th>3 WAS</th>
<th>6 WAS</th>
<th>9 WAS</th>
<th>12 WAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0N1</td>
<td>0.06 ± 0.00 a</td>
<td>0.16 ± 0.01 bcde</td>
<td>1.1 ± 0.24 bc</td>
<td>2.8 ± 0.36 c</td>
</tr>
<tr>
<td>P0N2</td>
<td>0.05 ± 0.01 a</td>
<td>0.17 ± 0.01 abcd</td>
<td>1.5 ± 0.18 b</td>
<td>3.4 ± 0.49 bc</td>
</tr>
<tr>
<td>P1N1</td>
<td>0.05 ± 0.02 a</td>
<td>0.02 ± 0.02 abc</td>
<td>1.4 ± 0.12 b</td>
<td>8.0 ± 0.20 ab</td>
</tr>
<tr>
<td>P1N2</td>
<td>0.07 ± 0.01 a</td>
<td>0.07 ± 0.03 de</td>
<td>0.61 ± 0.02 bc</td>
<td>3.1 ± 0.53 c</td>
</tr>
<tr>
<td>P2N1</td>
<td>0.02 ± 0.01 a</td>
<td>0.23 ± 0.03 ab</td>
<td>1.5 ± 0.13 b</td>
<td>8.6 ± 0.66 a</td>
</tr>
<tr>
<td>P2N2</td>
<td>0.06 ± 0.01 a</td>
<td>0.28 ± 0.05 a</td>
<td>3.5 ± 0.57 a</td>
<td>7.3 ± 0.65 a</td>
</tr>
<tr>
<td>R1N1</td>
<td>0.06 ± 0.01 a</td>
<td>0.05 ± 0.01 e</td>
<td>0.24 ± 0.03 c</td>
<td>0.49 ± 0.08 c</td>
</tr>
<tr>
<td>R1N2</td>
<td>0.06 ± 0.01 a</td>
<td>0.08 ± 0.01 de</td>
<td>0.36 ± 0.02 c</td>
<td>0.69 ± 0.08 c</td>
</tr>
<tr>
<td>R2N1</td>
<td>0.05 ± 0.01 a</td>
<td>0.09 ± 0.03 de</td>
<td>0.11 ± 0.01 c</td>
<td>0.08 ± 0.01 c</td>
</tr>
<tr>
<td>R2N2</td>
<td>0.07 ± 0.01 a</td>
<td>0.06 ± 0.01 de</td>
<td>0.08 ± 0.01 c</td>
<td>0.07 ± 0.02 c</td>
</tr>
<tr>
<td>W1N1</td>
<td>0.07 ± 0.02 a</td>
<td>0.09 ± 0.00 ced</td>
<td>0.15 ± 0.01 c</td>
<td>0.13 ± 0.04 c</td>
</tr>
<tr>
<td>W1N2</td>
<td>0.06 ± 0.01 a</td>
<td>0.06 ± 0.01 de</td>
<td>0.13 ± 0.01 c</td>
<td>0.27 ± 0.04 c</td>
</tr>
<tr>
<td>W2N1</td>
<td>0.06 ± 0.01 a</td>
<td>0.08 ± 0.01 de</td>
<td>0.08 ± 0.00 c</td>
<td>0.09 ± 0.01 c</td>
</tr>
<tr>
<td>W2N2</td>
<td>0.06 ± 0.00 a</td>
<td>0.06 ± 0.01 de</td>
<td>0.06 ± 0.00 c</td>
<td>0.06 ± 0.01 c</td>
</tr>
</tbody>
</table>

Means with different letters in a given column denote significant differences among the treatments at a given time (Tukey’s HSD_{0.05}, p ≤ 0.05). For treatment details, see section 3.2.4.

At 3 WAS, no significant difference was observed in wheat shoot and root biomass (Tables 5.5, 5.6). Highest shoot biomass was produced in the P2N2 treatment.
throughout the wheat growth period until final harvest (12 WAS) followed by P2N1, P1N1, P0N2, P1N2 and P0N1 (Table 5.5). The shoot biomass production in wheat grown on soils with crop residue amendments was not influenced by the crop residue type or P quantity in residues. Addition of P via inorganic P fertilizers (P2 and P1) increased wheat root biomass substantially (P ≤0.05) except in the P1N2 treatment that had significantly lower root biomass than other inorganic P fertilizer treatments throughout growth (Table 5.6).

Figure 5.3: Shoot P concentration in wheat at 0, 3, 6, 9 and 12 weeks after sowing in soils amended with inorganic P fertilizer or crop residues at low or optimal N fertilization. Mean ± SE, n=3. Different letters denote significant differences among treatment means (P <0.0001) at a given time (Tukey’s HSD_{0.05}). For treatment details, see Table 5.3.
5.3.1.4 Shoot P concentration in wheat

Phosphorus supplementation via either inorganic P fertilizers or crop residues increased shoot P concentration in wheat (Appendix 5.1), which declined with wheat growth. Significant interaction effects were observed among the P fertilization treatments and time (P <0.0001, see Appendix 5.1), whereas the quantity of N added had no significant effect.

Shoot P concentration was higher in wheat grown in soils amended with P in the form of inorganic P fertilizers than crop residues at 3 WAS (Figure 5.3). However, P concentration in wheat shoots gradually declined with time in treatments with inorganic P fertilizer or crop residues, except for the R1N1 and R1N2 (pea residue) treatments where shoot P concentration increased until 6 WAS and then declined with time (Figure 5.3).

Wheat grown in the R1N1 and R1N2 treatments had significantly higher shoot P concentration (from 6 WAS onwards) than P1N1 and P1N2 (inorganic P) (Figure 5.3). At 12 WAS, wheat shoot P concentration was significantly higher in the R1N2 than the other crop residue or inorganic P fertilizer treatments and the control. Even though soil N amendment had no significant effect on shoot P concentration among the treatments, optimal N fertilization (N2) increased wheat shoot P concentration significantly more than low N fertilization (N1) in soils amended with low P via pea residue (R1) or wheat residue (W1) (P <0.05, see Appendix 5.1).

5.3.2 Soil properties as influenced by inorganic P fertilizers or crop residues amendment followed by wheat growth at low and optimal N fertilization

5.3.2.1 Soil pH

The soil pH during wheat growth significantly varied with soil P amendment (either inorganic P fertilizers or crop residues) and N fertilization (Appendix 5.2). The pH of the initial soil declined during pre-incubation period of 3 weeks irrespective of the soil P amendments, except for soil amended with higher quantity of P (equivalent to 40 mg P kg⁻¹ soil in R2) via pea residues (Figure 5.4). Growth of wheat on pre-incubated soil had a significant effect on the pH together with the soil amendments varying considerably with time (P <0.0001, Figure 5.4). The soil pH decreased with
wheat growth in the P-zero control, inorganic P fertilizer (P1, P2) and wheat residues (W1, W2) amended treatments. In contrast, soil amendment with pea residues in R2 showed an increase in the soil pH (Figure 5.4).

Figure 5.4: Changes in the soil pH\textsubscript{water} in treatments with inorganic P fertilizer or crop residues followed by wheat growth at low and optimal N fertilization. Mean ± SE, n=3. IS = Initial soil. Tukey’s HSD\textsubscript{0.05} = 0.046 for the interaction effect between time and treatments (P <0.0001). For treatment details, see Table 5.3.

The soil pH peaked at 6.8 in the R2N2 and 6.5 in the R2N1 treatments at 6 WAS, the values that were significantly higher than in the other treatments throughout the wheat growth period (Figure 5.4). No significant effects were observed on the soil pH with low P amendment via either pea (R1N1, R1N2) or wheat (W1N1, W1N2) residues until 9 WAS. However, N fertilization had a significant effect on the soil pH in the R1 and W1 treatments with time (Figure 5.4). At 12 WAS, the soil pH in the control (P0), inorganic P fertilizer (P1, P2) and crop residues (R1, W1) treatments
receiving low N fertilization (N1) were significantly higher (P <0.01) than the optimal N fertilization (Figure 5.4).

Figure 5.5: Changes in available P (Colwell P) in treatments with inorganic P fertilizer or crop residues amendment at low or optimal N fertilization during 3-week pre-incubation (0 WAS) and during wheat growth (0 to 12 weeks). IS = Initial soil. Vertical bars indicate ± standard errors (n=3). Tukey’s HSD\_0.05 = 0.8448 for the interaction effect between time and treatments (P <0.0001). For statistical analyses, see Appendix 5.2.

5.3.2.2 Soil available phosphorus (Colwell P)

Availability of soil P was significantly influenced by the P amendments, P fertilization and wheat growth (Appendix 5.2). Pre-incubation of soil with inorganic P fertilizers or crop residues had a significant effect on soil P availability before sowing wheat crop (Figure 5.5). After 3 weeks of pre-incubation, supplementation of P via inorganic P fertilizers, especially in P2 (40 mg P kg\(^{-1}\) soil), increased soil P availability more than the crop residue treatments (Figure 5.5). Soil amendment with crop residues (especially pea residues) lowered soil P availability at the end of the pre-incubation period, whereas
wheat residues had no significant effect, except for the W2N2 treatment where an increase in available P was observed (Figure 5.5).

Soils amended with inorganic P fertilizers or wheat residues had a substantial amount of available P remaining in soil at 12 WAS in comparison to the initial soil (Figure 5.5). Availability of soil P was higher in soil amended with inorganic P fertilizers until 3 WAS followed by a gradual decline with wheat growth (Figure 5.5). However, P availability was significantly higher in the P2N2, P2N1 and P1N2 than the crop residue treatments and the P-zero control (Figure 5.5). Even though N fertilization had no significant effect on P availability, the inorganic P fertilization (P1, P2) treatments showed an increase in soil available P with optimal N fertilization (Figure 5.5). The growth of wheat had little or no significant effect on P availability in soils amended with crop residues (pea or wheat) (Figure 5.5).

5.3.2.3 Soil P fractions

Adding inorganic P fertilizers significantly increased labile inorganic P (labile Pi) in soil (Appendix 5.2). In contrast, adding crop residues low in P did not increase labile Pi in soil. Nitrogen fertilization had no effect on the labile inorganic P pool in soil. However, the significant interaction between added N and P fertilization was observed with growth of wheat (Appendix 5.2).

Soil pre-incubated with higher quantity of inorganic P fertilizers (P2) showed a significant increase in labile Pi that gradually declined with wheat growth, but still had a considerably larger labile Pi pool than other treatments even after 12 weeks of wheat growth (Figure 5.6). Similar addition of P via pea (R2) or wheat (W2) residue amendments had no significant effect on labile Pi at the end of soil pre-incubation and was not affected by wheat growth (Figure 5.6). Low P amendment (10 mg P kg\(^{-1}\) soil) as inorganic P fertilizer (P1) or wheat residue (W1) resulted in no significant increase in soil P (Figure 5.6). In contrast, low P amendment via pea residues significantly lowered labile Pi during 3-week soil pre-incubation, gradually increasing with wheat growth and was on par with the the P-zero control treatment at 12 WAS (Figure 5.6).

During the pre-incubation period (3 weeks), higher addition of P in the P2 treatments elevated the moderately-labile Pi pool in soil compared with the P1 treatments (Figure 5.6). In contrast, pre-incubation with crop residues (pea or wheat)
significantly decreased moderately-labile Pi in soil irrespective of the quantity of P added in the residues (Figure 5.6). The control soil with no added P (P0) showed a significant decrease in moderately-labile Pi with low N fertilization (N1), whereas the optimal N fertilization maintained moderately-labile Pi during the 3-week soil pre-incubation.

With a substantial increase in moderately-labile Pi via high inorganic P amendment (P2) during pre-incubation, growth of wheat decreased moderately-labile Pi in P2, but it still remained significantly higher than in the other inorganic P fertilizer (P1) or crop residue treatments (Figure 5.6). No significant change in the moderately-labile Pi pool during wheat growth was observed at low P addition via inorganic P fertilizers (P1) or wheat residues (W1). Growing wheat with optimal N fertilization (N2) decreased moderately-labile Pi significantly (P <0.05) compared with low N fertilization (N1) in treatments with pea residues (R1, R2), control (P0) or high P addition via wheat residue (W2) (Figure 5.6).

During soil pre-incubation the stable Pi pool in soil was similar across various soil amendments and was not affected by the amount of P added (Figure 5.6). However, soil amended with crop residues (especially wheat residues) showed a significant increase in stable Pi at the end of wheat growth (Figure 5.6). In particular, optimal N fertilization (N2) in the wheat residue (W1, W2) treatments increased significantly the stable Pi pool in soils with duration of wheat growth (P <0.001) compared with low N (N1), but not in soils with pea residue amendments (Figure 5.6). Low N fertilization (N1) in the control (P0) showed an increase, and optimal N fertilization (N2) a decrease, in the stable Pi pool in the P-zero control during wheat growth.

Low P amendment via either crop residues (R1, W1) or inorganic P fertilizer (P1) showed significant mobilization (P <0.0001) of soil residual P during the pre-incubation period (Figure 5.6). However, increased P supplementation through inorganic P fertilizer (P2) or crop residues (R2, W2) had no effect on the residual soil P (Figure 5.6). Nitrogen fertilization had a significant effect on mobilization of the native soil P in the control treatment (P0) (P <0.05, Figure 5.6). During pre-incubation, the control soil (P0) showed a significant decline in the residual P pool with low N fertilization (N1), and no variation with optimal N fertilization (N2) (Figure 5.6).
Figure 5.6: Dynamics of soil P pools in treatments with inorganic P fertilizer or crop residues amendment at low or optimal N fertilization during 3-week pre-incubation (0 WAS) and during the wheat growth (0 to 12 weeks). Mean ± SE, n=3. IS = Initial soil. Tukey’s HSD\textsubscript{0.05} = 0.97 (labile Pi), 3.30 (moderately labile Pi), 6.6 (stable Pi) and 1.59 (residual P) for the interaction effect between time and treatments (P <0.0001). For statistical analyses, see Appendix 5.2.
### Table 5.7: Correlation between wheat biomass production, shoot P concentration and soil properties at different stages of wheat growth

<table>
<thead>
<tr>
<th>Time</th>
<th>Parameters</th>
<th>Soil pH</th>
<th>Colwell P</th>
<th>Soil P fractions</th>
<th>Shoot biomass</th>
<th>Root biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Labile Pi</td>
<td>Moderately labile Pi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 WAS</td>
<td>Shoot biomass</td>
<td>ns</td>
<td>+**</td>
<td>+*</td>
<td>1</td>
<td>ns</td>
</tr>
<tr>
<td></td>
<td>Root biomass</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Shoot P</td>
<td>ns</td>
<td>+***</td>
<td>+***</td>
<td>+***</td>
<td>ns</td>
</tr>
<tr>
<td>6 WAS</td>
<td>Shoot biomass</td>
<td>-*</td>
<td>+**</td>
<td>+**</td>
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</tr>
<tr>
<td></td>
<td>Root biomass</td>
<td>-*</td>
<td>+*</td>
<td>+**</td>
<td>ns</td>
<td>1</td>
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<tr>
<td></td>
<td>Shoot P</td>
<td>-*</td>
<td>+*</td>
<td>ns</td>
<td>ns</td>
<td>+**</td>
</tr>
<tr>
<td>9 WAS</td>
<td>Shoot biomass</td>
<td>-*</td>
<td>+*</td>
<td>+**</td>
<td>+*</td>
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<td>+**</td>
<td>+**</td>
<td>ns</td>
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</tr>
<tr>
<td></td>
<td>Shoot P</td>
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<td>ns</td>
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<tr>
<td>12 WAS</td>
<td>Shoot biomass</td>
<td>ns</td>
<td>+**</td>
<td>+**</td>
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<td></td>
<td>Root biomass</td>
<td>ns</td>
<td>+**</td>
<td>+**</td>
<td>+**</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Shoot P</td>
<td>-**</td>
<td>ns</td>
<td>ns</td>
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</tbody>
</table>

The symbols + / - denote positive or negative correlation (r); *** significant at P ≤ 0.001; ** significant at P ≤ 0.01; * significant at P ≤ 0.05; ns not significant at P ≤ 0.05. Data for stable Pi and residual fractions of soil P were excluded from the table as no significant correlations were observed.

The residual P pool in soils during wheat growth was affected by the significant interaction between P and N rates added via soil amendments and varied with time. Soil with crop residue amendments showed a significant reduction in the residual P pool with wheat growth regardless of the residue type and the amount of P added (Figure 5.6). Conversely, soil amended with inorganic P fertilizers showed a decrease in the residual P with wheat growth in treatments receiving a higher amount of P (P2), whereas the residual P pool in soils with lower inorganic P amendment (P1) was not
affected by wheat growth (Figure 5.6). Residual P in the control soils (P0) was partially mobilized during wheat growth at optimal N fertilization (N2), whereas low N fertilization (N1) led to a build-up of residual P with wheat growth (Figure 5.6).

5.3.2.4 Correlation between changes in soil properties and growth of wheat

Growth of wheat shoots and roots was significantly reduced at 6 and 9 WAS with an increase in soil pH with addition of crop residues, subsequently affecting shoot P concentration in growing wheat (Table 5.7). A significant positive correlation between Colwell P (or labile P) and wheat biomass (shoot or root) production showed the importance of P supplementation for plant growth (Table 5.7). Shoot P concentration in wheat was correlated (P ≤0.05) with soil P availability until 6 WAS. Moderately-labile Pi had a strong positive influence on shoot dry matter production in wheat throughout the growth period (Table 5.7). The root biomass yield was correlated with moderately-labile Pi at 12 WAS. Shoot biomass was highly correlated with root biomass (P <0.0001) and shoot P concentration in wheat (Table 5.7). Shoot P concentration in wheat improved with an increase in root biomass at 6 and 9 WAS.

5.4 Discussion

Phosphorus supplementation via inorganic P fertilizers improved the growth and development of wheat more than the crop residue amendments used in the present study (Tables 5.5, 5.6). Despite the amount of P added being the same, P supplied in inorganic P fertilizers was available for plant uptake, whereas P added in crop residues even at a high rate of 40 mg P kg⁻¹ soil was not sufficient for wheat, limiting its growth. This might be due to low P content (Table 5.2) in pea and wheat residues, with 90 % of total plant P partitioned to grain (Batten et al., 1986; Smith, 1965) and thus removed at harvest.

Growth and development of wheat was significantly affected by amendment of low-P residues (Figure 5.1, Tables 5.5, 5.6). Post-harvest residues are generally of low quality because much of nutrients are partitioned into grains. Both pea and wheat residues with P content less than 2.4 mg P g⁻¹ would be considered low quality (Iyamuremye et al., 1996a). Figure 5.5 shows an increase in plant-available P in soil after pre-incubation (0 WAS) in the inorganic P fertilizer treatments (P1, P2) even at a
low addition of 10 mg P kg\(^{-1}\) soil. In contrast, addition of crop residues (pea or wheat) low in P (Table 5.2) led to immobilization of P released from crop residues (cf. Fuller et al., 1956; Iyamuremye et al., 1996a; Nziguheba et al., 1998) even at high P addition of 40 mg P kg\(^{-1}\) soil (R2, W2), thus decreasing plant availability of P (Figure 5.5). However, good crop growth in the inorganic P fertilizer treatments led to increased uptake of P, gradually decreasing available soil P, whereas poor P uptake in the crop residue treatments resulted in an increase in available P in soil, but the values remained lower than in the inorganic P fertilizer treatments. Previously, Bünemann et al. (2004), Iqbal (2009) and Alamgir et al. (2012) reported that a significant amount of P added through crop residues was immobilized by microbes within 10 days (Bünemann, 2003), decreasing P availability for plant uptake.

In a recent isotope-labelling study, Noack (2014a) observed no significant improvement in wheat dry matter yield at 60 and 80 DAS with addition of mature field pea residues (10 t ha\(^{-1}\)) that had similar C, N and P content as pea residues (0.7 mg P g\(^{-1}\)) used in the present study. Also, Noack (2014a) reported an increase in microbial P at day 60 with pea residue application (surface or subsurface) in comparison with the fertilizer treatment and eventually decreased P availability for plant uptake in the residue-amended treatments. Later, at 80 days, residues (surface-applied) supported greater wheat P uptake than fertilizer. Again, this suggested immobilization of P added in pea or wheat residues early in decomposition (3 WAS) and improved wheat P uptake later until 6 WAS in the low pea-residue treatment (Figure 5.3, 5.4) but not in the other residue treatments. This might be explained by low C:N of pea residues in comparison with high C:N wheat residues (Table 5.2) resulting in enhanced decomposition of pea compared with wheat residues (Baldock, 2007; Duong, 2009), the former releasing P in excess of microbial demand and thus making it available for plant uptake. Also, addition of less C in the low-residue treatments resulted in lower microbial P demand in comparison with the high-residue treatments where adding a large amount of C led to immobilization of P released from decomposing residues irrespective of C:N of added residues (Figure 5.3).

An adequate P supply is critical in the early phases of plant growth (Batten 1992; Grant et al. 2001). This is supported by a highly positive correlation of wheat biomass or shoot P concentration with plant-available P until 6 WAS, with no
significant correlation thereafter (Table 5.6). Hence, timing, quality and quantity of crop residue application are important considerations for efficient management of crop residues and P cycling.

Incorporation of crop residues low in P, regardless of quantity, decreased plant-available P in soil and thus affected wheat growth (Figure 5.1, 5.2; Tables 5.5, 5.6). In particular, incorporation of a large quantity of crop residues inhibited wheat growth, with plants exhibiting severe P deficiency (shoot P <1.6 mg P g\(^{-1}\) dry matter, cf. Marschner, 1995). However, incorporating a low amount of field pea residues did not decrease wheat growth, but increased shoot P concentration (Figure 5.3).

Shoot P concentration in wheat steadily decreased in both low and high inorganic P fertilizer treatments and in crop residue treatments as a result of dilution with an increase in wheat shoot biomass, except for low P applied via pea residues in the R1 treatments (Figure 5.3). Wheat grown on soils amended with field peas at a low rate (R1) showed an increase in P concentration at 6 WAS despite an increase in shoot biomass (especially under optimal N fertilization), but declined with time (Figure 5.3). This suggests increased availability of P released from decomposing crop residues. However, lower shoot biomass production in the low pea-residue treatment in comparison with the similar low P-fertilizer treatment would have contributed to higher shoot P concentration in the former due to minimal dilution of shoot P (Table 5.2, Figure 5.3).

Phosphorus uptake by wheat in soils amended with crop residues primarily depends on the decomposition rate governed by P and C content of crop residues and soil microbial activity (Dalal, 1979; Palm and Rowland, 1997; Trinsoutrot et al., 2000; Wang et al., 2004; Abiven et al., 2005; Betrant et al., 2006). Iqbal (2009) observed a positive correlation between plant dry matter yield and residue P content, and negative correlation with C:P, C:N and C in the crop residues. Results presented in Chapters 3 & 4 are consistent with previous reports that net P mineralization occurred with addition of high-P crop residues (≥2.4 mg P g\(^{-1}\)) in contrast to P immobilization with adding low-P residues (Fuller et al., 1956; Iyamuremey et al., 1996a; Nziguheba et al., 1998). In a review, Damon et al. (2014) suggested a threshold level for P mineralization of 2 to 3 mg P g\(^{-1}\) crop residues.
The amount of available P remaining in soils amended with inorganic P fertilizers steadily declined with time due to plant uptake (Dalal 1979), microbial immobilization (Bünemann et al., 2012) and soil P sorption (Barrow, 1983, 1999; Umrit and Friesen, 1994; Celi and Barberis, 2005; Jalali, 2009; Jalali and Ranjbar, 2009). In the present study, despite P uptake by wheat, addition of P without C in the high P-fertilizer treatment increased available P in soil, whereas a similar addition of P along with C in the crop-residue treatments decreased available P (Figure 5.5). Similar results were obtained by Chauhan et al. (1981), Hedley et al. (1982), Nwuke et al. (2004) and Iqbal (2009), where P added via crop residues was immobilized in microbes. However, soils amended with a low amount of pea or wheat residue showed similar P availability as that of the low-rate P-fertilizer treatment, except for optimal N fertilization that showed improved soil P availability. This might be due to lower microbial activity in the low P-fertilizer treatment immobilizing less P and N than the crop residue treatments (Figure 5.5).

Figure 5.3 shows enhanced P accumulation in shoots in the low-wheat residue treatment with optimal N compared with low N fertilization. Optimal N fertilization might have increased decomposition rate of wheat residues, releasing more P than in the low-N treatment. In the high-residue treatments, P availability was low (Figure 5.5) as soil P and P released from the added residues were not enough to meet the P demand of microbes (cf. Vrede et al., 2004) fuelled by a high quantity of C added (cf. Iqbal, 2009) in the high-residue treatments. However, re-mineralization of immobilized P occurs with time due to a decline in microbial activity (McLaughlin et al., 1988c; Kwabiah et al., 2003a; Oberson and Joner, 2005; Iqbal, 2009).

Mineralization of P added via crop residues is mainly determined by P content, decomposition rate and microbial activity (Stevenson and Cole, 1999). Noack et al. (2014b) reported that about 85% of total P in mature post-harvest wheat residues was water-extractable, mostly in inorganic form. This is in agreement with earlier studies by Birch (1961), Jones and Bromfield (1969) and Martin and Cunningham (1973) where 50-80% of P in mature crop residues was in readily-soluble inorganic forms. However, decomposition of mature residues is slower compared with young residues due to less water-soluble C and higher content of recalcitrant compounds in the former (Mary et al, 1992; Bertrand et al., 2006; Abiven et al., 2007; Duong, 2009; Iqbal, 2009).
High P concentration in crop residues enhances decomposition rate, with an increase in microbial activity resulting in net P mineralization (Tian et al., 1992). Incorporation of high C:P (>300) crop residues (Table 5.2) increased microbial activity (Dalal, 1977) and microbial P uptake (Iqbal, 2009), but not P uptake by wheat (Figure 5.3). Previous study by Nziguheba et al. (1998) reported a decrease in available P in soil amended with maize stover residues (C:P=633) and an increase with inorganic P fertilizers. However, Iqbal (2009) reported a decrease in plant-available P in soil amended with mature wheat and pea residues low in P (0.7 mg P g\(^{-1}\), C:P>300) than in the unamended soils for the first 6 weeks followed by an increase in plant-available P with microbial turn-over.

Crop residues added to acid soils increase soil pH (Kretzschmar et al., 1991; Noble et al. 1996; Tang et al. 1997, 1999; Tang and Yu 1999; Mokolobate and Haynes, 2003; Xu et al., 2006a, b). In the present study, an addition of high quantity of crop residues (mainly pea, R2) increased soil pH (Figure 5.4), whereas it declined with time in all the other P fertilizer and crop residue treatments. This might be due to a high amount of N (Yan et al., 1996; Pocknee and Sumner, 1997) and cations (Tang and Yu, 1999) added in the high-residue pea treatment (R2, Table 5.2).

Field-pea, being a legume contains a large amount of excess cations (Tang and Yu, 1999; Hardiputra, 2011) and its decomposition entails increased decarboxylation of organic anions, eventually increasing the soil pH. In addition, poor wheat growth in the R2 treatment would have led to lower plant uptake of NO\(_3^-\) ions and hence increased NO\(_3^-\) concentration in soil (cf. Pearse et al., 2006, 2007; Rose et al., 2010a). The H\(^+\) released via decomposition of pea residues as well as increased exudation of H\(^+\) from P-deficient wheat roots (Dinkelaker et al., 1989; Hinsinger, 2001) might have led to ammonification of that NO\(_3^-\) (Heylar and Porter, 1989; Khalil et al., 2005) and thereby an increase in soil pH (Tang and Yu, 1999; Hinsinger et al., 2003). However, the increase in soil pH did not lead to a significant increase in P availability in the high-residue treatments (R2, W2).

Increased P supplementation via inorganic P fertilizers (40 mg P kg\(^{-1}\) soil) increased the labile Pi pool in soil, which did not occur with the low P-fertilizer rate (10 mg P kg\(^{-1}\) soil). In contrast, higher P supplementation via pea or wheat residues in comparison with low P addition showed no significant increase in labile Pi (Figure 5.6).
Increased microbial activity with increased addition of C (cf. Chauhan et al., 1979; He et al., 1997; Vrede et al., 2004) in low-P residues, especially at a high rate, immobilized added P. Relatively weak immobilization of P added as inorganic fertilizer (and thus increased labile Pi) would be expected because no C was added with fertilizer (Figure 5.6).

An addition of a small amount of C in the low pea-residue treatment was accompanied by an increase in the labile Pi pool during wheat growth (Figure 5.6), indicating re-mineralization of added P from microbial lysis (cf. Oberson and Joner, 2005) with time. In contrast, Alamgir et al. (2012) reported that mineralized P from microbial turnover did not increase the labile Pi pool, but rather was converted into the less labile organic and inorganic P forms and later became stabilized in soil colloids (Kuono et al, 1995). This difference might be due to plants being grown in the present study, whereby the labile Pi pool decreasing with wheat P uptake was replenished by buffering action of the moderately labile Pi pool maintaining equilibrium between labile and stable P pools (Kuono et al, 1995). Iqbal (2009) concluded that addition of residues with low C:P increased availability of added P in the short-term, whereas residues with high C:P increased soil P mobilization in the long-term.

Application of P via inorganic P fertilizers in excess of wheat requirement transformed readily labile P into moderately-labile P pools (Figure 5.6) that might have become available later due to root growth and development (Li et al., 2009; Shimizu et al., 2004) and rhizosphere processes (Richardson, 1994; Richardson et al., 2000; Richardson, 2001; Chen et al., 2002; Kwabiah et al., 2003b; Li et al., 2004; Wang et al., 2007; George et al., 2008), including root exudation and microbial activity. Similarly, Zamuner et al. (2012) observed an increase in labile Pi and moderately labile Pi pools with fertilizer P application in excess of wheat requirement.

Even though the moderately-labile P pool increased initially during pre-incubation in the treatment with high P-fertilizer application, a gradual decrease in moderately-labile Pi pool with growth of wheat might have been caused by enhanced mobilization of P from that pool, thus increasing P availability for plant growth (Figure 5.6). This is in agreement with results of Alamgir and Marschner (2013) that the moderately-labile pools were mobilized into the labile Pi pool during wheat growth. However, application of P via inorganic P fertilizers at a low rate maintained
equilibrium between the moderately-labile and labile Pi pools (Zhang and McKenzie, 1997a, b), with no significant change in these Pi pools in the present study (Figure 5.6).

Even though growth of wheat was poor in the crop-residue treatments except for low P added via pea or wheat residues, the moderately-labile Pi pool in the crop residue treatments decreased with time (Figure 5.6). This suggests that P added via crop residues might have entered the microbial P pool due to increased microbial activity with addition of C via residues. Several studies have reported P immobilization by microbes with residue addition (Chauhan et al., 1979; Hedley et al., 1982; Bünemann, 2003; Nwuke et al., 2004, Iqbal, 2009). Even though re-mineralization of the immobilized P would occur with a decrease in microbial activity with time (Oberson and Joner, 2005), no significant increase in other P pools was observed in soils amended with crop residues (Figure 5.6). This might be due to plant uptake of mineralized P or fixation in soil. However, Kuono et al. (1995) reported that P uptake by plants was low, whereas microbial assimilation played a major role in P mineralization-immobilization processes. Bünemann (2003) noticed that plant P uptake did not increase with microbial turnover after 42 days, suggesting slow mineralization of initially immobilized P.

Even with poor growth of wheat in the high-residue treatments, the moderately-labile Pi pool did not increase with time, not even at the end of growth period (12 WAS, 15 weeks after residue incorporation). This finding might have been due to fixation of available P by soil clay minerals (White and Ayoub, 1983; Friesen and Blair, 1988; Umrit and Friesen, 1994; Celi and Barberis, 2005; Bah et al., 2006) and stabilization in poorly-labile soil P pools. However, the present study showed no increase in either plant P uptake or stable/residual soil P pools in the high-residue treatments, except in soils amended with wheat residues (Figure 5.6) that showed a slight increase in the stable-P pool. This suggests microbial immobilization of P released from added pea or wheat residues.

The native soil residual P was mobilized in the absence of P fertilization mainly due to microbial activity increasing with the addition of water (Stewart and Tiessen, 1987; Oberson and Joner, 2005) and leading to partial mobilization of stable soil P to meet a P demand of growing microbial populations. In the present study, P fertilization via inorganic P fertilizer or crop residue at a low rate (10 mg P kg⁻¹ soil) stimulated mobilization of native residual P by increased microbial activity (Figure 5.6). Similar
results were obtained by Alamgir et al. (2012) with soil incubation of crop residues low in P, whereby mineralization of residual P from the native soil occurred because the amount of P added in crop residues was too low to satisfy the microbial P demand.

Compared with low-rate P treatments, the higher rate of P application via either crop residues or inorganic P fertilizer did not resulted in mobilization of native residual P, with the higher amount of added P sufficient to meet the initial microbial demand in the high-rate residue treatments (Figure 5.6). However, enhanced wheat growth at high rate of inorganic P fertilization mobilized the residual soil P as the total P demand increased with increasing plant growth and root uptake.

Given poor wheat growth (and thus low P uptake) in the high-residue treatments, the residual soil P pool was mobilized mainly to meet the microbial demand (Table 5.5, Figure 5.6), with no increase in other soil P pools except for stable P. This might be due to prolonged microbial activity with addition of a high amount of C (Bünemann et al., 2004) via residues high in C:P. In addition, subsequently re-mineralized P during microbial turnover might have been sorbed to soil minerals and colloids (Tiessen et al., 1984), which in the absence of substantial P removal due to poor root growth and P uptake by wheat reduced the movement of P among soil P pools. Alamgir et al. (2012) reported that mineralized P was converted to stable P in soil without grown plants within the first 14 days.

Mobilization of residual P suggested mineralization of native soil P acting as a main source of P for plant uptake, whereas P added through external sources via inorganic P fertilizers or crop residues contributed to the various soil P pools (Noack, 2014) either decreasing or increasing P availability in soil depending on the quality and quantity of the P source. Therefore, inorganic P fertilizers and crop residues play a major role in maintaining soil P to sustain crop production.

Optimal N fertilization increased mobility of P in soil mainly due to improved plant growth (Figure 5.1). Results from the present study suggest that wheat P uptake improved under optimal N fertilization due to enhanced wheat growth (Table 5.3) increasing P accessibility to roots, and increased microbial activity enhancing mobilization of soil P.
5.5 Conclusions

Soil P plays a major role in crop P uptake and can be increased by addition of inorganic P fertilizers or crop residues. Adding low-quality residues immobilizes P, which is not beneficial for wheat because P deficiency in the early stages affects crop growth and nutrient uptake. However, combined application of inorganic P fertilizers and crop residues could reduce the requirement for inorganic P fertilizers, thus minimizing fertilizer costs. Hence, P cycling in soil can be sustained in the short- and long-term with integration of nutrient management practices that combine low quality post-harvest crop residues with fertilizer P, or include high quality crop residues (e.g. green manure crops) in a cropping system.
Phosphorus, a major essential element required for plant growth, is supplemented in agriculture as inorganic P fertilizers produced from rock phosphate and as organic P to a certain extent. Globally, P availability in soils is mostly low, increasing a demand for P fertilizers, which will become accentuated with intensification of agriculture due to increasing world population. Increased use of inorganic P fertilizers has depleted the global rock phosphate reserves, imposing a major threat to the future crop production.

Australian soils are generally low in P due to the highly weathered nature, making Australia the 5th largest importer of P fertilizers in the world (Cordell et al., 2013). Importantly, a majority of P fertilizers applied in crop production is fixed in soil or lost to surface and ground water (Cordell, 2010). Plant P uptake occurs via roots that absorb inorganic P only from soil solution. Hence, P supplementation is mainly focused on increasing the soil solution P.

**6.1 Phosphorus supplementation**

Though P can be supplemented via organic sources such as crop residues and animal manures, P fertilizers are widely used as sources of P in the farming systems globally. Transformations of P added via organic and inorganic sources have been a focus of many studies. The results presented in this thesis provide new knowledge on P cycling through incorporation of crop residues varying in quality and quantity in Western Australian soils.

**6.1.1 Fertilizer P versus crop residues**

Inorganic P fertilizers are a major source of P in crop production. The readily-available inorganic form of P fertilizers increases P availability in soil immediately after application (Chapters 3, 4 and 5). However, P added via fertilizers becomes unavailable with time due to soil adsorption, microbial immobilization and plant uptake.

Phosphorus uptake by plants is relatively small compared with soil and microbial fixation processes. The results from Chapter 4 show increased P uptake with higher rate of P application (50 mg P kg⁻¹ soil) than at lower rate (20 mg P kg⁻¹ soil), the latter not significantly different from the unamended control soils. Chapter 5 shows
similar results on P uptake by wheat with inorganic P fertilizer amendments at low and high rates.

A decrease in plant P availability at low rate of inorganic P fertilization might be due to (i) microbial immobilization of added P; (ii) crop P uptake, and (iii) P adsorption to minerals, mainly Fe and Al sesquioxides, due to slightly acidic nature of Bindoon soil (Chapter 3 and 5). Previous studies by Tisdale et al. (1993), Bolland and Gilkes (1998), Richardson et al. (2001) and Vu et al. (2008) reported that only 10-20 % of added P fertilizers are available for plant uptake in the first year, with a major part of added P fertilizers becoming unavailable due to soil fixation or microbial immobilization depending on the soil properties and the nature of crops grown. However, in soil amended with inorganic P, plant availability of added P declined with time, mainly due to plant uptake and soil adsorption processes because the microbial activity was low with no added C (Chapter 3). Relatively low respiration rates in soil amended with inorganic P fertilizer confirmed low microbial activity in comparison with the soils amended with crop residues where a significant amount of C was added (Chapter 3).

In the soil incubation study without plants (Chapter 3), a gradual increase in the soil residual P pool after 21 days with inorganic P fertilizer application suggested conversion of available to insoluble P, either strongly bound to Al and Fe oxides (Hedley et al., 1982; Haynes and Mokolobate, 2001) or immobilized in the recalcitrant soil organic matter (Tiessen and Moir, 1993; Cross and Schlesinger, 1995). Therefore, P availability in P-fertilizer-supplemented soils were governed mainly by the soil properties such as P sorption capacity (Singh and Gilkes, 1991; Sharpley, 1996) followed by P uptake efficiency of crops grown.

Soil incorporation of crop residues mobilized the residual P pool (Chapter 3). This might be due to decreased soil P sorption with addition of organic matter (Yusran, 2005) through crop residues. Furthermore, the release of organic acid anions (Richardson et al., 2009) with decomposition of crop residues might have led to the release of P adsorbed onto soil particles. Earlier studies by Ohno and Crannell (1996) and Ohno and Erich (1997) found that crop residues are effective in decreasing P sorption, whereas an addition of animal manure had no influence on P sorption. This suggested that crop residues would improve P availability more than animal manure.
However, such an outcome would depend on the quality and quantity of added crop residues.

Phosphorus supplementation in either inorganic form as P fertilizers or organic form as crop residues would improve P availability in soil. However, the efficacy of added P depends on transformations among soil P pools. Phosphorus fertilizers when added to soil resulted in increased P availability initially, and later became transformed to less labile and stable P pools (Chapter 3). In the course of time, P added via inorganic fertilizers became unavailable due to fixation in soil, increasing the residual P pool (Chapter 3) that is not accessible to plants. However, this inaccessible residual P pool can be mobilized by P-efficient crops (eg. legumes) or with incorporation of crop residues (Chapter 4). Therefore, inorganic P fertilization improved P nutrition of crop in the short-term at a relatively high cost, whereas incorporation of crop residues improved mobilization of initially inaccessible soil and residual P pools, likely reducing fertilizer costs in the long term.

6.1.2 Low P residues versus high P residues

Plant availability of P was higher after incorporation of crop residues with high P content (Chapter 4) than post-harvest crop residues low in P (Chapter 5). Mineralization of added P would occur when P content of added residues was above 2 to 3 mg P kg$^{-1}$ (Damon et al., 2014). Also, addition of higher quantity of post-harvest crop residues low in P diminished availability of added P in comparison with lower quantity of same residues (Chapter 5). Soil P availability with addition of crop residues was dictated mainly by the microbial processes of immobilization and mineralization determined by C availability (Bünemann et al., 2004; Iqbal, 2009). Microbial immobilization dominated due to high C availability with addition of high quantity of crop residues, whereas re-mineralization occurred with less C in the low-residue treatments due to relatively fast microbial turnover (Chapter 5).

Incorporation of high quality faba bean residues in the present study (>2.4 g P kg$^{-1}$) (cf. Iyamuremye and Dick, 1996) enhanced P uptake by the subsequent wheat crop due to an increase in available P in soil, whereby the products of decomposition such as organic acid anions would have facilitated mobilization (Hue, 1991) of native P and residual fertilizer P (Chapter 4). Without crop growth, P supplementation via crop
residues increased soil P availability, especially high-quality fresh residues (green manure) (Chapter 3) in comparison with inorganic P fertilizer; in other studies, an addition of organic matter also decreased P sorption (cf. Halajnia et al., 2009) and increased microbial activity (cf. Bünemann et al., 2004a; Iqbal, 2009; Alamgir et al., 2012) mineralizing P in crop residues. Hence, inclusion of crop residues may be more advantageous than inorganic P fertilization in improving P cycling and enhancing mobilization of native soil P as well as residual fertilizer P.

6.1.3 Fresh versus dry residues

Fresh crop residues (green manure) increased plant-available P in soil to a greater extent than dry residues (Chapter 3, cf. Hardiputra, 2011). In other studies, crop residues high in P content resulted in net P mineralization increasing P availability in soil (Fuller et al., 1956; Singh and Jones, 1976; Bumaya and Naylor, 1988; Iyamuremye et al., 1996a). High content of water-soluble C, N and P (Iqbal, 2009) as well as high content of dissolved P and organic compounds such as phenolics in fresh than oven-dried residues (Dou et al., 2000; Hardiputra, 2011) would favour rapid decomposition (Iqbal, 2009), increasing available P in soil. This might be a result of net P mineralization and a decrease in soil P sorption (Easterwood and Sartain, 1990; Hue et al., 1994; Iyamuremye and Dick, 1996), with the products of residue decomposition such as organic acid anions (Richardson et al., 2011) releasing P from adsorption sites (McDowell and Condron, 2001). Hence, inclusion of green manure crop every few years in the rotation in the cropping systems would improve nutrient cycling and enhance soil fertility, reducing the demand for P fertilizers.

6.2 Integrated management practices

Wheat growth and P uptake were improved with incorporation of faba bean shoot residues (10 g kg\(^{-1}\) soil) together with whole roots in soil containing residual P fertilizer (Chapter 4). Returning shoot residues to soil enhanced P cycling to a greater extent than adding root residues alone (Chapter 4). Incorporating residues to soil mobilized native soil P (Chapters 3, 4 and 5) as well as residual fertilizer P from a previous crop (Chapter 4), reducing the P fertilizer requirement of subsequent crops. Also, incorporation of fresh roots together with dry shoot residues enhanced P uptake by wheat, reflecting
higher P availability due to (i) net P mineralization of high-P residues, especially due to high P in fresh roots together with high water-soluble C and N, resulting in enhanced decomposition, and (ii) soil P mobilization facilitated by the products of decomposition of added residues.

Incorporation of high-P residues (eg. green manure crops) involves a cost of production, partly offsetting the benefits of restored soil fertility. In contrast, incorporation of post-harvest residues low in P is unlikely to involve any significant costs, but such practice is not efficient in improving crop productivity in the short term due to a decrease in P availability, but may improve soil fertility in the long term. However, this initial reduction in P availability can be off-set through addition of inorganic P fertilizers in lower amounts than if the fertilizers were the only P addition.

6.3 Conclusions

Incorporation of crop residues high in P, especially as fresh residues (eg. green manure crops), is beneficial in increasing the labile soil P pool accessible to plants in comparison with incorporation of dry crop residues low in P. Hence, inclusion of green manure crops would promote P cycling in addition to recycling other essential nutrients and restoring soil fertility, particularly in low-nutrient soils with continuous crop cultivation where removal of nutrients occurs through harvest of grains. However, this occurs at a cost which reaps benefits in long-term.

Improving soil fertility in the long-term can be achieved by increasing soil organic matter by effective management of crop residues retained in the field after grain harvest. Incorporation of post-harvest crop residues, even when low in P, would improve the efficacy of subsequently added fertilizers by decreasing P sorption and mobilizing native soil P and residual fertilizer P. Therefore, integrated nutrient management practices, including crop residues (eg. green manuring, combined application of organic and inorganic P), could reduce the P-fertilizer demand and the associated costs, which is especially important considering depletion of rock phosphate reserves in the world.
6.4 Future research directions

Incorporation of crop residues may augment P cycling in soil and enhance P availability by improving mobilization of native soil P and added fertilizer P. However, this depends on the P content of added crop residues and the soil properties and is influenced by climatic factors and management practices. Hence, there is a great need for research on soil P cycling from crop residues taking into account the above mentioned factors, which could be addressed as indicated below.

1. Wide range of crop residues, soil types and climatic factors require variations in management practices in farming systems. However, most studies on transformations of P added in crop residues have been conducted in controlled environments via incubation and glasshouse experiments. In contrast, field studies involving crop residues varying in quality in different soil types and cropping systems would provide a better understanding of soil P transformations of added P and mobilization of soil residual P accumulated with previously added P fertilizers in a particular area, allowing development of strategies for integrated nutrient management practices in the farming systems.

2. Availability of soil P was improved more by incorporation of fresh than dry residues because of rapid P release in the early stages of decomposition (Chapter 3). The rapid release of P from fresh residues after soil incorporation could enhance P nutrition of subsequent crops. Hence, further research should be conducted in the field to characterize the efficacy of green manure in improving soil P cycling and enhancing P availability to the subsequent crops. Thereby, introducing green manure crops in a cropping system can restore soil fertility in the long term.

3. Research and development trials should be conducted on the farmers’ fields focusing on integrated management practices (e.g., inorganic P + crop residues, green manuring, minimal tillage to incorporate crop residues in otherwise no-till systems) that would improve P cycling in the farming systems considering soil, crop and residue properties along with climatic factors. This could help farmers understand P cycling through crop residues and underpin adapted management practices that could minimize fertilizer P requirement in crop production,
thereby reducing the future threat of diminished P reserves, allowing cheaper crop production in addition to sustaining soil fertility in the cropping systems.
REFERENCES


References


References


References


References


130

References


References


References


References


LECO CHN-1000 autoanalyzer, LECO Corporation, St Joseph, MI, USA.


References


References


Marschner B and Noble AD (2000). Chemical and biological processes leading to the neutralization of soil acidity after incubation with different litter materials. Soil Biology and Biochemistry, 32:805-813.


Myers RJK, Palm CA, Cuevas E, Gunatilleke IUN and Brossard M (1994). The synchronization of nutrient mineralization and plant nutrient demand. In: Woomer PL, Swift MJ (eds.), The biological management of tropical soil fertility, John Willey and Sons, Baffins Lane, Chichester, West Sussex PO19 IUD, United Kingdom, 81-116.


Nutrient source specifics, Fact sheet No. 21, Ref 11082, International Plant Nutrition Institute, Georgia, USA.


References


Palm CA, Myers RJK and Nandwa SM (1997). Combined use of organic and inorganic nutrient sources for soil fertility maintenance and replenishment. In: Buresh RJ,
Sanchez PA, Calhoun F (Eds.), Replenishing Soil Fertility in Africa, SSSA, Special Publication No. 51:193-217.


References


Ryan MH and Ash J (1999). Effects of phosphorus and nitrogen on growth of pasture plants and VAM fungi in SE Australian soils with contrasting fertilizer histories


References


References


References


References


References

herbaceous legume cropping and rock phosphate treatments. Soil Biology and Biochemistry, 32:2079–2090.


by NMR and wet-chemical analysis. Soil Biology and Biochemistry, 36(12):2045-2058.


References


APPENDICES

Appendix 3.1: Summary of statistical analyses of soil pH, Colwell P and P fractions in the soil incubation treatments (inorganic P fertilizer or crop residues) over time (0, 21, 42, 63, 84 and 126 days).

<table>
<thead>
<tr>
<th>Source</th>
<th>Soil pH</th>
<th>Colwell P</th>
<th>Labile Pi</th>
<th>Moderately-labile Pi</th>
<th>Stable Pi</th>
<th>Residual Pi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Treatment</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Time * Treatment</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Appendix 4.1: Summary of statistical analyses of dry weight and wheat P concentrations (Control – C0W1, previously fertilized with inorganic P – C1W2, freshly fertilized with inorganic P – C0W3, faba bean roots amendment – R1W2 and faba bean whole roots+shoots amendment – R2W2) over time (14, 28, 42, 56 and 77 days after sowing).

<table>
<thead>
<tr>
<th>Source</th>
<th>Shoot dry weight</th>
<th>Root dry weight</th>
<th>Shoot P concentration</th>
<th>Root P concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
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<tr>
<td>Treatment</td>
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<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Time * Treatment</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>
Appendix 4.2: Summary of statistical analyses of nutrient concentrations in wheat shoots and roots (Control – C0W1, previously fertilized with inorganic P – C1W2, freshly fertilized with inorganic P – C0W3, faba whole root amendment – R1W2 and faba whole root + shoot amendment – R2W2) at time (14, 28, 42, 56 and 77 DAS).

<table>
<thead>
<tr>
<th>Time</th>
<th>Nutrient concentration</th>
<th>N</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>S</th>
<th>Zn</th>
<th>Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14 DAS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>ns</td>
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<td>0.002</td>
<td>ns</td>
<td>0.002</td>
<td>0.004</td>
<td>&lt;.0001</td>
<td></td>
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<tr>
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<td>0.021</td>
<td>ns</td>
<td>0.002</td>
<td>ns</td>
<td>&lt;.0001</td>
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<td>0.002</td>
<td>0.034</td>
<td>0.006</td>
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<tr>
<td>Shoot</td>
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<td>ns</td>
<td>ns</td>
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</tr>
<tr>
<td>Shoot</td>
<td>&lt;.0001</td>
<td>0.004</td>
<td>&lt;.0001</td>
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<td>ns</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
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<td>0.038</td>
<td>0.049</td>
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<td>ns</td>
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<td></td>
</tr>
<tr>
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<td>&lt;.0001</td>
<td>&lt;.0001</td>
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<td>0.050</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
<td>&lt;.0001</td>
<td>0.001</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>0.001</td>
<td>&lt;.0001</td>
<td></td>
</tr>
<tr>
<td>Shoot</td>
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<td>0.0007</td>
<td>0.002</td>
<td>0.010</td>
<td>0.003</td>
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<tr>
<td>Shoot</td>
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<td>ns</td>
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<td>ns</td>
<td>0.024</td>
<td>&lt;.0001</td>
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</tr>
</tbody>
</table>
Appendices

Appendix 4.3: Growth of wheat in soils amended with inorganic P fertilizer (prior to the legume phase - C1W2 - or prior to the cereal phase – C0W3), faba bean residues (roots only – R1W2 - or roots+shoots – R2W2) and in the non-amended control (C0W2) at 14 (top row) and 77 days after sowing (bottom row).

14 DAS

77 DAS
Appendix 5.1: Summary of statistical analyses of shoot and root biomass and shoot P concentration.

<table>
<thead>
<tr>
<th>Source</th>
<th>Shoot biomass</th>
<th>Root biomass</th>
<th>Shoot P concentration</th>
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</thead>
<tbody>
<tr>
<td>P amendment</td>
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<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>P rate</td>
<td>0.113</td>
<td>0.180</td>
<td>0.035</td>
</tr>
<tr>
<td>N rate</td>
<td>0.915</td>
<td>0.379</td>
<td>0.079</td>
</tr>
<tr>
<td>P rate * N rate</td>
<td>0.082</td>
<td>0.1480</td>
<td>0.035</td>
</tr>
<tr>
<td>Time</td>
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<td>&lt;.0001</td>
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<tr>
<td>Time * P amendment</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Time * P rate</td>
<td>0.057</td>
<td>0.478</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Time * N rate</td>
<td>0.968</td>
<td>0.031</td>
<td>0.320</td>
</tr>
<tr>
<td>Time * P rate * N rate</td>
<td>0.123</td>
<td>0.424</td>
<td>0.221</td>
</tr>
</tbody>
</table>

P amendments – Control (P0), inorganic P fertilizers (P1, P2), pea straw residues (R1, R2) and wheat straw residues (W1, W2); P rates – Low (10 mg P kg\(^{-1}\) soil) and high (40 mg P kg\(^{-1}\) soil); N rates – Low (75 mg N kg\(^{-1}\) soil) and optimal (300 mg N kg\(^{-1}\) soil); and Time – 0, 3, 6, 9 and 12 WAS.
Appendices

Appendix 5.2: Summary of statistical analyses of soil pH, Colwell P and P fractions.

<table>
<thead>
<tr>
<th>Source</th>
<th>Soil pH</th>
<th>Colwell P</th>
<th>P fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Labile Pi</td>
</tr>
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<td>P amendment</td>
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<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>P rate</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>N rate</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>P rate * N rate</td>
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<td>ns</td>
</tr>
<tr>
<td>Time</td>
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<td>&lt;.0001</td>
<td>0.002</td>
</tr>
<tr>
<td>Time * P amendment</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Time * P rate</td>
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<td>0.0002</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Time * N rate</td>
<td>0.007</td>
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</tr>
<tr>
<td>Time * P rate * N rate</td>
<td>ns</td>
<td>ns</td>
<td>0.001</td>
</tr>
</tbody>
</table>

P amendments – Control (P0), inorganic P fertilizers (P1, P2), pea straw residues (R1, R2) and wheat straw residues (W1, W2); P rates – Low (10 mg P kg\(^{-1}\) soil) and high (40 mg P kg\(^{-1}\) soil); N rates – Low (75 mg N kg\(^{-1}\) soil) and optimal (300 mg N kg\(^{-1}\) soil); and Time – 0, 3, 6, 9 and 12 WAS