Effect of Crop Residue Qualities on Decomposition Rates, Soil Phosphorus Dynamics and Plant Phosphorus Uptake

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Dedicated to my parent

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Abstract

Phosphorus (P) is an essential plant nutrient that may limit plant growth and agricultural productivity if not available for crop plant uptake in sufficient quantities at the time required. Many Australian soils are deficient in available P, despite a long history of P fertilizer application, and this is due to fertilizer P rapidly becoming unavailable largely through biochemical fixation. The resulting low P fertilizer efficiency, coupled with rapidly rising cost of fertilizers, has increased interest in biological cycling of P from sources such as crop residues. However, to date, much of the Australian research has focussed on soils with relatively high organic matter content (> 2%) and relatively heavy texture i.e. medium to high clay content. Furthermore, although there is information on pasture residue decomposition and P release for sandy soils with low organic matter in Australia, a recent shift to continuous cropping systems means that information for a range of crop residues is required but is not currently available. Therefore the aims of the work described in this thesis were to (i) increase the efficiency of P use when crop residue P are applied to crops and (ii) determine the effect of crop residue biochemical quality on decomposition rates, soil P dynamics and plant P uptake in light textured sandy soils with low organic matter which are typical of a large proportion of the southern Australian wheat growing area. A further aim was to investigate the effects of combined additions of plant residue and P fertilizer on P cycling in these soils, a scenario highly relevant to farming systems.

A series of soil incubation and plant growth experiments were undertaken to characterize P dynamics in soil following addition of a wide range of crop residues (total 15) collected from agricultural sites throughout South Australia. The residues, differing in age and biochemical quality, were young shoots of canola, lupin, pea, lucerne and lentil; mature shoot residues of

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canola, lupin, pea and wheat and mature root residues of wheat, canola and lupin. The

concentration of total and water soluble P, C, and N in the residues was measured using

standard wet chemical analyses and the carbon chemistry was determined by NMR

spectroscopy. Decomposition of crop residues was continuously monitored over a period of up

to 140 days by measuring soil respiration. Available P and microbial biomass P and C were

also assessed at different times during the incubations. The total P in residues ranged from

0.16% to 0.32% and 0.05% to 0.08% in young and mature shoots, respectively. Water-soluble

P was related to residue total P and ranged from 29% to 81% and 13% to 29% of total P in

young and mature shoots, respectively. The C: P ratio ranged from 133: 1 to 253: 1 and 504: 1

to 858: 1 in young and mature shoots, respectively.

Phosphorus availability and microbial P uptake differed between soils amended with crop

residues and soluble P fertilizer as triple super phosphate (TSP). Soil respiration rates were

significantly higher in soils amended with crop residues than in the soils amended with TSP or

the unamended control in the first 58 days of incubation. In an experiment in which residues

and TSP were added at a rate of 10 mg P kg^{-1}, available P was greater for TSP than residue-

amended soil, whereas microbial P showed the opposite trend. Respiration rate and microbial

P were positively correlated with C addition rate, which was highest in mature wheat residue

because it had the lowest P concentration.

In order to assess when P released from the residues is available for plants, wheat was grown

over three consecutive crop periods with each period lasting for 4 weeks. Young residues with

high content of water soluble P, C, N and amide and low lignin and phenolic content

decomposed faster than mature residues. The C type and amount added with residues

controlled the dynamics of P availability. Surprisingly, canola mature root increased available P

and plant growth as much as young shoot residues while root residues of wheat and lupin resulted in P immobilization and low plant growth. Compared to canola young shoot, canola mature root has a higher total P concentration and a lower C: P ratio. Plant P uptake was positively correlated with residue total and water-soluble P content and negatively correlated with residue C: P and C: N ratio and amount of C added with the residues. In another experiment where residue was added at 2.5 g C kg^{-1} soil and compared with TSP (4 and 10 mg P kg^{-1} soil), available P and plant P uptake decreased in the following order: TSP-10P > canola root $\geq$ young shoot $\geq$ TSP-4P > control > mature shoot.

Microbial P was greater with residue addition than with TSP and in the control. Residues with low total P and high C: P ratio resulted in P immobilisation in the microbial biomass. Therefore, residues with high total P and low C: P ratio can be an important source of P for plants. Net P immobilisation of mature wheat residues (0.07% P) was significantly reduced by combining wheat residue (C: P ratio 615: 1) with TSP leading to a C: P ratio of 155: 1 to 310: 1. Furthermore, the combination of wheat residue with TSP increased available P in residue and TSP-amended soils by 3.0 mg P kg^{-1} soil, which was shown to be sufficient to support wheat growth in the early stages of development in the other experiments.

Although water-soluble P fertilizers provide plants with immediately available P, a large proportion becomes unavailable over time. Addition of low C: P residues on the other hand, may not result in high amounts of immediately available P, but the P supply is more sustained due to P release from decomposing residues and turnover of microbial biomass P. Phosphorus immobilization after addition of residues which have high C: P ratio (615: 1) may be offset when residue is applied together with inorganic P fertilizer if the resulting C: P ratio is 300: 1 or less.

Overall, this study has highlighted the potential role that crop residues, either alone or in combination with inorganic P, can play in increasing P availability in the light textured, low organic matter, P-limited soils typical of many southern Australian farming systems. The results provide important quantitative information on the potential of a wide range of crop residues to supply wheat with P, and how additions of inorganic P interact with residue decomposition and influence available P supply. This quantitative information will be valuable for the construction or validation of mechanistic models of residue decomposition relevant to low organc matter light textured soils in farming systems of southern Australia, and will ultimately assist in the development of economic management strategies for minimizing P fertilizer inputs and maximizing the benefits of biological cycling of P.

Declaration

I declare that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution. To the best of my knowledge and belief, this work contains no material previously published or written by another person, except where due reference is made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying subject to the provisions of the Copyright Act 1968.

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Chapter 1

General introduction

Phosphorus (P) is an important plant macronutrient that is limiting for plant growth in many soils. Although Australian soils contain large amounts of total P, plant P availability is generally low. Phosphorus requirement for optimal plant growth ranges from 0.3% to 0.5% of the dry matter. Adequate P supply enhances many physiological processes such as photosynthesis, flowering, fruiting and seed formation. Phosphorous deficiency affects root growth and reduces leaf surface area (Brady and Weil 2000; Marschner 1995). Besides these, P plays an important role in metabolic processes as ATP and in cell division as component of DNA and RNA (Schachtman *et al.* 1998).

Phosphorus in the soil solution is the more labile than other pools such as microbial biomass, exchangeable inorganic P and labile organic P (Bunemann and Condron 2007; McLaughlin *et al.* 1988c). Plants only take up P in the form of phosphate ions from the soil solution in which P concentrations rarely exceed 10 µm (Schachtman *et al.* 1998).

Inorganic P (P_i) mostly consists of poorly soluble Ca phosphates and Fe and Al phosphates respectively in alkaline soils and acid soils (Marschner *et al.* 2005). Most recent studies have focused on inorganic P fertilizers as a P source for plants. In many agricultural systems, fertilizer has been applied to overcome the P deficiency for better plant growth, however, the recovery of P by the plants from applied fertilizer is low, only 10%-20% in the year of application, because a large amount of applied P becomes unavailable for plant uptake due to adsorption, precipitation, or transformation into the organic pool (Richardson 2001; Schachtman *et al.* 1998).

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In Australian soils, a significant proportion (20 to 80%) of total soil P is organic P (P_o) which includes mainly phytate (inositol phosphates), phospholipids and nucleic acids (Dalal 1977; Schachtman *et al.* 1998; Tate 1985). Pools of P_o include soil organic matter, crop residues, and microbial biomass. Organic P concentration can be high (100-400 kg P ha^{-1} 10 cm^{-1}) and could be a potential source of P for plant growth by supplying the plant with available P_i after mineralization (Blair and Boland 1978; Richardson 1994). The importance of this P_o pool is likely to depend on its rate of mineralization and the factors influencing mineralization such as microbial activity, soil moisture, temperature and pH (Blair and Boland 1978; Richardson 1994; Tiessen *et al.* 1994). For example, in a fertile pasture soil up to 29 kg P ha^{-1} was found to be released from labile P_o and microbial P pools in late spring (Richardson 1994).

Organic P can be an important P source for plants where application of inorganic fertilizer P is not economic in highly P adsorbing soils. The potential of the P_o pools to supply P to plants through mineralization is poorly understood and the role of P_o in plant P uptake is unclear. Added crop residues and microbial activity increase the availability of P but it remains to be assessed how much P can be released from crop residues and the microbial biomass and how much this contributes to plant uptake in soils from Southern Australia. There was some work done in South Australia by McLaughlin and Alston (1986a) that looked at the pasture residues using ^{33}P. Although the work was done on residues, over the past twenty years there has been a shift towards more continuous cropping and so the information is now needed for crops. Little is known about the interactions between inorganic P fertilizers and organic P sources and their effect on plant P uptake in low organic matter content South Australian soils. Therefore, more research on P_o is essential to maximize release of P from organic sources as a P source for

plants. We hypothesize that available P supply will change in relation to type of organic P amendments, microbial biomass P and C, soil type and extent of organic matter decomposition.

This study was designed to focus on biological and biochemical aspects of P cycling in relation to available P supply and plant P uptake in light textured sandy soils with low organic matter which are typical of a large proportion of the southern Australian wheat growing area. The principal objective of the study described in this thesis was to determine the effect of crop residue biochemical quality on decomposition rates, soil P dynamics and plant P uptake. A further aim was to investigate the effects of combined additions of plant residue and P fertilizer on P cycling in these soils, a scenario highly relevant to farming systems.

Chapter 2

Literature review

2.1 Introduction

Phosphorus (P) is an essential mineral nutrient in all living systems. As a component of nucleic acids (DNA and RNA), phosphate esters (e.g. sugar phosphates), and phosphate compounds which are attached by energy-rich pyrophosphate bond (e.g. ATP), P plays a vital role in carrying and translating genetic information, in energy transfer for metabolic processes including ion uptake and their transport within the plant as well as for synthesis of carbohydrates (Marschner 1995).

Although large amounts of total P can be present in soils, only a small proportion of P is immediately available to plants (~$1\mu M$) (Richardson 1994). Plants exclusively take up phosphate ions from the soil solution. Therefore soil solution P must be replenished to supply sufficient P for plants (Richardson 1994; Richardson 2001).

Inorganic P (P_i) occurs in soils in different forms: as minerals, adsorbed, and precipitated. Phosphorus forms poorly soluble minerals with Ca, Fe and Al. Phosphorus can be adsorbed onto the surface of positively charged minerals such as Fe and Al oxides (Brady and Weil 2000; Hinsinger 2001; Sanyal and Datta 1991).

Organic P (P_o) in soil is converted to P_i through mineralization. Phosphatases excreted by plants and microorganisms mediate the mineralization of P_o. Different pools of P_o such as crop residues, native organic matter, and microbial biomass have different mineralization rates and

therefore supply P_i at different rates (Blair and Boland 1978; McLaughlin *et al.* 1988c; Nziguheba *et al.* 1998).

The microbial biomass has a significant role in the transformation of soil P_o and also plays an important role as a source and sink of available P (Kwabiah *et al.* 2003a; Oberson and Joner 2004). In most agricultural soils the amount of microbial P varies between 1 and 10% of total soil P (Richardson 1994).

The soil P cycle involves soil, plants, and microorganisms. Major processes of the P cycle are uptake of solution P by plants and microorganisms, recycling of P through plant residues, microbial P turnover through mineralization and immobilization, fixation of P on clay and Fe, Al oxides/hydroxides, and solubilization of P (Stevenson and Cole 1999). Pools and transformations are affected by temperature, moisture, pH, microbial activity, and C: P ratios of residues and biomass.

The aim of this review is to provide an overview of soil P pools (P_o and P_i) and the role of P_o which includes crop residues, native organic matter, and microbial biomass in plant P nutrition.

2.2 Soil inorganic phosphorus

The total amount of P in soils depends upon the parent material, soil genesis and physicochemical and environmental factors (Matar *et al.* 1992). In general, the total P concentration in Australian soils is about 422 mg P kg^{-1} soil (Brady and Weil 2000). About 50 to 200 kg P ha^{-1} (0-10 cm) of the total soil P is inorganic P (P_i) which includes adsorbed, precipitated P and P minerals (Richardson 1994). Soil P_i is P not bound with organic material. The amount and forms of P_i compounds present in soil vary with soil pH, clay contents and

mineral types (Brady and Weil 2000). About 200 different phosphate-bearing minerals are known (Tate 1985).

In most soils, native P is derived from soil-forming parent materials such as strengite ($FePO_4 \cdot 2H_2O$) or variscite ($AlPO_4 \cdot 2H_2O$) and apatite (Brady and Weil 2000; Stevenson and Cole 1999). These P bearing minerals release P during weathering and soil development and this P can be subsequently taken up by plants and soil organisms and recycled, incorporated into the organic matter and be adsorbed or occluded in soils and sediments or precipitate with Ca, Al or Fe to form secondary P minerals (Matar *et al.* 1992; Stevenson and Cole 1999).

Phosphorus fertilizers are the main source of P_i in agricultural ecosystems. Australian soils naturally have low P status compared to European and US soils. To cope with this deficiency, P fertilizers (mainly super phosphates) have been used for more than 100 years in Australia (Bertrand *et al.* 2003). The consumption of P fertilizers in Australian agriculture in 1997 was about 450,000 tonnes (Glendinning 2000).

2.2.1 Speciation of P in the soil solution

In general, P ions derive from the dissociation of phosphoric acid. Speciation of P in the soil solution mainly depends on soil pH (Hinsinger 2001; Stevenson and Cole 1999). $H_2PO_4^-$ and HPO_4^{2-} are the dominant P ions at soil pH between 4 and 10. HPO_4^{2-} is the major species at pH above 7.5 (Brady and Weil 2000; Hinsinger 2001). Plants take up P_i from the soil solution mainly as $H_2PO_4^-$ (Marschner 1995).

2.2.2 Physico-chemical reactions influencing the P concentration in solution

Most soils contain comparatively low P_i concentrations in the soil solution while most P_i is more or less strongly bound to soil minerals (Hinsinger 2001). Phosphate ions rapidly react with soil components. Physicochemical reactions involved in controlling the P concentration in soil solution include: adsorption, desorption, precipitation, and dissolution (Hinsinger 2001; Matar *et al.* 1992; Sanyal and Datta 1991). These reactions are affected by soil components (e.g. clay content, organic matter/organic anions, Fe and Al hydroxides), concentration of supporting electrolytes, and pH (Hinsinger 2001; Sanyal and Datta 1991). Adsorption generally predominates at low P concentration. In contrast, precipitation is dominant at higher concentrations that are produced by the application of P fertilizer (Matar *et al.* 1992).

2.2.2.1 Adsorption of P

Adsorption causes soluble phosphate ions in the soil solution to become poorly available for plants (Sanyal and Datta 1991). Major P adsorbents contain either hydroxyl groups (Fe and Al oxides), carboxyl groups (organic matter) or silanol (clay) groups (Hinsinger 2001; Samadi and Gilkes 1998; Sanyal and Datta 1991). In general, P is adsorbed to Al and Fe hydroxides in acid soils and to calcite in alkaline soils (Samadi and Gilkes 1998; Sanyal and Datta 1991).

2.2.2.2 Desorption of P

Desorption of adsorbed P generally occurs through ligand exchange reactions (Hinsinger 2001). Decreased P concentration in the soil solution and increased concentration of competing anions change the adsorption-desorption equilibrium and increase P desorption (Hinsinger 2001; Sanyal and Datta 1991). High concentrations of competing organic ligands such as oxalate and citrate can desorb significant amounts of P (Hinsinger 2001).

2.2.2.3 Precipitation of P

Precipitation is the formation of insoluble P minerals when the P concentration in solution is high. In neutral to alkaline soils, P ions generally precipitate as Ca phosphates: dicalcium or octacalcium phosphates, and apatite (Bertrand *et al.* 2003; Hinsinger 2001; Samadi and Gilkes 1998; Sanyal and Datta 1991). In acid soils, P ions precipitate as several Al and Fe phosphates such as variscite and strengite (Hinsinger 2001; Sanyal and Datta 1991).

2.2.2.4 Dissolution of P minerals

The solubility of Al and Fe phosphates increases with increasing pH up to 6.5. In contrast, Ca phosphate solubility decreases with increasing soil pH above 7 (Hinsinger 2001; Sanyal and Datta 1991). The slow dissolution rate of Al, Fe, and Ca phosphate cannot maintain the concentration of P in soil solution at levels sufficient for plants and microorganisms (Sanyal and Datta 1991).

2.3 Soil organic phosphorus

Soil organic phosphorus (P_o) is P bound to organic compounds. It is converted to P_i by mineralization, which can then be taken up by plants and microorganisms. The dynamics of P are defined as the movement of P among the different pools. Stewart and Tiessen (1987) stated that a complete P cycle included mineralization, immobilization and redistribution of P (Figure 2.1).
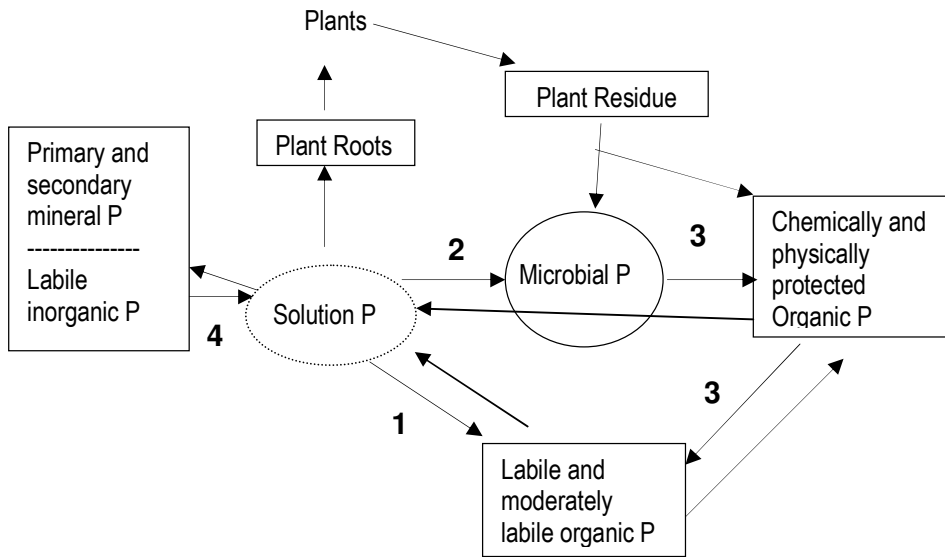
Figure 2.1 Phosphorus cycle and processes involved such as mineralization (1), immobilization (2), transformation (3) and solubilization (4). From Stewart and Tiessen (1987).

The P_o concentration in soils may vary from 4% of total P in podzols to 90% in alpine soils and in pasture soils it may be 50-84% of total P (Blair and Boland 1978; Dalal 1977). In the soil surface horizon, P_o varies from 20 to 90% of the total P (Dalal 1977; Tate 1985). In Australian soils, the amount of P_o is 40-900 mg kg^{-1} while it can be 120-1360 mg kg^{-1} in New Zealand soils (Stevenson and Cole 1999). This variation is due to several factors such as soil texture, soil pH, temperature, organic C content, mineralization and immobilization, which will be discussed later. Organic P may be derived from plant residues, soil organic matter and/or microorganisms (Sanyal and Datta 1991).

2.3.1 Chemical extraction of organic phosphorus

Different pools of P_o differ in their availability to plants. Some may be rapidly mineralized and transformed into available P_i for plants and microbes and some will remain unavailable. Based on the extractability of P from soil in a sequence of different extractants (Hedley *et al.* 1982),

Tiessen and Moir (1993) developed an extraction scheme for characterization of P_i and P_o. Resin extractable P and 0.5 M $NaHCO_3$ extractable P are labile P in exchangeable form considered to be plant available, 0.5 M $NaHCO_3$ extractable P_o is labile P_o associated with organic surfaces, humic and fulvic acids (Hedley *et al.* 1982c). Organic P more strongly associated with humic and fulvic acid is extractable with 0.1 M NaOH and is moderately labile. Hot concentrated HCl-extractable P is poorly available P_i and stable P_o. Stable and residual P_o can be extracted by digestion with concentrate H_2SO_4 and H_2O_2. Magid et al.(1996) noted that $NaHCO_3$ extractable P_o is easily mineralizable and labile and potentially available for plants and NaOH extractable P_o can contribute to plant available P after a long-term mineralization of P_o compounds.

For measuring the amount of P held in the microbial biomass, Kouno *et al.* (1995) described a fumigation-extraction method by anion exchange resin (AER). The principles of this method were (1) the soil microbial P is released from the cells through cell lysis with chloroform, and (2) the AER strips bind P_i in the soil extracts. The microbial biomass P is calculated from the difference between P extracted from fumigated and un-fumigated soils after corrections for P sorption and P recovery during fumigation. Using hexanol for fumigation has been found to be as effective as chloroform (McLaughlin *et al.* 1986b) while chloroform may gradually dissolve AER (Bünemann 2003).

2.3.2 Chemical nature of soil organic P

The main fraction of soil P_o occurs as mono- and diesters (Tate 1985) and their availability varies with their chemical structure (Magid *et al.* 1996; Tate 1985). Soil P_o can be classified into three major groups, (i) inositol phosphate, (ii) phospholipids, and (iii) nucleic acids (Dalal 1977; Sanyal and Datta 1991; Stevenson and Cole 1999). In addition to these three classes, small

amounts of other organic phosphates have been detected in the form of phosphoprotein, sugar phosphates, phosphonate, glycerophophate, polyphosphate and pyrophosphate (Dalal 1977; Magid *et al.* 1996; Tate 1985). Inositol phosphate is the predominant form among the identified soil P_o compounds. The inositol phosphate content in soil ranges from 0.4 to 83% of total soil P_o (Dalal 1977; Stevenson and Cole 1999). The amount of inositol P in Australian soils is 0.4 to 38% of total P_o which is lower than the British soils (24-58%) (Dalal 1977). Phospholipid P in soil varies from 0.5 to 7.0% of total soil P_o and this small amount is due to their rapid degradation (Dalal 1977). A small fraction, less than 3% of soil P_o, occurs as nucleic acids or their derivatives (Dalal 1977; Stevenson and Cole 1999). In microorganisms, more than 60% of microbial intracellular P is in the form of nucleic acids (Stewart and Tiessen 1987). Despite the relatively large amounts of nucleic acids added to soil from decomposed microbial, plant and animal residues, the amount of nucleic acids in soil is very low due to rapid mineralization and incorporation into microbial biomass (Dalal 1977; Sanyal and Datta 1991).

2.4 Dynamics of phosphorus

The dynamics of P in soil depends on the interactions between microbes, organic matter and plants in the processes of mineralization, immobilization and redistribution (Stewart and Tiessen 1987). Phosphorus immobilization is the biological incorporation of P into the living microbial biomass, thus reducing P_i in the soil solution. Redistribution of P is the release of P from disrupted microbial cells followed by transfer into other P pools (Oberson and Joner 2004). Both mineralization and immobilization can occur simultaneously in soil and the amount of P_o and P_i can change both in the short- and the long-term (Dalal 1977; McLaughlin *et al.* 1988c; Oberson and Joner 2004; Stewart and Tiessen 1987). The P cycle and the different P pools are shown in Figure 2.2.

Microbial P can be a potential source of P for plants. A large amounts of P_o in Australian soil is found in the microbial biomass (10-30 kg ha^{-1} 10cm^{-1} depths), which is 3% higher than in the plant residues (7-10 kg ha^{-1} 10cm^{-1} depths) (Richardson 1994). Therefore, soil microorganisms play a central role in soil P dynamics as a labile reservoir of P_o, as well as by microbial uptake of P, mineralization and solubilization of P_i (Magid *et al.* 1996; McLaughlin and Alston 1986a; Richardson 1994; Stewart and Tiessen 1987). A large proportion of P from added pasture residues is taken up into the microbial biomass and remains there up to 40 days (McLaughlin and Alston 1986a). The cell contents of dead microorganisms and the products released by bacterial grazers, such as protozoa, and nematodes contain P_o and P_i (Stewart and Tiessen 1987; Tate 1985).

In P dynamics, the fate of fertilizer-supplied P_i is mainly determined by the P content of the soil, size and activity of the microbial biomass, organic matter content, presence of plants, P adsorption capacity, and soil properties such as pH, moisture, temperature, and clay content (Blair and Boland 1978; Iyamuremye *et al.* 1996a; Iyamuremye *et al.* 1996b; McLaughlin and Alston 1986a; Nziguheba *et al.* 1998).

Inorganic P and P_o added to soil interact. Addition of plant residues tends to increase fertilizer P uptake by the microbial biomass by 6.2% while plant P uptake was decreased by 4.3% (McLaughlin and Alston 1986a). Plants and microbial biomass compete for applied fertilizer P. In the absence of crop residues, the fraction of applied fertilizer P taken up by the plants (23.5%) and in the microbial biomass (22.6%) was not significantly different after 40 days (McLaughlin and Alston 1986a).
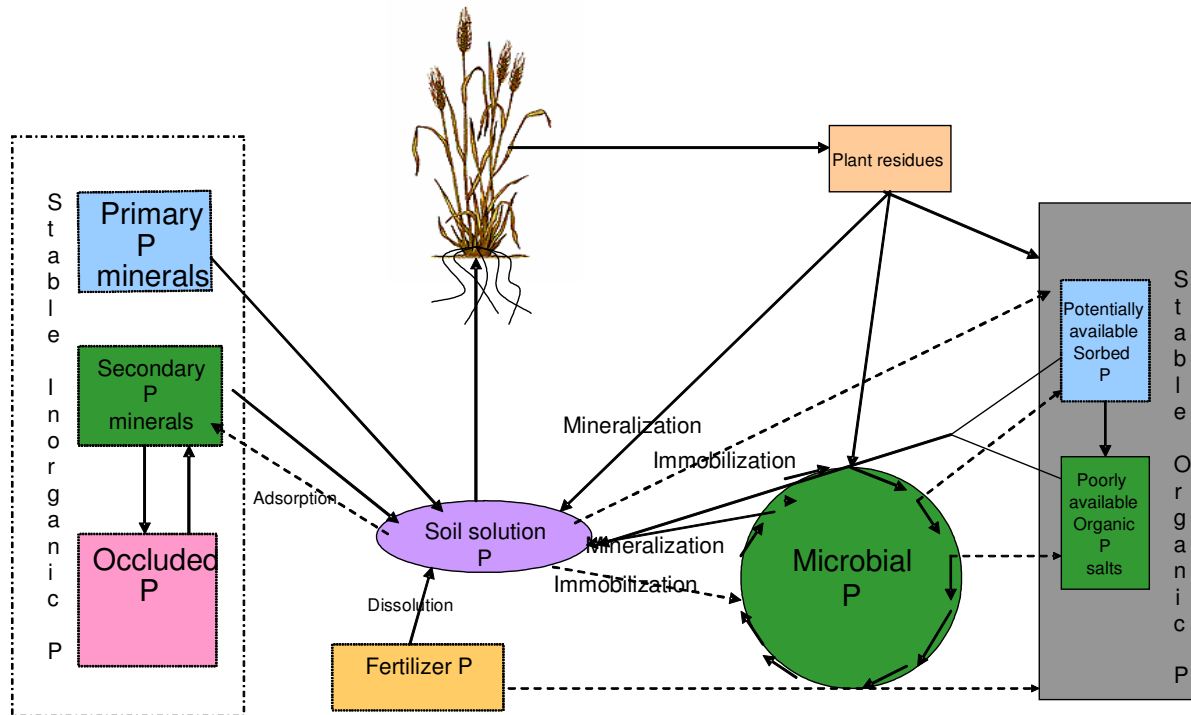
Figure 2.2 Phosphorus cycle based on (McLaughlin *et al.* 1988c; Sanyal and Datta 1991; Tate 1985)

Both native soil P and P added with plant residues contribute to plant P uptake (Blair and Boland 1978; McLaughlin et $al.$ 1988a). Phosphorus uptake by plants is significantly higher from added plant residues in a high available P soil compared to a low P soil (Blair and Boland 1978). Decomposition of residues is required for the P to become plant available. In a field study, McLaughlin et $al.$ (1988a) reported that the proportion of plant P derived from added plant residue gradually increased to 7% after 18 days and then remained constant over 61 days. Application of plant residues high in P increased P availability in soil to a similar or greater extent than P_i fertilizer when the same amount P was added as fertilizer or residue P (Nziguheba et $al.$ 1998).

In Australian soils, about 100-400 kg P ha^{-1} $10cm^{-1}$ is stable P_o and can be a potential source of P for plants after mineralization (Richardson 1994). Organic P compounds with high charge densities are rapidly stabilized and accumulate in soil. Organic P compounds with low charge densities are more accessible to microorganisms and consequently low amounts are found in soils (Stewart and Tiessen 1987). Stable P_o is mainly involved in the long-term mineralization of P in soil and therefore, the P in the stable P_o become more available to plants over time (Magid et $al.$ 1996; Richardson 1994). The mineralization rate is influenced by numerous factors such as soil texture, pH, moisture, temperature, microbial activity, C and P ratio of soil, cultivation, aeration, and P addition (Dalal 1977; Richardson 1994; Stewart and Tiessen 1987; Tate 1985).

2.4.1 Role of crop residues in soil P dynamics

Crop residues are an important source of plant nutrients and can return nutrients to soil as they decompose. Crop residues provide food for soil microorganisms and stimulate microbial activity and microbial growth that leads to increase biological cycling of nutrients in the soil (Ros et $al.$ 2003; Tate 1985; Varinderpal-Singh and Rengel 2007). Addition of plant residues to soil

strongly affects on soil microbial activity. The changes of microbial activity after residue addition are affected by the types and quality of residue and generally, microbial activity is higher in soil amended with young leaves and shoot residues than in soil amended with mature shoot and root residues (Abiven *et al.* 2005; Bertrand *et al.* 2006; Wang *et al.* 2004). In the plant material, the soluble carbon compounds including metabolic carbohydrates, amino acids are the first compounds to decompose. On the other hand, lignin, cellulose and polyphenols are considered as the less degradable compounds that decompose in the late stage of decomposition (Abiven *et al.* 2005; Jensen *et al.* 2005; Palm and Rowland 1997; Wang *et al.* 2004). Addition of crop residues also affects the soil microbial community composition. Changes in microbial community structure in soil have been reported to be higher in residue-amended soil than the control and fertlizer-amended soil and it is affected by the residue types and the amount of residues incorporate in soil over a long period (Cookson *et al.* 2005; Nelson and Mele 2006). As a result of residue addition, the ratios of gram-positive to gram negative bacteria and of bacteria to fungi become higher than the fertilizer-amended soil (Marschner *et al.* 2003) and the changes in microbial community may also affect the N and P immobilization/mineralization in soil (Bünemann *et al.* 2004; Cookson *et al.* 2005; Nelson and Mele 2006). Organic residues can reduce P sorption and increase P availability to plants. Organic acids produced during mineralization of organic matter components compete with phosphate anions for binding sites in the soil (Iyamuremye *et al.* 1996a; Nziguheba *et al.* 1998). Decomposed organic matter can affect the availability of P by changing the rate of uptake of P_i by the microbial biomass and affecting the microbial activity (Kwabiah *et al.* 2003b). The rate of decomposition of crop residues is an important factor to determine the value of crop residues as a P source for plants (Dalal 1979; Fuller *et al.* 1956). Residue quality and biochemical composition such as lignin, soluble C, soluble polyphenolics, α-cellulose, and ash greatly affect residue decomposition and P release (Abiven *et al.* 2005; Kwabiah *et al.* 2003b; Nwoke *et al.* 2004; Wang *et al.* 2004).

Crop residues with higher P content (> 0.24% P) increased net P mineralization while crop residues with low P content (< 0.07% P) resulted in net P immobilization (Fuller *et al.* 1956; Iyamuremye *et al.* 1996a; Nziguheba *et al.* 1998). Therefore, less fertilizer P is required for crop when soils are amended with crop residues with high P content (> 0.24% P) (Iyamuremye *et al.* 1996a). Residue quality is dependent on plant species, part and maturity as well as on nutrient availability during plant growth and chemical composition changes over time during decomposition (Wang *et al.* 2004). In Australian soils, the relationships between crop residue quality and dynamics of available P and plant P uptake have not been studied over a wide range of different crop residues available to Australian farmers. Most studies on residue decomposition and plant P uptake were done to monitor residue decomposition and plant P uptake with plants planted at the beginning of the decomposition process and thus ageing during plant residue decomposition (Blair and Boland 1978; Dalal 1979; McLaughlin *et al.* 1988a). Older plants may not respond to nutrients released in the later stages of decomposition because their nutrient demand is lower than in young plants. Moreover, it has been found that addition of residues with inorganic P fertilizer increases not only available P and plant P uptake compared to residue alone but also microbial P (Bah *et al.* 2006; McLaughlin and Alston 1986a; Reddy *et al.* 2005). In wheat growing South Australian soils, wheat residues (0.07% P) are added after grain harvest and also costly inorganic P fertilizers are added every year but very little is known about the effect of wheat residues in combination with inorganic P fertilizer.

2.4.2 Microbial biomass and turn over of microbial P

The soil microbial biomass is an important pool of P, and acts as a major source and sink of available P and transformer of soil P_o (Kwabiah *et al.* 2003a; Oberson and Joner 2004). The microbial biomass constitutes a significant amount of P in most agricultural soils and ranges between 1 to 10% of total soil P (Richardson 1994). Environmental factors and soil

management practices influence the contribution of microbial P to total soil P (Oberson and Joner 2004; Richardson 1994; 2001). The microorganisms that are considered as an important driving force for P turnover in soil include microflora and microfauna (Oberson and Joner 2004), such as bacteria, fungi, algae, protozoa and nematodes (Oberson and Joner 2004; Richardson 2001; Stewart and Tiessen 1987). Most of the microbial P is contained in bacteria and fungi. Although protozoa and nematodes contribute little to the microbial P pool, they may be important for the net mineralization of P by stimulating the turnover of bacteria and fungi (Oberson and Joner 2004; Tiessen *et al.* 1994). The concentration and forms of P in microorganisms vary with growth stage and activity of the microorganisms (Oberson and Joner 2004). Of the intracellular P, over 60% are nucleic acids, 20% are acid soluble P-esters and 5-20% are phospholipids (Stewart and Tiessen 1987).

Microbial turnover of P in soil is the sum of all types of P transformations that are mediated by microorganisms and the related microbial P fluxes in soil (Oberson and Joner 2004). Soil microorganisms can increase P supply to plants by solubilization, mineralization and re-mineralization of P_i and P_o, and can decrease P_i in the soil solution by immobilization (Oberson and Joner 2004; Richardson 1994). Re-mineralization occurs by releasing P_i from microorganisms after their death (Oberson and Joner 2004).

2.4.2.1 Solubilization of P_i and mycorrhizal association

A wide range of soil bacteria and fungi (e.g. *Bacillus*, *Pseudomonas*, *Penicillium* and *Aspergillus*) can solubilize various forms of precipitated P_i and can increase plant P uptake (Oberson and Joner 2004; Richardson 2001). Phosphorus solubilizing microorganisms can comprise up to 40% of the cultured microorganisms in a soil sample (Richardson 2001). The

general mechanism of P solubilization by microorganisms is the excretion of low molecular weight organic acids that either directly dissolve P minerals and/or decrease soil pH and chelate associated cations (Oberson and Joner 2004; Richardson 1994; Richardson 2001).

The symbiotic association between plant roots and mycorrhizal fungi is an important mechanism which enables plants to take up more P from soil (Richardson 2001). Arbuscular mycorrhizae (endomycorrhizae) establish a symbiotic relationships with most terrestrial plant species (Oberson and Joner 2004). The plant provides carbohydrates to the fungus which results in an extensive hyphal network and a substantial increase of nutrient absorbing surface area (Richardson 2001). Plants with mycorrhizal roots and non-mycorrhizal plants can access similar soil P pools but the increased root-hyphal surface area allows exploration of a larger volume of soil and uptake of greater amounts of soil P by mycorrhizal roots (Richardson 1994; Richardson 2001). Mycorrhiza can also produce phosphatases that can enhance mineralization, and they can excrete organic acids (Oberson and Joner 2004; Richardson 2001).

2.4.2.2 Mineralization of P_o

Mineralization of P_o takes place mainly from three P_o sources: microorganisms, soil organic matter and freshly added organic materials (e.g. plant residues, animal manure, composts) (Blair and Boland 1978; McLaughlin *et al.* 1988c; Oberson and Joner 2004) and consists of different processes such as flush effects, basal mineralization and re-mineralization (Oberson and Joner 2004). Flush effects are caused by drying-wetting or freezing-thawing cycles that result in microbial death and a return of P to the soil solution (Oberson and Joner 2004; Stewart and Tiessen 1987). Basal P mineralization is the gross mineralization of soil organic matter excluding freshly added organic matter (Oberson and Joner 2004). Mineralization of P_o

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includes biological and biochemical processes. Biological mineralization is the release of P_i from organic matter during oxidation of C, which provides energy to microorganisms. Biochemical mineralization is the release of P_i from organic compounds through hydrolysis by extracellular enzymes (Oberson and Joner 2004; Stewart and Tiessen 1987). Therefore, biochemical mineralization of P_o is not closely linked to C turnover. It is controlled by the supply of and need for P rather than C requirement (Richardson 1994; Stewart and Tiessen 1987). A range of phosphatases mineralize various P_o substrates both in microbial and plant materials. Although major phosphatases are of microbial origin, plant roots also contribute to phosphatase activity, which is higher in the rhizosphere than in bulk soil (Oberson and Joner 2004; Richardson 1994). Soil microorganisms appear to be the major source of phytase in soils (Richardson 2001).

2.4.2.3 Immobilization and re-mineralization of P

A substantial amount of native P_i enters the P_o (including microbial P) pool over the year (Dalal 1977). Within 10 days after addition of residues, the soil microbial P content can increase by 2.8 mg kg^{-1} (Bünemann 2003). In soils low in native P such as Australian soils, application of P fertilizer results in a build up of the P_o pool (Dalal 1977). As stated above, about 22.6% of the applied fertilizer can accumulate in the microbial biomass (McLaughlin and Alston 1986a). Therefore, availability of P_i for plant uptake decreases when microbial P increases (Bünemann 2003). In tropical pasture and forest soils microbial P increases and available P decreases during the rainy season (Bünemann 2003; Oberson and Joner 2004). Thus, microorganisms maintain the balance of both P_i and P_o in soil solution and influence the P availability (Richardson 2001).

Oberson and Joner (2004) defined re-mineralization of P_o as the release of microbial P caused by microbial death, predation and mineralization of released P_o. In a field study on a solonized brown soil, the percentage of microbial P derived from the added crop residues remained constant at 14 to 15% between 7 and 95 days after residue addition due to a balance between mineralization, uptake and re-mineralization (McLaughlin *et al.* 1988b). Phosphorus added with plant residues may transiently be immobilized but can be re-mineralized over time (Oberson and Joner 2004). Overall, immobilization and re-mineralization of soil P depends on size, activity and composition of the microbial biomass, soil type, soil moisture, temperature, energy supply and the interactive effects of soil microflora and microfauna (Bünemann 2003; Oberson and Joner 2004; Richardson 1994; Stewart and Tiessen 1987).

2.5 Factors affecting the availability of organic phosphorus

2.5.1 Microbial activity

The size as well as the activity of microorganisms play an important role in mineralization of P_o in soil (Chen *et al.* 2002; Dalal 1977). Biological activity mediates the mineralization of soil P_o by phosphatases excreted by plants or microorganisms (Magid *et al.* 1996). Phosphatase excretion by microbes and plants is enhanced under low P_i availability (Richardson 1994; Stewart and Tiessen 1987). Inorganic P may be taken up by microorganisms or plants. Phosphorus taken up by microorganisms may be lower, equal to or greater than mineralized P_o (Oberson and Joner 2004). Immobilization of P is regulated by the growth and death cycle of microorganisms (Kwabiah *et al.* 2003a; McLaughlin *et al.* 1988c).

2.5.2 Temperature

Mineralization and immobilization of soil P_o is strongly influenced by temperature (Tate 1985). The optimum temperature for P_o mineralization is 35 ºC (Dalal 1977). The availability of P derived from crop residues for plant uptake is affected by temperature (McLaughlin *et al.* 1988a). At high temperature and adequate soil moisture, crop residues can be a significant source of P for crops (Tate 1985). Low soil temperature (8ºC) was found to delay mineralization of manure and release of P_i in an European soil (Blake *et al.* 2000).

2.5.3 Soil moisture

Moisture is relatively more important than temperature in determining the size and activity of the microbial biomass and is the cause of seasonal changes in microbial activity and growth (Chen *et al.* 2003). Microbial biomass P was decreased by 22 to 64% when soil moisture declined over summer in a pasture soil in the UK (He *et al.* 1997). Microbial P was linearly related to soil moisture in soils with added crop residue and it increased more than five fold (3.7 to 19.5 kg ha^{-1}) after 7 days of favorable moisture conditions (McLaughlin *et al.* 1988b; McLaughlin *et al.* 1988c). Thus, adequate moisture has a significant role in mineralization of P_o from organic matter and decomposing crop residues (Dalal 1977). Alternate wetting and drying influence the mineralization of organic matter and can ultimately lead to maximum mineralization of P_o and partial mineralization of organic C and N (Blair and Boland 1978; McLaughlin *et al.* 1988c; McNeill *et al.* 1998; Tate 1985).

2.5.4 Soil pH

Soil pH strongly influences the availability of P_o in soil. At high pH, OH^- ions compete with PO_4^{2-} for P adsorption sites and release P into the soil solution (Iyamuremye *et al.* 1996a;

Stewart and Tiessen 1987). In acid soil, microbial activity and P_o mineralization increases with increasing soil pH (Richardson 1994; Stewart and Tiessen 1987).

2.5.5 Aeration

Aeration is essential for microbial breakdown of organic matter and mineralization of P_o (Glendinning 2000). Aeration increases the rate of organic matter decomposition by increasing microbial activity (Dalal 1977; Tate 1985).

2.5.6 Soil texture

Variation of soil texture can influence the relative amounts of P_i and P_o on the same field site (Stewart and Tiessen 1987). Phosphorus content increases with clay content because generally clay soils have higher organic matter content than coarse textured soils (Sanyal and Datta 1991). Mineralization of P_o was greater in sandy loam soil (27% of P_o) compared with clay and loam soils (17-18%) (Magid *et al.* 1996).

2.5.7 Carbon-Phosphorus ratio

The C: P ratio of the soil microbial biomass and plant residues is an important factor that controls the availability of P in soil. As the microbial C: P ratio decreases, microbes have a higher potential to release P into the soil solution during mineralization and microbial turnover (He *et al.* 1997). The C: P ratio in the microbial biomass in pasture soils was found to vary from 9 to 276:1 (He *et al.* 1997). Plant residues can have a wide range of C: P ratios, depending on plant species and P nutrition, and will differ in P mineralization rate (McLaughlin *et al.* 1988c). Plant residues with a C: P ratio of 123: 1 increased 0.5 M $NaHCO_3$ extractable P_i whilst the

plant residues with a C: P ratio of 506: 1 decreased it. Residue with low C: P ratio (123: 1)
released about 2 kg P ha^{-1} in 15 cm soil during 42 days (White and Ayoub 1983).

2.5.8 Addition of P_i

Addition of P_i can result in increased mineralization of P_o. This may be due to increased
solubility of P_o. Inorganic P competes with P_o for binding sites and thus can potentially release
bound P_o (Dalal 1977; Nziguheba *et al.* 1998) and an increase of P_o has been found after P_i
addition in temperate and tropical soils (Magid *et al.* 1996; Reddy *et al.* 2005). Addition of P_i
can stimulate plant P uptake from crop residues (McLaughlin and Alston 1986a).

2.5.9 Cultivation

The forms and amounts of soil P_o can be affected by long-term cultivation (Magid *et al.* 1996).
Cultivation increases soil aeration and promotes microbial activity which subsequently
increases decomposition of organic matter (Dalal 1977; Raiesi 2006). Cultivation of pasture
soils markedly increases mineralization of P_o resulting in a rapid depletion of the total P_o and an
increased availability of stable P_o (Richardson 1994).

Besides the factors discussed above, there are other factors that influence the availability of P
in soil solution such as soil salinity, fertilizers other than phosphorus and presence of plants. In
an incubation study, Zahoor *et al.* (2007) showed that soil salinity suppressed P availability and
plant P uptake and the suppressing effect of Cl salt was more severe than SO_4 salt. The
deficiency of C, N or S may retard the decomposition of organic matter or mineralization of P
(Dalal 1977; McGill and Cole 1981; Wang *et al.* 2004). In such cases, addition of inorganic N or
S can increase residue decomposition and can increase P availability but it depends on the

amount of N and S addition (Dalal 1977). In a pot study on residue-amended soil, Dalal (1979) reported that both the P_i and P_o concentration in soil decreased considerably due to presence of plants.

2.6 Plant P acquisition and the role of the rhizosphere in P availability

Plants have developed mechanisms to acquire P from the soil solution which include (i) root morphological characteristics such as greater root length, the rate of root growth, root density, the abundance and distribution of root hairs, root hair length, mycorrhizal association, secreted enzymes and H^+ (Gahoonia and Nielsen 1996; Richardson 1994; Richardson 2001), (ii) the kinetics of P uptake by the roots (Richardson 1994; Richardson 2001). Phosphorus availability to plants is also influenced by root-soil interactions in the rhizosphere that include the rate of diffusion of phosphate anions within soil and rhizosphere, equilibrium concentration of phosphate anions and the rate of replenishment (Chen *et al.* 2002; Kwabiah *et al.* 2003b; Richardson 1994; Richardson 2001).

In addition, biological and biochemical processes occur at the root surface that influence the availability of soil P to plants (Richardson 2001). The concept of bioavailability of nutrients includes three components: root interception, mass flow, and diffusion (Hinsinger 2001; Marschner 1995). When roots grow into the soil, they reach areas containing available nutrients. Thus, roots can intercept nutrients but only a small portion of total nutrients will come into root contact by interception. Through mass flow, which is stimulated by transpiration, P ions in the soil solution move to the root surface (Hinsinger 2001; Marschner 1995). Only a small proportion of phosphate ions (about 5% of the plant P uptake) is transported by mass flow (Hinsinger 2001). The quantity depends on the concentration of phosphate ions in the soil solution and the rate of transpiration (Marschner 1995). Diffusion is the most important process

of the movement of P ions to the root surface (Marschner 1995; Smith 2002). The decrease in

P concentration in the rhizosphere by P uptake causes a concentration gradient that is the

driving force for the diffusion of phosphate ions to the root surface (Hinsinger 2001; Smith

2002). However, diffusion is a comparatively slow process in most soils and results in depletion

of phosphate in soil solution around the root surface (Hinsinger 2001; Smith 2002). Any

changes in diffusion conditions close to roots can influence the net P influx into the root cell

(Gahoonia and Nielsen 1996). Extension of root hairs as well as mycorrhizal hyphae can

increase the volume of soil exploited (Gahoonia and Nielsen 1996; Richardson 2001; Smith

2002).

Plant rhizospheres contain a large number of soil microorganisms and a wide range of plant

and microbial exudates and metabolites. Organic acids are common exudates and effective in

releasing P (Richardson 2001). Simultaneously released H^+ ions change the ratio between

$H_2PO_4^-$ and HPO_4^{2-} and lead to relatively more $H_2PO_4^-$ in the soil solution. The solubility of Ca

phosphates increases with decreasing pH (Gahoonia and Nielsen 1992; Hinsinger 2001;

Richardson 2001). Hydrogen ion (H^+) release can be increased by P deficiency (Hinsinger

2001). Organic acids in the rhizosphere play an important role in displacing adsorbed

phosphate through ligand exchange reactions and thus enhance solubility of P_i (Chen *et al.*

2002; Richardson 2001). Low molecular organic acids such as dicarboxylic (oxalic, tartaric,

malic, malonic) and tricarboxylic (citric) acids can chelate Ca, Al, and Fe and increase P

availability and thereby improve the acquisition of P ions by the root (Hinsinger 2001;

Richardson 2001). The exudation of organic acids/anions increases in response to low P

supply (Hinsinger 2001). Organic acids may derive from microorganisms or plant roots (Chen

et al. 2002; Richardson 2001). Organic acid exudation from roots varies both quantitatively and

qualitatively depending on genotype, distance from root tip and environmental factors

(Gahoonia and Nielsen 1996; Hinsinger 2001). For example, citric acid is the dominant organic acid exuded by white lupin and alfalfa roots, while malic acid is dominant in wheat and tomato (Hinsinger 2001).

2.7 Soil P tests and phosphorus responsiveness of wheat

Generally, soil P tests are measured by 6 preocedures: (i) Bray P, 0.5 M NH_4F extractable P is measured as described by Bray and Kurt (1945); (ii) Olsen P, 0.5 M $NaHCO_3$ (pH 8.5) extractable P is measured after Olsen *et al.* (1954) in 1: 20 soil: extractant with 30 minutes of shaking; (iii) Colwell P is measured as described by Colwell (1963) using 0.5 M $NaHCO_3$ (pH 8.5) extractant in 1: 100 soil: extractant with 16 hours of shaking; (iv) Resin extractable P (Resin P) is determined using anion exchange resin membrane (Kouno *et al.* 1995); (v) P E-value, the isotopically exchangeable P is measured by the isotopic dilution method as described by Hamon and McLaughlin (2002) and (vi) the P DGT (Diffusive Gradients in Thin Films) technique is used as described by Zhang and Davison (1995) with mix binding layer (MBL) for the measurement of P (McBeath *et al.* 2007). In order to better predict the P responsiveness of wheat, different soil P tests (Bray P, Colwell P, Resin P, P E-value and P DGT) were carried out with wide range of South Australian soils followed by determining the effect of P fertilizer addition on wheat growth (McBeath *et al.* 2005; McBeath *et al.* 2007). They reported that available P measurement by resin method was better than all other soil test methods although the resin P values varied for alkaline calcareous soils and alkaline non-calcareous soils. Moreover, Bertrand *et al.* (2003) showed that in calcareous soils, Colwell P may overestimate the availability of P because the Colwell reagent is likely to dissolve the Ca-P that are not plant available whereas the resin P method more accurately estimated the amount of labile P. Resin P is generally the best compared to other P for predicting the yield response

of wheat to applied P and the resin P concentration 9 mg kg^{-1} soil is considered to be adequate for wheat growth (McBeath $et\ al.$ 2005; McBeath $et\ al.$ 2007).

2.8 Aims of the study

Although Australian soils contain large amounts of total P, P availability for plant uptake is generally low. Adequate concentrations of available P from different sources are necessary to achieve and maintain a high level of productivity. Addition of P_i can increase plant P uptake but this supply is often not sufficient for high yields because most of the applied P is immobilized by adsorption to soil minerals or uptake by soil microorganisms. Organic P can represent a large proportion of total soil P. Organic P is a potential P source that becomes available to plants through mineralization mediated by the soil microbial biomass but the role of P_o in plant P uptake is not yet clear. Pools of P_o include recently added plant residues, native organic matter, and the soil microbial biomass. The microbial biomass influences P supply to plants through mineralization, immobilization and turnover. The microbial biomass contains a significant amount of soil P, which can potentially become available to plants after cell death. However, how much P can be released from microbial biomass or mineralized by microorganisms from soil P_o and its contribution to plant uptake is still largely unknown. In addition, the potential of crop residues for plant P supply is not well understood. While added crop residues can increase the availability of P, it is poorly understood how much P from added crop residues becomes available and how much P is released through short or long-term mineralization. The C: P ratio of crop residues appears to be important but there may be other properties that also play a role. Little is known about the interactions between P_i fertilizers and P_o sources and their effect on plant P uptake. More information is needed about mineralization and immobilization processes to maximize the biological release of P from organic sources. The broad context of this study is a focus on the biological cycling of P for managing P supply to grain crops and

maintaining productivity. This includes microbial turnover, organic matter quality, amount of P in different organic pools, and mineralization rate. The aims of this study are: (i) to determine the effect of crop residue biochemical quality on decomposition rates, soil P dynamics and plant P uptake and (ii) to investigate the effects of combined additions of plant residue and P fertilizer on P cycling in these soils, a scenario highly relevant to farming systems. The outcomes of this study will provide a more detailed picture of P dynamics in light textured low organic matter soils after addition of crop residues.

Chapter 3

General materials and methods

The materials and methods that were frequently used in the studies explained in this thesis are described in this chapter. Further details and modifications in individual experiments are provided in the relevant chapters.

3.1 Materials

3.1.1 Soils used in the study

Soils for the experiments were collected from two different locations of South Australia. For the study described in Chapter 4, the soil selected for higher P availability was collected from a long-term crop rotation trial at Waite Agriculture Research Institute. For the other studies a P deficient soil was collected from Monarto, South Australia. The description of the soils can be found in Chapters 4 and 5.

3.1.2 Pre incubation of soils

In all the studies described in Chapters 4 and 5, 6 and 7 soils were pre incubated for 9-14 days with 65%, 80% and 85% water holding capacity respectively before the start of the actual experiment. The bulk density of the soils used in the studies described in Chapters 4 and 5, 6 and 7 were 1.3 and 1.6 g cm^{-3}, respectively. This pre-incubation period under moist conditions ensured that microbial activity was high and had reached a steady state (Oehl *et al.* 2001b).

3.1.3 Crop residues used in the studies

Different types of crop residues were used in this work and collected from different locations of South Australia. The crop residues were chosen because they are commonly grown in South Australia in various crop rotations. Three crop residues; wheat, canola and pea were used for the work described in Chapter 4 (section 4.2) and those were collected from different fields at Roseworthy (South Australia) in May 2004. Crop residues used for the works described in Chapters 5, 6 and 7 were collected in December 2004 from different locations in South Australia and have different growth and fertilization history (Table 3.1).

Table 3.1 Crop residues used in experiments described in Chapters 4, 5, 6 and 7 and amount of fertilizers applied.

Chapter. No.	Residues	Locations	Growth stages	Fertilizers applied (kgha^{-1})
4	**Canola MS**	Roseworthy (C6)	Straw after harvest	93 TSP
4	**Pea MS**	Roseworthy (E5)	Straw after harvest	93 TSP
4	**Wheat MS**	Roseworthy (N1)	Straw after harvest	93 TSP
5, 6	**Pea YS**	Mid North	Pre flowering	50 TSP
5	**Pea MS**	Mid North	Straw after harvest	50 TSP
5, 6	**Canola YS**	Yorke Peninsula	Pre flowering	100 DAP
5	**Canola MS**	Yorke Peninsula	Straw after harvest	100 DAP
5, 6	**Canola MR**	Yorke Peninsula	Root after harvest	100 DAP
5, 6	**Lupine YS**	Yorke Peninsula	Pre flowering	100 DAP
5, 6	**Lupine MS**	Yorke Peninsula	Straw after harvest	100 DAP
6	**Lupine MR**	Yorke Peninsula	Root after harvest	100 DAP
5, 6, 7	**Wheat MS**	Yorke Peninsula	Straw after harvest	100 DAP
6	**Wheat MR**	Yorke Peninsula	Root after harvest	100 DAP
5	**Lentil YS**	Waite trial (plot32)	Pre flowering	50 DAP
5	**Lucerne YS**	Roseworthy (N2a)	Vegetative	60 MAP

YS, Young shoot; **MS**, Mature shoot; **MR**, Mature root

After collection, crop residues were dried at room temperature for 7 days. Before being chopped to <5.0 mm size the residues were dried in an oven at 30 ºC for 7 days and then for 20 hours at 36 ºC to remove any residual moisture.

3.1.4 Plant selection, seed treatment and germination

For the studies described in Chapters 5 and 6 carried out in 2005, the wheat cultivar, Yitpi (*Triticum aestivum* L. cv. Yitpi) was selected because it has been used in a previous study to examine P uptake efficiency (McBeath *et al.* 2005) and because it matures early (McBeath *et al.* 2005). Yitpi is a popular wheat variety in South Australia. Wheat seeds for this study were harvested in 2002 and obtained from the Wheat and Barley Unit, Waite Agriculture Research Institute.

Before each germination, uniform seeds were selected and surface sterilized in 1.6% sodium hypochlorite solution for disinfections. Seeds were soaked in 300 ml 1.6% sodium hypochlorite solution for 10 minutes in a beaker and then kept under a fume hood for debubbling so that all seed surface could come in contact with sodium hypochlorite solution. Then seeds were washed with running reverse osmosis (RO) water for 15-20 minutes. After sterilization, seeds were germinated on moist filter papers in petridishes for 3 days in the dark at 25 ºC.

3.1.5 Nutrient solutions

Nutrient solutions were used for the plants grown in the study described in Chapter 5 and 6. Nutrient solution was added following Zhu et al. (2001) to ensure that P was the main limiting to plant growth. Since the total N content in the soil was very low an additional 50 mg N kg^{-1} soil was added as $Ca(NO_3)_2$. The amounts of different nutrients added are shown in Table 5.3.

3.2 Methods

3.2.1 Soil analyses

3.2.1.1 Determination of available P (P_{resin}) and microbial biomass P (P_{mic})

Resin extractable available P (P_{resin}) and microbial P (P_{mic}) were determined by the method of Kouno et al. (1995) as modified by Bünemann et al. (2004). Three sets of moist soil sub samples (equivalent to 2.0 g of air-dry basis soil) were shaken horizontally at 120 rpm for 16 h. The first set of sub samples were shaken with 30 ml RO water and one anion exchange resin membrane (6x2 cm) (AEM; Product 55164 2S; BDH laboratory supplies, Poole, Dorset, BH15 1TD, England) in bicarbonate form to measure P_{resin}. The second set contained 30 ml RO water, one resin membrane and 1 ml of hexanol as the fumigant to measure P released from the microbial biomass by hexanol. Hexanol has a similar effect as chloroform to release microbial P during fumigation (McLaughlin *et al.* 1986b). Compared to hexanol, chloroform causes rapid deterioration of the resin and is more hazardous. The third set of sub samples contained 30 ml RO water, one resin membrane and an inorganic P spike equal to 10 mg P kg^{-1} soil in order to estimate the recovery of P during fumigation extraction. After shaking, the resin strips were washed with RO water to remove adhering soil particles. P adsorbed by the resin membrane was eluted with 30 ml of 0.1 M NaCl/HCl by shaking for 2 hours. P concentration in all extracts was measured by the ammonium molybdate-ascorbic acid method described by Murphy and Riley (1962) at 712 nm wavelength (Shimadzu UV 1601,Shimadzu Corporation, Tokyo, Japan). P_{mic} was calculated from the difference between fumigated and unfumigated samples with a correction for the recovery of P adsorption from the spike during the extraction period, which ranged from 78 to 117%. In the study described in Chapter 7, P_{mic} was calculated from the difference between fumigated and unfumigated samples without correction for P recovery, as recovery in that study was >95%.

3.2.1.2 Determination of microbial biomass C and N

The amount of C and N in the microbial biomass was determined using the method of Vance et al. (1987) with two sub samples per replicate, using moist soils (10.0 g of air dry basis) of which one was immediately extracted with 40 ml of 0.5 M K_2SO_4 for 1 h and another one was fumigated with ethanol-free chloroform for 24 h before extraction. Extracts were stored at 4 °C until measurement. Before measurement of total organic carbon (TOC), the stored samples were defrosted at room temperature over night and diluted to 20% with ultrapure water. TOC and TON in the extract was measured by using Formacs^{HT}-TOC Analyzer MDL Spec (Skalar, 4328 AA Breda, The Netherlands). Microbial C and N released during chloroform fumigation (C_{mic}, N_{mic}) were calculated from the difference between extractable C and N in fumigated and unfumigated samples. No conversion factors were used to calculate P_{mic}, C_{mic}, and N_{mic} values to microbial biomass.

3.2.1.3 Measurement of soil respiration

The respiration rates described in Chapter 4 were measured following the method of Alef (1995). Soil respiration rate was measured in four replicates for each treatment. The CO_2 evolved from soil equivalent to 30 g of air-dry soil (36.3 g moist soil) was trapped in 10 ml of 0.5 M NaOH and measured by back titration with 0.5 M HCl after addition of 4 ml of 1.5 M $BaCl_2$ and 2-3 drops of phenolphthalein solution. The NaOH traps were exchanged every week in the first 4 weeks and every 12 days in the remaining period. The respiration data was converted into CO_2-C (mg C kg^{-1} soil day^{-1}) released during incubation by calculating the amount of CO_2 trapped in NaOH and multiplying by the atomic weight of C (12) and dividing by the soil weight and time.

The respiration rates in the studies described in Chapter 5, 6 and 7 were measured by using an Infrared Gas Analyzer (Servomex 1450 series, Servomex Group Ltd, Crowborough, East Sussex, TN6 3DU, England). This method is less labor-intensive, more sensitive and accurate than the NaOH trap. The measurement was carried out in triplicate for each treatment. An equivalent to 20 g of air-dry soil (23 g moist soil) and 15 ml RO water, each in separate plastic containers were incubated in a closed 1l glass jar in a constant temperature (25 ºC) room. The plastic vial with water was placed into the glass jar to humidify the air and thus minimize the moisture loss from the soil. The jar lid was fitted with a rubber septum The CO_2 evolved from the soil was measured by injecting a needle connected with the gas analyzer into the glass jar (Figure 3.1). Headspace gas was withdrawn through the gas analyzer by a vacuum pump until a maximum value on the LCD indicator was reached. Then the valve between the gas analyzer and the jar was closed. The CO_2 measurement in six standard gas jars was also included during every measurement to calculate the correlation between CO2 concentration and voltage. After each measurement for time one (T1) the sample jar was opened in the fresh air for 5 minutes. After closing of the jars the initial CO_2 in the sample jars and standard gas jars was measured for time zero (T0). The CO_2 evolved from the soil was calculated from the difference between T1 and T0 of the previous sampling time. The calculation of respiration rates measured by gas analyzer is shown in section 3.3. The CO_2 evolved from the soil was measured every 3 days in the first 4 weeks and every 7 days for the remainder of the incubation period.

Figure 3.1 Measurement of respiration

3.2.2 Crop residue analysis

Most of the residue characteristics were determined using the chopped residues (5.0 mm) except for total P (P_{tot}) for which the dried crop residues were further ground to < 0.5 mm. Water extractable P (P_{H2O}), C (C_{H2O}) and N (N_{H2O}) were determined after shaking 0.1 g dry residue with 30 ml RO water in a horizontal shaker at 120 rpm for 16 h and vacuum filtration through Whatman no. 42. Resin extractable P (P_{resin}) was determined by anion exchange membrane method as described by Bünemann et al. (2004), in which 0.1 g of dry residue was shaken for 16 h in a horizontal shaker (120 rpm) with 30 ml RO water and an anion exchange resin membrane. Hexanol was added to half of the samples to determine the amount of P released from the residues by hexanol. If large amounts of P in the residues were hexanol-soluble this would lead to an overestimation of soil microbial P, since this is determined by shaking the soil with hexanol. Resin strips were processed as described above (Section 3.2.1.1) for resin and microbial P. Total P (P_{tot}) was determined by the method by Hanson (1950): 0.25 g of residue dry matter (0.5 mm in size) was digested with 7 ml nitric-perchloric acid (6: 1) at 160 ºC for 3 hours and at 180 ºC for 1 hour (AIM500 programmable digestion

block, AI Scientific Ltd, Clontarf, Qld, Australia). The volume of the digest was made up to 50

ml by adding RO water and was filtered through Whatman no. 42. Phosphorus in the aliquot

was measured colorimetrically using the ammonium molybdate-ascorbic acid method. Total C

and N in the crop residues were determined by a combustion method (Rayment and Higginson

1992) in which 1.0 g of residues was ignited in LECO CN2000 analyzer at 1200 ℃. Total C and

N were measured by an infrared detector cell and expressed as %C or %N on an oven dry

basis. Microbial C and N in residues were determined by the similar method to soil microbial C

and N analyses (see section 3.2.1.2), using 0.1 g of residue. Water-soluble C (C_{H2O}) and N

(N_{H2O}), and microbial biomass C (C_{chl}) and N (N_{chl}) in the extract was measured by using

Formacs[HT]-TOC Analyzer MDL Spec (Skalar, 4328 AA Breda, The Netherlands).

The biochemical composition (C-chemistry) of the crop residues was assessed with solid-state

[13]C nuclear magnetic resonance (NMR) spectroscopy. A Varian Unity 200 spectrometer

operating at a ^{13}C frequency of 50.3 MHz was used for all analyses. A pre-weighed residue

(260-360 mg) was packed in a 7 mm diameter cylindrical zirconia rotor with Kel-F end-caps and

spun at 5000+100 Hz in a Doty Scientific magic angle-spinning (MAS) probe. A cross

polarisation (CP) analysis was completed using a standard CP pulse sequence with a contact

time of 1 ms for all samples. Before completing a CP analysis, the value of T_1H for the sample

being analyzed was quantified using an inversion recovery pulse sequence. The recycle delay

set up into the CP pulse sequence was 10 times (10 s) the derived T1H value, which confirmed

the use of recycle delays ranging from 1 s to 6 s. For each CP analysis, transients were

collected between 1000 and 2000. Background signal intensities derived from an empty rotor

were always ≤ 1% of the total signal intensity obtained for the sample. Therefore no corrections

were applied for background signals. The free induction decay (FID) for each analysis was

acquired using a sweep width of 40 kHz. Over an acquisition time of 15 ms, 1216 data points

were collected. All spectra were zero filled to 8192 data points and processed with a 50 Hz Lorentzian line broadening and a 0.005 s Gaussian broadening. At 17.6ppm, chemical shifts were externally referenced to the methyl resonance of hexamethylbenzene. The CP spectra were baseline corrected between –100 and 300 ppm after Fourier transformation of the acquired FID. The spectra were divided into the chemical shift regions identified by Baldock and Smernik (2002), and the signal intensity linked with each region determined by integration. The name of the major types of carbon associated with each chemical shift region and the calculation used to determine the percentage of total signal intensity (300 ppm to –50 ppm) were as presented by Baldock and Smernik (2002).

3.2.3 Plant harvest and analysis

Plant shoots and roots were separated by cutting above the soil surface of the pots. Fresh shoot weight was measured immediately. Roots were cleaned by washing with tap water and manual removal of adhering soil particles. Plant shoots and roots were dried at 60 ºC in an oven for 7 days. Dried plant material was ground and used for nutrient analysis.

Phosphorus concentration in plant shoot and roots were determined according to the method by Hanson (1950) that is described in 3.2.2. Due to low amount of plant shoot and root dry matter, approximately 0.1 g of ground shoot dry matter and 0.05 g of ground root dry matter were used for measuring P concentration in shoot and root respectively.

3.3 Calculations

Respiration rate was expressed as mg C kg^{-1} soil day^{-1} equivalent to µg C g^{-1} soil day^{-1}. The respiration rate measured by the gas analyzer was calculated by the following equation;

$$Re\,spiration\,Rate = \frac{Re\,al_{CO_2} \times 1000 \times 1000 \times Atomic\,weight\,of\,C}{V_{CO_2} \times Time\,(d) \times Soil\,weight\,(g)}$$

Where $Re\,al_{CO_2} = (T1 - T0)\% \times V_{jar}$ and where T1 is the percent CO_2 evolved from the sample at time one (T1) and T0 is the percent CO_2 evolved at time zero (T0). V_{jar} is the volume of the jar (ml). V_{CO_2} is the volume of 1 mole CO_2 that was equal to 24465.30 ml at 25 ºC at 1 atmosphere pressure. Time is the day and soil weight is the unit g soil.

The apparent percentage of added C decomposed was calculated by the following equation and expressed as a percentage.

$$\%Decomposition = \frac{CumCO_2(residue) - CumCO_2(control)}{AddedC} \times \frac{100}{1000}$$

Where *CumCO$_2$ (residue)* is the cumulative CO_2 release (mg C kg^{-1} soil) from residue amended soil and $CumCO_2$ (control) is the cumulative CO_2 release from the control soil. Added C is expressed by the unit g C kg^{-1} soil. The decomposition rate described above is calculated disregarding the priming effect of CO_2 release. The term "priming effect" was defined by Bingeman et al. (1953) as "a greater loss of soil organic matter, in a soil receiving an organic amendments, than the loss of organic matter in an untreated soil". In addition, referring to the positive and negative priming effect, a new definition given by Kuzyakov et al. (2000) is "strong short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil".

3.4 Statistical Analysis

Data were analyzed statistically in a completely randomized design (CRD) using ANOVA, Genstat 6th edition, Release 6.1 (2002), Lawes Agricultural Trust (Rothamsted Experimental Station). Tukey's multiple range tests were used to compare means among the treatments.

Chapter 4

Decomposition of crop residues after addition to a red-brown Chromosol and temporal changes in available P and microbial P

4.1 Introduction

Phosphorus is a growth-limiting factor in many Australian soils due to the low availability of soil P sources and fertilizer P rapidly becoming unavailable through fixation. Bertrand *et al.* (2003) found that most of the total P in South Australian calcareous soils was present as P-Ca compounds formed from P fertilization in previous years. Crop residues could be a potential alternative P source for plants. It has been shown in several studies in other countries that the addition of crop residues can improve the physical and chemical properties of the soil, including water holding capacity, structure, pH, microbial biomass and activity, soil organic matter and availability of plant nutrients (Iyamuremye *et al.* 1996a; Kwabiah *et al.* 2003a; Nziguheba *et al.* 2005). Previous studies have found that P from residues can enter various soil P pools. Thus crop residues can play a significant role in the soil P cycle (Kwabiah *et al.* 2003b; McLaughlin and Alston 1986a; Nziguheba *et al.* 1998). Nziguheba *et al.* (1998) showed in a P-deficient African soil that addition of 5.5 Mg ha^{-1} of crop residues with a high P content (e.g. *Tithonia diversifolia,* 0.27% P) can supply similar amounts of P to plants as 15 kg ha^{-1} of TSP.

In Australian farming systems, an unknown proportion of crop roots as well as shoots remain in the soil and are mixed throughout the plowing layer during cultivation. It is well established that crop residues can be an important source of N. Evans *et al.* (2001) reported that about 15-30 kg N ha^{-1} of the mineralized N from the legume residues are taken up by wheat which provide

11%030% of total demand for N. While it can be assumed that N and P mineralization are linked, little is known about P release from residues in Australian soils.

The aim of the work described in this chapter was to: (a) measure the effect of addition of three different crop residues or inorganic P on available P, microbial P and the respiration rate as an indication of decomposition rate and microbial activity over time in soil that is wide-spread in southern Australia: a red-brown Chromosol.

4.2 Materials and methods

The soil chosen for the work described in this chapter was collected from the wheat-2 years pasture-fallow (W2PaF) rotation of the Permanent Rotation Trial at the Waite Agricultural Research Institute in South Australia (latitude 34°96′ S, longitude 138°63′ E and elevation 115m) that was established in 1925. The rotation was in the fallow period when the soil was collected. The soil is classified as red-brown chromosol (Isbell 1996) of the non-sodic Urrbrae series (Grace *et al.* 1995) and is a Rhodoxeralf in the USDA classification (Soil Survey Staff 2003). The climate of this site is Mediterranean with hot dry summers and cool moist winters. The soil was collected in April 2004 (end of summer with mean summer rainfall and temperature at this site 28 mm and 21 ºC). Because the soil was very dry, the area that was to be sampled was wetted one day before collection. Soil samples were collected from 0-10 cm soil depth from seven different locations within the plot and thoroughly mixed to give a composite sample. The soil was sieved to 2 mm to remove stones and plant debris. The soil was loamy and slightly acidic with high organic matter content. The properties of the soil are shown in Table 4.1.

Table 4.1 Soil properties

Soil properties			
Sand (%)	50	Total Organic Carbon (%)	1.6
Silt (%)	36	Total N (%)	0.13
Clay (%)	14	Total P (%)[a]	0.05
pH_w (1:5)	5.1	P_{resin} (mg kg^{-1})[b]	23
pH $_{CaCl2}$ (1: 5)	4.6	P_{mic} (mg kg^{-1})[b]	2.5
EC (dS m^{-1})	0.81	Exchangeable Ca (cmol$_c$ kg^{-1})	3.6
Gravimetric water content at –33kPa (%)	21	Exchangeable Mg (cmol$_c$ kg^{-1})	1.13

soil properties without notation were estimated by MIR (Janik *et al.* 2007)

[a] soil total P concentration determined after Hanson (1950)

[b] soil P_{resin} and P_{mic} concentration measured by (Bünemann *et al.* 2004)

About 6 kg of soil was pre-incubated at 65% water holding capacity (210 g kg^{-1} soil) at 25 ºC for 14 days in the dark in 5 perforated polyethylene bags before addition of crop residues to obtain a steady state in microbial activity (Oehl *et al.* 2001b).

After the pre-incubation period, the 5 treatments were imposed, consisting of 3 crop residues (Table 4.2), one rate of inorganic P fertilizer and the control without amendment. The residues and the inorganic P fertilizer were added at the rate equivalent to total P addition by 4 Mg ha^{-1} wheat straw i.e. 0.702 mg P kg^{-1} soil. Due to differences in total P among the residues (Table 4.2), the amount of crop residues added for wheat, canola and pea were 3.1, 1.4 and 1. 9 g DM kg^{-1} soil respectively.

The chopped shoot residues (passed through a 5.0 mm sieve) of mature wheat (*Triticum aestivum* L.), canola (*Brassica napus* L.) and pea (*Pisum sativum* L.) were mixed thoroughly in the pre-incubated soil. A further treatment was the soluble P fertilizer triple super phosphate (TSP 19.2% P, 13.6 % Ca, 1.8 % Fe, 1.2% Al). The control treatment was soil without

amendment. To ensure a homogenous distribution of the small amount of TSP fertilizer (4 mg kg^{-1} soil), the fertilizer was dissolved in 3 ml RO (reverse osmosis) water and then mixed thoroughly in the soil. There were 4 replicates per treatment and sampling date.

During the incubation period, crop residue and TSP-amended soils and non-amended control soil were kept in 250 ml plastic pots (about 125 g of soil per pot) at 25 ºC in the dark at 65% water holding capacity (at –33kPa). Five small (35 ml) plastic vials with 15 ml water were placed around the pots containing soil to reduce the water loss. Water was added to the soil twice a week to maintain 65% water holding capacity with an average weight loss was 6.0 g per week per pot. At the sampling on day 112, soil samples were moister than the other sampling dates. Any emerging seedlings in the pots were removed. Soils were sampled before the application of treatments and after 7, 21, 42, 63, 84, 112 and 140 days of incubation for analysis of resin extractable P and microbial P. Microbial C and N were measured on days 21 and 42 but data are not presented here due to large variation. At each sampling date, 1 pot of the each treatment was removed and divided into 4 sub samples for analysis. Soil respiration was determined separately with four replicates of each treatment (equal to 30 g of air dry soil) using NaOH traps (see section 3.2.1.3).

4.3 Results

4.3.1 Properties of crop residues

The three crop residues varied with their properties (Table 4.2). Total P in the crop residues decreased in the following order: canola > pea > wheat residue. The total P content of pea residues was twice as high as in wheat. The total C and N content among the residues were also significantly different. The highest total N content was found in the pea residue and lowest

in canola residues. The C: P ratio in the crop residues was in the order of canola < pea < wheat. The C_{H2O}: P_{H2O} was higher in canola residue than in pea and wheat residues.

Table 4.2 Properties of mature crop shoot residues used for the experiment in this chapter

Characteristics	Wheat	Canola	Pea
C_{tot} (%)	42.6b	43.2c	41.5a
N_{tot} (%)	0.5b	0.4a	0.7c
P_{tot} (%)	0.02a	0.05c	0.04b
P_{H2O} (%)	0.002a	0.012c	0.003b
C_{H2O} (%)	0.6a	0.7a	0.8a
N_{H2O} (%)	0.03a	0.04a	0.05b
C_{tot} : N_{tot}	87b	109c	57a
C_{tot}: P_{tot}	1856c	868a	1152b
C_{H2O}: P_{tot}	27b	15a	21ab

Within a row, means followed by the same letter are not significantly different by the least significant difference ($LSD_{0.05}$).

4.3.2 Soil respiration

Soil respiration rates were significantly higher in soils amended with crop residues than in the soils amended with TSP or the control in the first 58 days of incubation (Figure 4.1a). In the residue-amended soils, respiration peaked on day 10, when it was three to five times higher than in the control. Respiration then decreased rapidly in the residue-amended soils while it remained more or less unchanged in the control and the TSP amended soil. There were no significant differences among the treatments after day 84. Among the residue-amended soils, respiration was higher for wheat residue-amended soil than for pea and canola residue-amended soils thus showing similar trends as P_{mic} (see section 4.3.4).

The cumulative CO_2 release was highest in soil amended with wheat residues followed by soils amended with pea and canola residues and was lowest in TSP amended soil and the control

(Figure 4.1b). After 58 days of incubation, 40-50% of added C had been mineralized from residue amended soils (see section 3.3 for calculation of % mineralization of added C). This percentage only slightly increased from days 58 to 98 when 50-56% of C added with residues had been mineralized. By the end of incubation, the percentage of added C mineralization was higher (only 20 mg C g^{-1} added C) in canola residue-amended soil than wheat and pea residue-amended soil.
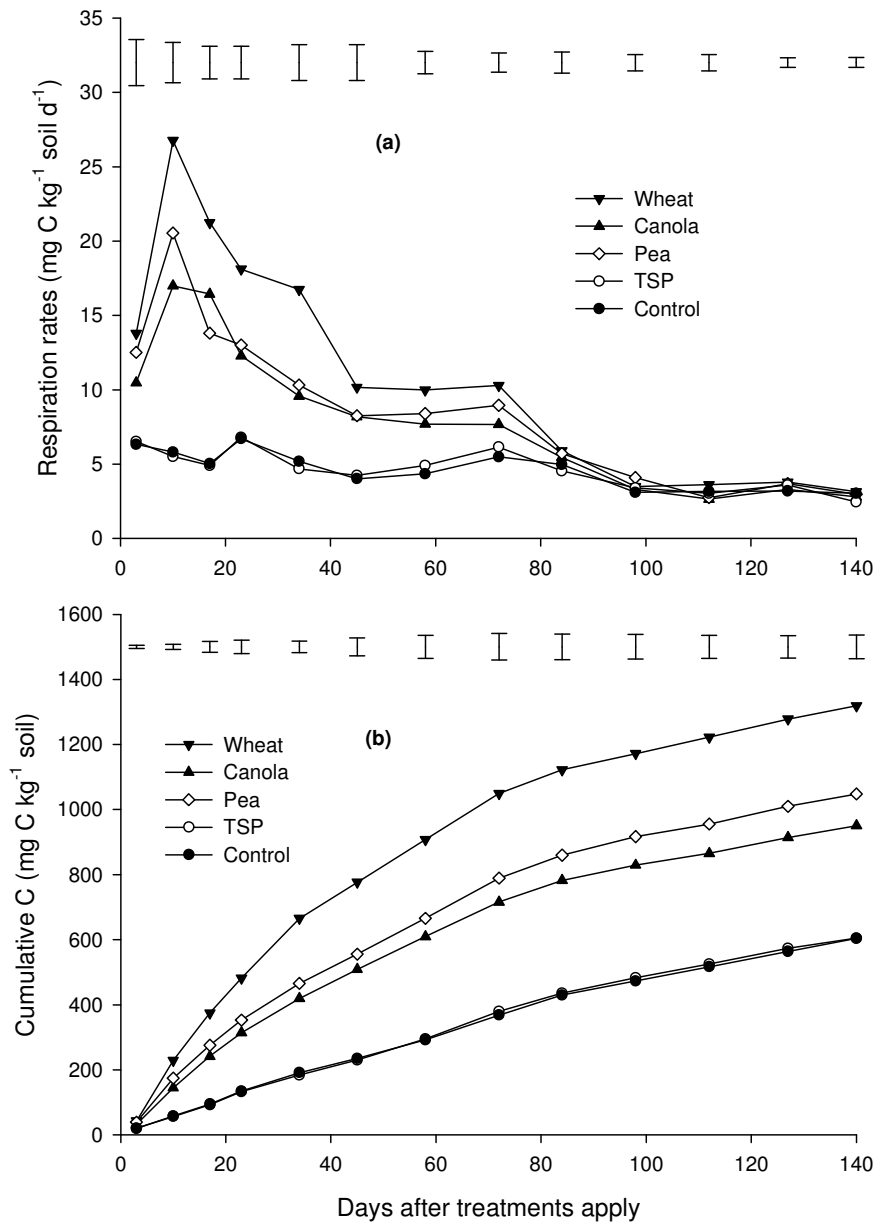
Figure 4.1 Respiration rates (a) and cumulative C release (b) from soil amended with wheat, pea and canola residues or TSP and non-amended soil over time. Means of 4 replicates, vertical bars indicate least significant differences (LSD $_{0.05}$).

4.3.3 Available P (P_{resin}) concentration in soil

Despite similar amounts of P added from organic and inorganic sources, P_{resin} concentration in TSP-amended soil was significantly higher than in soil amended with wheat, canola and pea residues but P_{resin} concentration in TSP-amended soil did not differ from the control in the first week (Figure 4.2a). Addition of wheat and pea residues resulted in a significant reduction in P_{resin} concentration during the first 21 days as compared to the control. On day 21, P_{resin} concentration in canola-amended soil was similar to TSP-amended soil and the control but significantly higher (by 1.6 mg P kg^{-1} soil) than the soils amended with wheat and pea residues. On day 42, P_{resin} concentration in the TSP-amended soil was significantly higher than in the other treatments. The P_{resin} concentration increased in all treatments from day 42 to day 63 and on day 63, it was significantly higher in TSP-amended soil than in wheat residue-amended soil and the control. From day 63 to day 84, P_{resin} concentration decreased in all treatments except in the control. On day 112, the very high P_{resin} concentration could be due to an analytical error. After 140 days of incubation, there was no significant difference in P_{resin} concentration among the treatments.

4.3.4 Microbial P (P_{mic}) concentration in soil

The treatment differences were more obvious for P_{mic} concentration than for P_{resin} concentration (Figure 4.2b). The P_{mic} concentration in soil amended with wheat residues was significantly higher than the control until day 84. Seven days after residue addition, P_{mic} concentration was significantly higher in soils amended with wheat, canola and pea residues compared to the control while it was lower in TSP-amended soil. The P_{mic} concentration increased from day 7 to day 21 in the soils amended with wheat, pea residues and TSP while it remained unchanged in canola residue-amended soil and the control. The highest P_{mic} concentration on day 21 was

measured in the soil amended with wheat residue, which was 5.2 mg P kg^{-1} soil higher than the control. The P_{mic} concentration decreased from day 21 to day 42 and then stabilized in the residue and TSP-amended soils. In the control, P_{mic} concentration increased until day 84 and then decreased. On day 42, P_{mic} concentration decreased in the following order: wheat > canola $\geq$ pea > TSP and control. In crop residue-amended soil, the increase of P_{mic} concentration compared to the control was greater (3 to 29 fold) than the reduction of P_{resin} concentration (Figure 4.2) during 42 days of incubation. On day 63, P_{mic} concentration was similar in the control and pea residue-amended soil whereas P_{mic} concentration was significantly lower in the soil amended with canola residue and TSP than in the control. On day 84, P_{mic} concentration in the residue-amended soils was significantly higher than TSP-amended soil and the control. With the exception of soil amended with canola residue, P_{mic} concentration decreased in all treatments from day 84 to day 112. In TSP and canola residue-amended soils, the decrease of P_{mic} concentration was equal to the increase of P_{resin} concentration on day 84 (Figure 4.2). On day 112, P_{mic} concentration in the soil amended with residues was higher than in the TSP-amended soil and the control. From day 112 to 140, P_{mic} concentration increased 0.2-1.3 mg P kg^{-1} soil except for the canola residue-amended soil while P_{resin} concentration decreased 5.2-6.4 mg P kg^{-1} soil. Compared to the control, P_{mic} concentration was higher (0.3-0.4 mg P kg^{-1} soil) only in wheat and pea residue-amended soil. Over the incubation period, the highest P_{resin}: P_{mic} ratio in the residue-amended soils was found in canola residue-amended soil (9-42) while the lowest P_{resin}: P_{mic} ratio was found in wheat residue-amended soil (4-19).
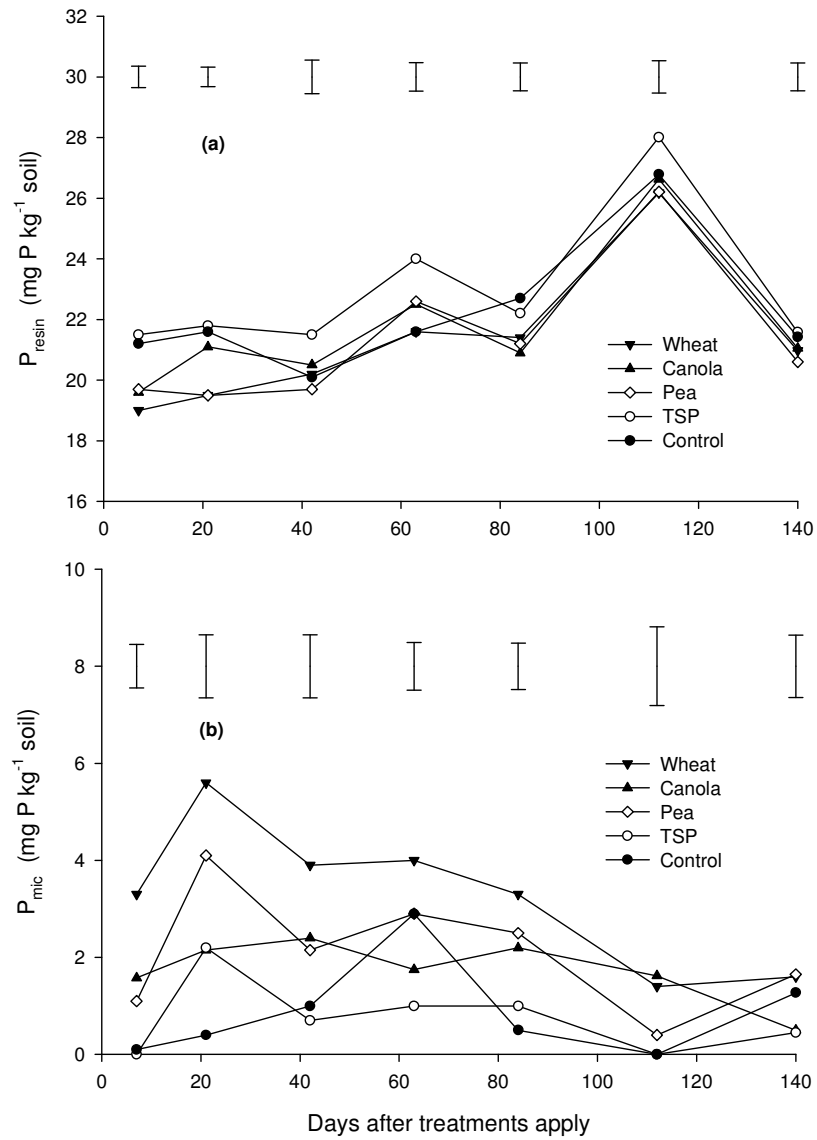
Figure 4.2 Available P (P_{resin}) (a) and microbial P (P_{mic}) (b) in soil amended with wheat, pea and canola residues or TSP and non-amended soil over time. Means of 4 replicates, vertical bars indicate least significant differences (LSD $_{0.05}$).

4.4 Discussion

Based on the soil respiration rates and consequently C mineralization, the decomposition process during the incubation showed 3 distinct phases (Figure 4.1).

4.4.1 Phase 1 (day 0 to 42)

Phase 1, consists of the first 42 days of incubation and is characterized by higher respiration rates than in the rest of the time. In the phase 1, the respiration rates were in the order of wheat > pea $\geq$ canola > TSP, control. Initially, the highest respiration rate was found 10 days after addition of crop residues. The respiration rate was 36% and 23% higher in soil amended with wheat than the pea and canola respectively whereas the amount of C added was 1.6, 0.6 and 0.8 g kg^{-1} soil with wheat, canola and pea residues respectively. Hence, during phase 1, respiration rates were significantly and positively correlated (r² = 0.92-0.99, in 42 days of incubation) to the amount of C added with crop residues. Respiration rates in phase 1 had moderate to strong correlation with water-soluble C (C_{H2O}) of the residues (r² =0.66-1.0, in 42 days) except on day 17. Therefore, the higher respiration P rates in residue-amended soils than TSP-amended soils and the control in phase 1 can be explained by the high concentration of water-soluble C and easily degradable compounds and sufficient N for initial break down of crop residues. This is in agreement with the previous studies which suggested that the initial decomposition of residue is correlated with water-soluble C and N content of residues (Abiven *et al.* 2005; Trinsoutrot *et al.* 2000). Consequently, cumulative C mineralization was highest during the first phase of decomposition for soil amended with crop residues. Similarly, Raiesi (2006) found higher cumulative C mineralization during the first 40 days of incubation at 25 °C in soils amended with alfalfa and wheat residues compared to the unamended control. In the present study 41%, 45% and 40% of added C was respired in the first 42 days from wheat,

canola and pea residue-amended soils respectively. The highest decomposition rate found in soil amended with canola residues may be due to the lower C: P ratio (Table 4.2) compared to the other crop residues. The percentage of residues decomposed in the present study are in agreement with Abiven *et al.* (2005) who reported that 42% of added C from wheat residues (added at the rate of 3.2 g DM kg^{-1} soil) was mineralized in 45 days. The cumulative C mineralization in the present study was positively and significantly correlated (r^2 = 0.77 - 1.0, in 42 days of incubation) with the water-soluble C of the crop residues confirming the results of other studies (Abiven *et al.* 2005; Bünemann 2003; Dalal 1979; Jensen *et al.* 2005).

Addition of crop residues had a great impact on available P (P_{resin}) and microbial P (P_{mic}) dynamics during the first phase of decomposition. The P_{resin} concentration was lower in residue-amended soil than in the control with the decrease being greater in wheat and pea residues than in canola residue-amended soil (Figure 4.2a). The P_{resin} concentration in TSP-amended soil was similar to that of the control but significantly higher than in wheat residue-amended soil which had the highest C: P ratio (1856: 1). Nziguheba *et al.* (1998) also found higher P_{resin} concentration for TSP than maize stover with C: P ratio 633: 1. This suggests that residues such as those used in the present study with C: P ratios of 868-1856: 1 led to a decrease in P availability in the early stages of decomposition. The P_{resin} concentration was positively correlated (r^2 = 0.60 - 0.80) with crop residues total P only in the first 21 days. Therefore, the release of P from crop residue may not always be correlated to the total P content of residues. Other residue properties such as C and N content may be involved in P release from crop residues. Nwoke *et al.* (2004) also did not find a relation between the total P of residues and P_{resin} concentration during 49 days.

On the other hand, P_{mic} concentration increased compared to the control in the following order: wheat > pea ≥ canola amended soil, which follows a similar trend as the respiration rates. The initial increase of P_{mic} concentration is likely caused by the addition of C which enhanced microbial growth, and consequently microbial P uptake. McLaughlin *et al.* (1988b) found that 25% of the ^{33}P added with medic residues was detected in the microbial biomass in 7 days. In phase 1, P_{mic} showed a significantly positive correlation (r² = 0.78 - 0.90) with C addition and C: P ratio of the crop residues. This suggests that crop residues with high C: P ratio increased microbial P uptake (Figure 4.2b). Dalal (1977) also discussed that addition of organic material with high C: P ratio leads to increased microbial activity and immobilization of P. In phase 1 in the present study both the total P and C: P ratio of residues effect on soil P_{mic} concentration and P_{mic} was strongly positively correlated with C: P ratio and negatively correlated with total P of residues (data not shown). However, the result in this study shows a disagreement with the findings of Kwabiah *et al.* (2003a). They reported that the C: P ratio of residue was less important than the total P of the residues for microbial P uptake.

The P_{resin} concentration in the residue-amended soil decreased in the first 7 days of incubation as a result of microbial P uptake, which was enhanced by the addition of C with residues. Hence, P_{resin} concentration negatively correlated with P_{mic} concentration in phase 1 (r^2 = 0.84, P = 0.05). In this phase, the increase of P_{mic} concentration was greater than the decrease of P_{resin} concentration in residue-amend soils. This additional P_{mic} indicated that microbes utilized P from residues, or previously unavailable P released from soil due to carboxylates or phosphatases produced during decomposition (He and Zhu 1998; Iyamuremye *et al.* 1996a; Marschner 2007; McGill and Cole 1981). This is in agreement with previous findings which reported an additional 2-3 mg P kg^{-1} soil increase in P_{mic} concentration than the P addition rate during the first 7 days following C addition (Chauhan *et al.* 1979; Nziguheba *et al.* 1998).

4.4.2 Phase 2 (day 42 to 84)

Phase 2, from day 42 to day 84 is characterized by lower respiration rates than phase 1. In phase 2, respiration rate was higher in residue-amended soils with wheat > pea, canola > TSP, control (Figure 4.1a) up to day 72 but at the end of phase 2 there was no significant difference between the treatments. The decrease of respiration rates compared to phase 1 was greatest in wheat residue-amended soil (42%) than canola and pea residue-amended soils (33% and 31% respectively). Hence, wheat residue had more C to be mineralized. The decline of respiration rates during phase 2 is probably due to the decrease in C availability. The lower available C in phase 2 than phase 1 can be explained by (i) less total C remaining and (ii) easily degradable compounds having been decomposed in phase 1, leaving only more recalcitrant compounds. The lower respiration rates may also be explained by the lower microbial biomass as suggested by the lower microbial biomass P (P_{mic}). Similar to phase 1, the respiration rate was higher in soil amended with wheat residues than with canola and pea residues. Thus, respiration rates were significantly and positively correlated ($r^2 = 0.88$-0.99, in 42 to 84 days of incubation) to water-soluble C of crop residues and the amount of C added with crop residues. Cumulative C mineralization increased in the order of wheat > pea ≥ canola > TSP, control (Figure 4.1b). As in the phase 1, cumulative C mineralization was significantly positively correlated with the amount of C added with crop residues ($r^2 = 1.0$, in 42 to 84 days of incubation) and moderately positively correlated with water-soluble C of crop residues ($r^2 = 0.70$, in 42 to 84 days of incubation). Dalal (1979) also reported that the cumulative C mineralization was correlated with water-soluble C of clover shoot residue during 72 days. On the other hand, cumulative C mineralization was not related to amount of C added in the study by Raiesi (2006). Raiesi (2006) found significantly higher C mineralization in alfalfa than wheat residue-amended soil during 60 days of incubation although the amount of C added as well as

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lignin and cellulose was greater in wheat than in alfalfa. During phase 2, the decomposition rates of crop residues were slower than the phase 1. Only an additional 12%, 13% and 14% of added C was mineralized from wheat, canola and pea residue-amended soils respectively by 84 days of incubation. Abiven *et al.* (2005) also reported an additional 8% of added C mineralization in wheat residues-amended soil from day 45 to 85.

During phase 2, P_{resin} concentration was higher than in phase 1 and reached similar levels in the residue-amended soils as in the control (Figure 4.2a). This suggests that the P_{resin} concentration increase by 84 days of incubation may be due to mineralization of residue P or decrease of microbial P uptake since microbial activity decreased. On the other hand, in phase 2, P_{mic} concentration in residue-amended soils was lower than phase 1 probably due to lower C availability compared to phase 1 but higher than in the TSP-amended soil. Therefore, the differences in P_{mic} concentration among residue-amended soils were smaller than phase 1. However, the increase in P_{resin} concentration was higher than the decrease of P_{mic} concentration. This indicates that the increase of P_{resin} concentration in phase 2 is probably due to mineralization of P from crop residues or solubilization of soil P. In phase 2, compared to the control, the increase in P_{resin} concentration by 2.4 mg P kg^{-1} soil in TSP-amended soil was greater than the amount of P added (0.7 mg P kg^{-1} soil) but P_{mic} was similar as in the control. This could be due to increased mineralization of P_o or solubilization of P. Addition of P_i can replace P_o bound to Fe and Al oxides (Sanyal and Datta 1991). This released P_o could be mineralized thus increasing available P (Dalal 1977). On day 63, P_{mic} was very high in the control compared to the other dates, suggesting that this high value may be due to an analytical error. In phase 2, P_{resin} and P_{mic} were negatively correlated, but the relationship was weaker ($r^2 = 0.68\text{-}0.74$) than in phase 1.

The increase of P_{resin} concentration by 1.3 to 2.4 mg P kg^{-1} soil in the residue-amended soils from day 0 to day 84 could potentially increase the yield of wheat. In glasshouse experiments with wide range of Australian soils, McBeath *et al.* (2005; 2007) showed that the wheat yield responded to P addition at P_{resin} concentrations of 1.0 to 9.0 mg P kg^{-1} soil. The resin P values required for optimal crop yield were about 2.5 and 5.0 mg P kg^{-1} soil in non-calcareous alkaline soil and acidic soil, respectively.

4.4.3 Phase 3 (day 84 to 140)

Phase 3, between days 84 to 140, is characterized by lower respiration rates in residue-amended soils compared to phases 1 and 2. During phase 3, the respiration rate in residue-amended soils did not differ from that in the control. In phase 3, there was no significant correlation between respiration rates and amount of C added. Thus, the correlation coefficient between C addition and respiration rates decreased as decomposition proceeded. The low respiration rates at phase 3 can be explained by the smaller amounts of total C, and microbial biomass as indicated by the lower microbial biomass P, being left than in phase 2, and presumably that the remaining C was more recalcitrant. In phase 3, only an additional 2% of added C was mineralized from soil amended with wheat and pea residues compared to phase 2 probably because higher C: P ratio of wheat and pea residues than canola residue and higher recalcitrant C compound prolonged residue decomposition. Similar findings were reported in the study by Abiven *et al.* (2005). They reported that an additional 5% of added C was mineralized from day 85 to 150 for wheat residues. However, the percentage of added C mineralized in canola residue-amended soil did not increase from day 84 to 140 (Figure 4.1b) probably due to low C and N availability and decrease of microbial growth as suggested by low P_{mic} (Figure 4.2b). This shows that a low C: P ratio but high C: N ratio in canola residue may impede the decomposition in the longer term and limiting microbial growth and activity.

Trinsoutrot et $al.$ (2000b) also reported that residue decomposition can be affected by the availability of N because the biomass C: N ratio can be changed by the residues nutrients.

In phase 3, P_{resin} was similar in the control and in the residue amended soils and in general, P_{resin} was higher than in phase 2 whereas P_{mic} was lower. This shows that over time P immobilization decreased in residue-amended soils. Hence, this suggests that C limitation resulted in a reduction in the size of the microbial biomass and that P released from the microbial biomass can increase available P or can be fixed in the soil. Bünemann et al (2004) also reported an increase of P_{resin} in low quality (0.06% P, C: P 633: 1) maize stover-amended soil after 120 days of incubation while P_{mic} decreased very little. In phase 3, P_{resin} in TSP-amended soil remained higher than in residue-amended soil compared to the control but the difference was smaller than phase 2, whereas P_{mic} was lower than in residue-amended soils. This suggests that addition of TSP in a non-P limited soil can provide higher available P and lower P immobilization than addition of low quality crop residues (< 0.05% P) which may result in longer P immobilization. However, Nziguheba et $al.$ (1998) found in a Kenyan P fixing soil that P_{resin} in maize stover-amended soil was lower than TSP-amended soil but higher than the control from day 84 to 112 while P_{mic} was higher than TSP-amended soil and the control. In phase 3, the correlation between P_{resin} and P_{mic} was less significant ($r^2 = 0.39\text{-}0.68$) than in phase 1 and 2. On day 112, the very high P_{resin} concentration in all treatments could be due to analytical error. Compared to day 84, the decrease of P_{resin} and P_{mic} concentration on day 140 in residue-amended soils suggests that any P released from the microbial biomass was fixed in to the soil.

In summary, crop residues with low P content and wider C: P ratio (Table 4.2) resulted in initial P immobilization. Although the same amount of P was added as crop residues or as TSP, there

was a perceptible difference between treatments in P_{resin} and P_{mic}. Addition of C as crop residues enhanced microbial activity and increased microbial P uptake. In this study, however, different amounts of C were added with the three different crop residues. Respiration and microbial P uptake was higher in treatments where more C was added. It is therefore not clear if the differences in respiration, P_{mic} and P_{resin} between the residue treatments are caused by the different amount of C addition or by other residue biochemical quality factors such as lignin, polyphenols, O-alkyle and amide. Residue biochemical quality may play an important role in the decomposition process and P dynamics and further investigations are therefore worthwhile. This study using only 3 mature crop shoot residues demonstrated P immobilization for at least 6 weeks period which may be crucial to plant available P supply. However, no plants were grown. Plants generally compete with microbes for available P (He *et al.* 1997; Kouno *et al.* 2002) and therefore P concentration in different P pools can be changed. Crop residues at different growth stages have different nutrient content and differ in biochemical quality (Bertrand *et al.* 2006; Wang *et al.* 2004) thus would have different decomposition rates and a variable effect on P availability in a P-limited soil. Therefore it is important to investigate the P dynamics with more crop residues at different growth stages and with different plant parts. Although the results of this study showed that the residues from mature plants with C: P ratio 868 (canola) to 1856 (wheat) resulted in P immobilization, other residue types with lower C: P ratio or different biochemical quality, for example young shoot, mature root may be a potential P source. Previous studies showed (Dalal 1979; Kwabiah *et al.* 2003b; Nziguheba *et al.* 1998; White and Ayoub 1983) that residues with high P content (0.2%) and low C: P ratio (252: 1) can increase P availability and plant P uptake.

Chapter 5

Effect of residue quality on decomposition rates, soil P dynamics and plant P uptake. I. Residue addition based on the same amount of P

5.1 Introduction

The previous study in Chapter 4 showed that mature crop residues with high C: P ratio (868-1856) caused P immobilization for 42 days. Respiration rates and microbial P uptake were related to C addition. The rate of decomposition of crop residues is an important factor to determine the value of crop residues as a P source for plants (Dalal 1979; Fuller *et al.* 1956). It is therefore important to determine which residue properties have the greatest effect on decomposition and P release. Among these, the quality or biochemical composition of crop residues are particularly important (Abiven *et al.* 2005; Kwabiah *et al.* 2003a; Nwoke *et al.* 2004; Wang *et al.* 2004). A minimum set of parameters influencing the decomposition and nutrient release that has to be considered to characterize crop residue quality is recommended by Palm and Rowland (1997). This minimum data set includes: total P, total N, total C, lignin, soluble C, soluble polyphenolics, α-cellulose, and ash. Based on these quality parameters of crop residues, numerous studies have been conducted on residue decomposition and P mineralization (Abiven *et al.* 2005; Kwabiah *et al.* 2003a; Kwabiah *et al.* 2003b; Nwoke *et al.* 2004; Nziguheba *et al.* 2005). Although apparently well studied, the relationships between crop residue quality and dynamics of available P and plant P uptake have not been studied in Australian soils which are often characterized by low P availability over a wide range of different plant residues available to Australian farmers. It is also not well known if the relative importance of the parameters discussed by Palm and Rowland (1997) changes during the decomposition process. Soil P availability and P uptake by the microbial biomass changes during decomposition and therefore plant P uptake may change at different stages of residue

decomposition. For a better picture of P availability it is important to measure it several times during decomposition. Determination of P availability by chemical methods may not be a very good indicator of plant P uptake. Therefore, chemical determination of P availability should be combined with plant P uptake studies. Plant P demand is highest in the early stages of growth. So it is useful to use young plants in such studies.

The objectives of this study were to investigate the relationship of residue characteristics (a different range of nutrient contents, carbon quality, maturity) with residue decomposition, soil P availability and plant and microbial P uptake. A wide range of residues was used to compare the decomposition rate and P release from different plant parts (young and mature shoots, and mature root) when added to soil at equivalent amounts of P. Three consecutive crop periods were used to elucidate the effect of residue P on plant growth and plant P uptake during the decomposition process.

5.2 Materials and methods

For this study, a P-deficient soil was collected from Monarto, South Australia (latitude 35°05´ S, longitude 139°06´ E and elevation 166 m), 60 km south-east from Adelaide and located on the eastern margins of the southern Mount Lofty Ranges (Chittleborough 1978). When the soil was collected, the land was fallow and the previous crop was wheat. The soil is classified as red Chromosol (Isbell 1996) or Rhodoxeralf in the USDA soil classification system (Soil Survey Staff 2003). The soil is a colluvium and alluvium red duplex soil (Chittleborough 1978). The climate at this site is Mediterranean with hot dry summers and moist winters. The soil was collected in April 2005 (end of summer with mean rainfall and temperature at this site was 29 mm and 23 ℃). Soil samples were collected from 0-20 cm soil depth from two different locations within the paddock and thoroughly mixed to give a composite sample. Plant debris

and stones were removed from the soil. After air-drying, the soil was sieved to pass through a 2 mm sieve. The soil is a sandy loam with moderate organic matter content. The properties of the soil are shown in Table 5.1.

A soil with low P availability and low P fixing capacity was chosen to obtain the greatest effect of crop residues on P availability, microbial P and crop growth. Furthermore, agricultural soils with low P availability are not uncommon. Previous studies (Mason and McNeill 2008; McBeath *et al.* 2007) reported that a number soils with low P availability (ranging from 1.0 to 46.8 mg kg^{-1} soil Resin P) across the cropping regions in southern Australia.

Before the start of the experiment, the soil was pre-incubated at 80% water holding capacity for 9 days at 18°C/24°C. This was done to allow for the flush of microbial activity when air-dried soil is moistened prior to treatments being imposed (Brookes *et al.* 1982; Oehl *et al.* 2001b). Oehl *et al.* (2001a) showed that microbial activity is stable after 10 days of moist incubation.

Table 5.1 Soil properties

Soil properties			
Sand (%)	69	Total Organic Carbon (%)	0.9
Silt (%)	11	Total N (%)	0.04
Clay (%)	20	Total P (%)[a]	0.009
pH$_w$ (1:5)	7.1	P$_{resin}$ (mg kg^{-1})[b]	1.3
pH $_{CaCl2}$ (1: 5)	6.7	P$_{mic}$ (mg kg^{-1})[b]	4.6
EC (dS m^{-1})	0.1	Exchangeable Ca (cmol$_c$ kg^{-1})	4.0
Water holding capacity at –10kPa (%)	18	Exchangeable Mg (cmol$_c$ kg^{-1})	1.7

soil properties without notation were estimated by MIR (Janik *et al.* 2007)

[a] soil total P concentration determined after Hanson (1950)

[b] soil P$_{resin}$ and P$_{mic}$ concentration from (Bünemann *et al.* 2004)

Ten crop residues were used: wheat (*Triticum aestivum* L.), pea (*Pisum sativum* L.), canola (*Brassica napus* L.), lupin (*Lupinus albus* L.), lucerne (*Medicago sativa* L.) and lentil (*Lens culinaris* L.), resulting in 5 young shoot, 4 mature shoot and 1 mature root residues (Table 5.2). The residues were selected to encompass a wide range of quality factors such as C: P, C: N ratios and biochemical composition (Table 5.4). Residue properties were determined as described in Chapter 3 (see section 3.2.2). Crop residues or an inorganic P source, TSP (triple super phosphate) were added at a rate of 10 mg P kg^{-1} soil and mixed thoroughly with the pre-incubated soil. The amount of crop residues and TSP as well as C added per kg soil is shown in Table 5.2. Mature crop residues were applied to soil at rates at least 10-fold higher than the current agricultural practice to ensure measurable differences between treatments and the control. Triple super phosphate, TSP was dissolved before addition. An unamended control treatment was also included and mixed in the same manner as the other treatments.

Table 5.2 Crop residues, TSP and C added to an addition rate of 10 mg P kg^{-1} soil

Residues and TSP	Amount of added residue and TSP (g DM kg^{-1} soil)	C added (g kg^{-1} soil)
Canola YS	6.14	2.52
Pea YS	3.09	1.32
Lupin YS	4.96	2.13
Lucerne YS	4.23	1.79
Lentil YS	3.83	1.65
Canola MS	20.01	8.74
Pea MS	11.82	5.04
Lupin MS	17.44	7.65
Wheat MS	14.13	6.15
Canola MR	4.47	1.92
TSP	0.05	0.0

YS, Young shoot; MS, Mature shoot; MR, Mature root

Nutrients other than P were added to the residue and TSP amended soils and the unamended control soil, following Zhu et al. (2001) but with an additional 43 mg N kg^{-1} soil due to low N content of the soil. Macronutrient solutions were prepared separately, whereas there was a single solution for all micronutrients (Table 5.3).

Table 5.3 Nutrients added in the form of nutrient solution

Macro nutrient compounds	Amount of compound (g kg^{-1} soil)	Micro nutrient compounds	Amount of compound (mg kg^{-1} soil)
NH_4NO_3	0.429	FeEDTA	0.4
$Ca(NO_3)_2.\ 4H_2O$	0.422	$CuSO_4.\ 5H_2O$	2.0
K_2SO_4	0.174	$MnSO_4.\ 4H_2O$	0.6
$MgSO_4.\ 7H_2O$	0.379	$CoCl_2.\ 6H_2O$	0.34
		H_3BO_3	0.5
		$Na_2MoO_4.\ 2H_2O$	0.7
		$ZnSO_4.\ 7H_2O$	2.2

Amount of macro nutrient (mg kg^{-1} soil)					Amount of micro nutrient (mg kg^{-1} soil)						
N	K	Ca	Mg	S	Fe	Cu	Zn	Mn	Mo	B	Co
150.0	78.0	71.5	37.0	81.9	0.04	0.05	0.50	0.15	0.30	0.09	0.08

A total of 1.15 kg of moist soil of each treatment was weighed into a plastic pot of 115 mm height and 120 mm diameter. There were 3 crop periods of wheat (*Triticum aestivum* cv. Yitpi) (crop periods 1, 2, and 3) each lasting for 28 days. Hence, crop period 1 was grown from day 0 to day 28, crop period 2 from day 28 to day 56 and crop period 3 from day 56 to day 84. The pots used for the later crop periods were left unplanted until the start of the given period. This experimental design was chosen to have plants at a similar growth stage and thus nutrient demand at the different stages of residue decomposition. The number of pre-germinated seeds planted at the start differed between the crop periods, being 5 for crop period 1 and 3, and 4 for crop period 2. The pre-germinated seeds were transplanted in each pot at 1.0-1.5 cm depth. The seedlings were thinned to 3 per pot after 4 days of transplanting. The pots were placed in

the glass house at 10°C/18°C, 8 h night/16 h day and ranging from 25 to 5000 lux light intensity. The pots were watered with RO water daily to maintain 85% water holding capacity. During crop period 2, there were more days with lower light intensity and temperature than in the other crop periods. All pots were arranged in a completely randomized design with 4 replicates and re-randomized at every 2 weeks.

Soil respiration rates of the different treatments were determined separately. After mixing the soil as described above, 23 g of moist soil were removed for separate analyses of soil respiration. The soil samples were incubated in sealed glass jars and CO_2 release was measured using an infrared gas analyser (see section 3.2.1.3).

During the incubation, soil samples were taken from each pot with the aid of a stainless steel corer (diameter ~15 mm) on days 7, 17, 28, 35, 45, 56, 63, 73, and 84 days after transplanting (Figure 5.1) for P_{resin} and P_{mic} (See section 3.2.1.1) while for C_{mic} (See section 3.2.1.2) samples were taken only on days 7, 35, and 63. Soil samples on days 28, 56 and 84 were taken immediately after the plants were harvested (Figure 5.1). The wheat plants were harvested by cutting the stem at the soil surface. The roots were carefully removed and washed to remove the adhering soil. The plant samples (shoot and roots) were dried at 60°C, weighed and ground for P determination (see section 3.2.2).
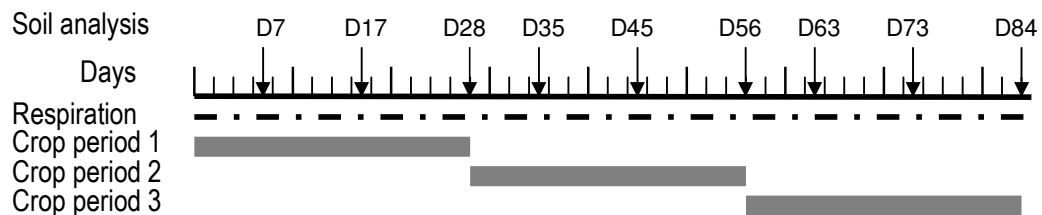
Figure 5.1 Layout of the experiment

5.3 Results

5.3.1 Residue properties

The crop residues were selected to achieve a wide range of chemical properties (Table 5.4). The total P content of the residues ranged from 0.5 g kg^{-1} in canola MS to 3.2 g kg^{-1} in pea YS. The total P concentration in the crop residues was affected by growth stage (young shoot > mature shoot) and in canola by plant part (mature shoot < mature root). Among the young crop residues, pea YS had the highest total P concentration while canola YS had the lowest (1.6 g kg^{-1}). Among mature residues, pea MS had a high total P concentration (0.8 g kg^{-1}) and it was low in canola MS (0.5 g kg^{-1}). The canola MR had a higher total P concentration (2.3 g kg^{-1}) than canola YS and canola MS. Water-soluble P (P_{H2O}) ranged from 0.1 g kg^{-1} (pea MS) to 1.3 g kg^{-1} (canola YS), representing 13% to 81% of the total P respectively (data not shown). Generally P_{H2O} in the crop residues decreased in the following order: young shoots $\geq$ mature root > mature shoot and was positively correlated with total P (r^2 =0.66, $P \leq 0.05$).

The total C concentration in the crop residues varied little, ranging from 411g kg^{-1} DM in canola YS to 439 g kg^{-1} DM in lupin MS. Water soluble C (C_{H2O}) was greater in young shoot residues compared to the mature shoots and root residues. The highest C_{H2O} was measured in the lupin YS (140 g kg^{-1} DM) and the lowest in canola MR residues (9.7 g kg^{-1} DM). The C: P ratio of the crop residues ranged from 133 (pea YS) to 858 (canola MS). The C: P ratio of the mature shoot residues was 2-6 fold higher than in the young shoot and canola MR residues. Canola MR residue had the lowest C_{H2O}: P_{H2O} ratio (9) while pea MS had the highest (419). Total N concentration of the residues ranged from 5.4 g kg^{-1} DM for wheat MS to 35.8 g kg^{-1} DM for pea YS and the C: N ratios ranged from 12 (pea YS) to 84 (canola MS). Total N content of the residues was significantly positively correlated with total P of the residues (r^2 =0.73, $P \leq 0.01$).

Water-soluble N (N_{H2O}) ranged from 1.5 g kg^{-1} DM to 12.6 g kg^{-1} DM in canola MS and lupin YS residue, respectively and was positively correlated with total N (r^2 = 0.79, $P \leq$ 0.001, data not shown).

Among the biochemical characteristics of residues determined with NMR spectroscopy, lignin content was highest in canola MR residues (45.5 g kg^{-1}DM) while it was lowest in lupin YS (27.7 g kg^{-1} DM). The O-alkyl content (mainly cellulose) ranged from 19.3 g kg^{-1} in canola MS to 59.3 g kg^{-1} in lucerne YS residue. Aryl-C, representing mainly phenolic compounds ranged from 11.1 g kg^{-1} DM in lucerne YS residue to 19.8 g kg^{-1} DM in canola MR residue. Amide content in residues showed a similar trend as total N and ranged from 17 to 46.5 g kg^{-1} DM in wheat MS residue and Pea YS residue, respectively.

O-alkyl and amide content were moderately positively correlated with total P content of the residues (r^2 =0.52, $P \leq$ 0.01 and r^2 =0.57, $P \leq$ 0.05, respectively), but were strongly positively correlated with total N content of the residues (r^2 =0.85, $P \leq$ 0.001 and r^2 =0.93, $P \leq$ 0.001, respectively). However, phenolics had only a weak negative correlation with total N content of the residues (r^2 = 0.42, $P \leq$ 0.05). There was no significant correlation between C compounds determined by NMR spectroscopy and total C of the residues.

Table 5.4 Chemical and biochemical properties of the crop residues determined by chemical analysis and NMR spectroscopy

Residues	Total P	Total C	Total N	P_{H2O}	C_{H2O}	N_{H2O}	Lignin[1]	O-alkyl	Phenolic	Amide	C: P	C: N
						g kg^{-1} DM						
Shoot												
Canola YS	1.6 c	411.0 a	14.6 c	1.3 g	63.4 d	8.0 b	31.5	23.0	14.9	27.2	253 e	28 c
Pea YS	3.2 g	427.7 cd	35.8 f	1.1 e	69.7 d	12.1 cd	33.1	59.0	12.4	46.5	133 a	12 a
Lupin YS	2.0 d	429.3 cd	21.9 d	1.2 f	140.0 g	12.6 d	27.7	41.5	11.4	40.1	213 d	19 b
Lucerne YS	2.4 e	423.4 b	30.4 e	0.7 c	79.6 e	11.0 cd	28.0	59.3	11.1	45.2	179 bc	14 a
Lentil YS	2.6 f	431.2 d	30.3 e	1.0 d	98.4 f	10.2 bc	35.2	51.0	12.8	41.0	165 b	14 a
Canola MS	0.5 a	436.8 e	5.6 a	0.1 ab	16.4 a	1.5 a	40.4	19.3	15.5	19.6	858 i	84 g
Pea MS	0.8 b	426.5 bc	12.8 b	0.1 a	27.0 bc	2.4 a	32.8	40.4	12.7	31.2	504 f	33 d
Lupin MS	0.6 a	438.8 e	14.4 c	0.1 ab	32.4 c	3.6 a	40.6	23.0	18.7	31.3	781 h	30 cd
Wheat MS	0.7 ab	435.3 e	5.4 a	0.2 b	24.5 b	1.6 a	38.9	21.9	16.3	17.0	615 g	80 f
Root												
Canola MR	2.3 e	429.4 cd	11.4 b	1.1 de	9.7 a	2.2 a	45.5	21.1	19.8	24.7	192 cd	38 e

YS, MS and MR indicated Young Shoot, Mature Shoot and Mature Root respectively. Within columns, means followed by the same letter are not significantly different ($P < 0.001$) by Tukey's multiple range test. [1] NMR was carried out on one composite sample (n=1).

5.3.2 Soil respiration

The respiration rate in residue-amended soil was higher than in the TSP-amended soil and the control during the entire incubation period. The ranking of crop residues for the amount of C mineralized during the incubation changed over time. In general, the respiration rate was in the following order: mature shoot residues $\geq$ young shoot residues > mature root > TSP $\geq$ control (Figure 5.2). Because the same amount of P was added, the C addition rates differed significantly which affected on the respiration rates. Among the young shoot residue-amended soils; the release of CO_2 on day 1 was highest in lupin YS residue-amended soil (251 mg C kg^{-1} soil day^{-1}) and lowest in pea YS and canola YS-amended soil (Figure 5.2a). Respiration rates in all young shoot-amended soils decreased very rapidly, decreasing by 65%-86% within 10 days. The decrease in respiration rates was least pronounced in canola YS residue-amended soil. On day 1 in mature shoot-amended soil, the highest respiration rate was in lupin MS and the lowest in wheat MS-amended soil with 260 and 147 mg C kg^{-1} soil day^{-1}, respectively. The decrease in respiration rate in mature shoot-amended soil was slower than the young shoot-amended soils, decreasing by 46%-64% in the first 10 days. The decrease of respiration rate in canola MR-amended soil was slower than shoot-amended soils, decreasing by only 38% within 10 days. From day 12 to 35, the respiration rate in canola YS-amended soils was higher than other young shoot-amended soils. From day 12 onward, the respiration rate was significantly higher in canola MS-amended soil than all other treatments (Figure 5.2b). From day 41 to 62 there were fewer differences between young shoot residues than there were between mature shoot-amended soils. From day 62 onward, there was no significant difference between young shoot and canola MR-amended soil and the differences in respiration rates between mature shoot-amended soils decreased. The respiration rates in canola residue-amended soils decreased in the order of canola MS > canola YS > canola MR during the entire incubation period.

The cumulative CO_2 release from residue and TSP-amended soils and the unamended control was clearly divided into 3 groups (Figure 5.3): mature shoot residue > young shoot and mature root > TSP and control. Among the young shoot-amended soils, cumulative CO_2 release was highest in lupin YS amended soil (248 mg C kg^{-1} soil) up to day 7. From day 27 onward, cumulative CO_2 release was in the order: canola YS, lupin YS > lentil YS, lucerne YS > pea YS-amended soil (Figure 5.3a). Cumulative CO_2 release was significantly higher in lupin MS-amended soil (2450 mg C kg^{-1} soil) than other mature shoot-amended soils up to 41 days. At day 84, cumulative CO_2 release was highest in canola MS-amended soil (2974 mg C kg^{-1} soil), followed by lupin MS and wheat MS (Figure 5.3b) and then pea MS residue-amended soil (2204 mg C kg^{-1} soil). The cumulative CO_2 release in canola MR-amended soil was significantly lower than young and mature shoot-amended soil until day 12 but from day 24 onwards, it was similar to that of pea YS-amended soil (Figure 5.3).

Carbon added with young shoot residues decomposed faster than the C added with mature shoot and root residues (Table 5.5). In young shoot-amended soils, 39-47% of added C was decomposed by 27 days. Afterwards, the decomposition of added C was slower with 44-52% and 46-54% of added C being decomposed by 55 days and 84 days respectively. The highest decomposition rate of added C was in lentil YS-amended soil. On the other hand, in mature shoot-amended soils, 19-28% of added C was decomposed by 27 days and 26-34% and 30-37% was decomposed by 55 and 84 days respectively. In canola MR-amended soil, 31% of added C was decomposed by 27 days. The decomposition of added C in canola MR-amended soil was approximately 1.5 times higher than canola MS-amended soil from day 27 onwards. As mentioned in Section 3.3, this calculation does not take priming effects into account.
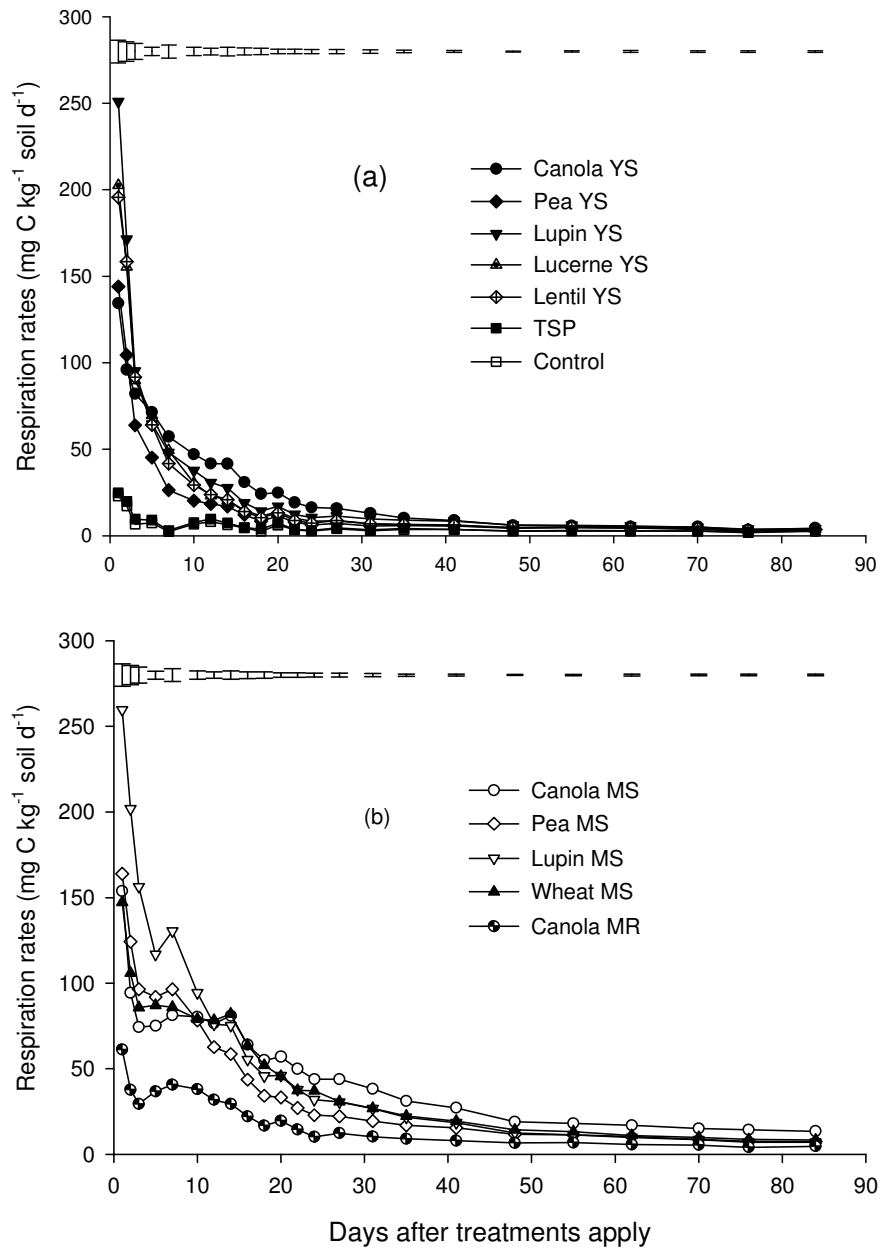
Figure 5.2 Respiration rates in soils amended with different crop residues; young shoot (a), mature shoot and root (b), TSP and unamended control over time. Means of 4 replicates, vertical bars indicate least significant difference ($LSD_{0.05}$).
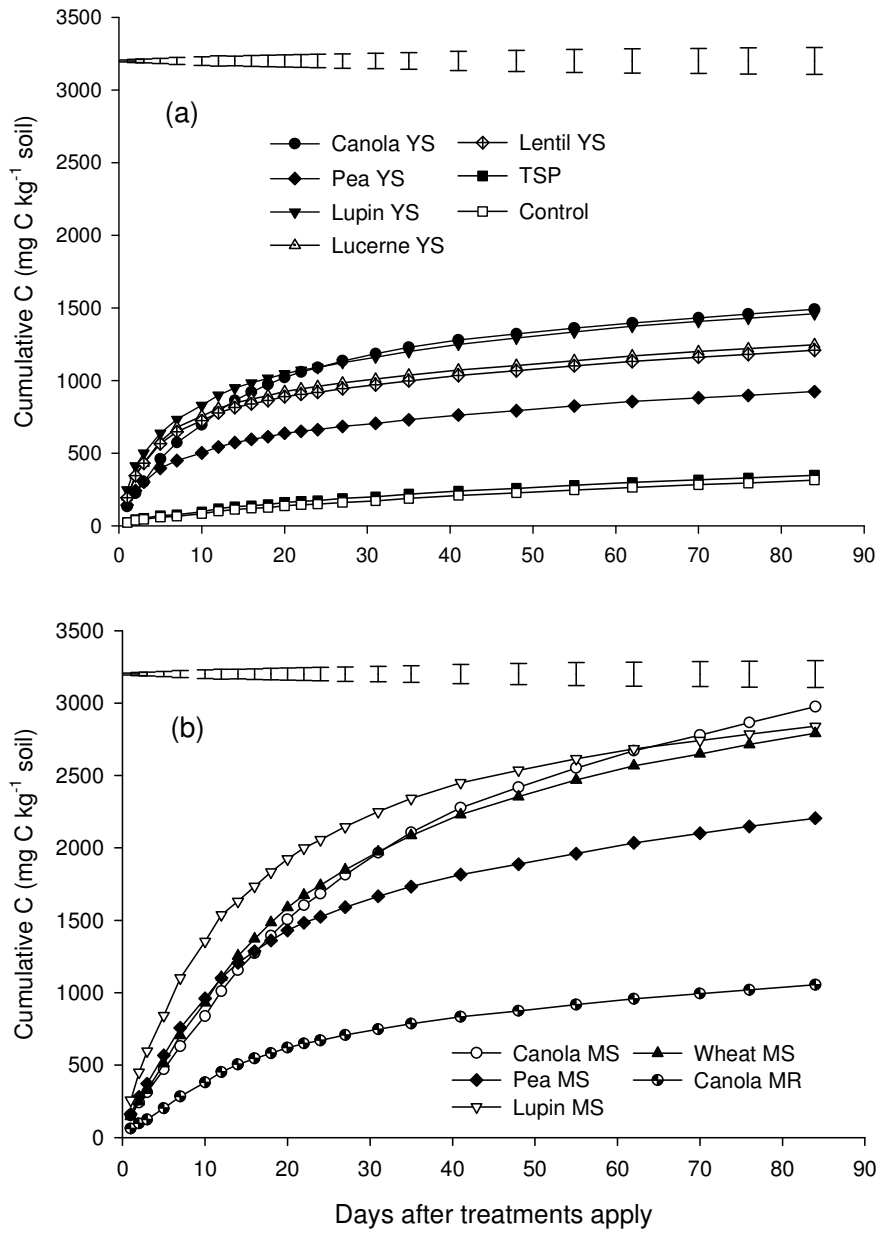
Figure 5.3 Cumulative C released from soils amended with different crop residues; young shoot (a), mature shoot and root (b), TSP and unamended control over time. Means of 4 replicates, vertical bars indicate least significant difference (LSD$_{0.05}$).

Table 5.5 Percentage of added C decomposed

Treatments	Day 7	Day 27	Day 55	Day 84
Canola YS	20.1 d	38.5 d	44.2 def	46.6 cde
Pea YS	29.0 e	39.4 de	43.7 de	46.0 cd
Lupin YS	31.3 ef	45.2 ef	51.1 fg	53.7 de
Lucerne YS	34.2 fg	46.0 f	49.8 efg	52.1 de
Lentil YS	35.3 g	47.4 f	51.7 g	54.0 e
Canola MS	6.5 a	18.9 a	26.4 a	30.4 a
Pea MS	13.7 c	28.3 bc	34.0 bc	37.4 ab
Lupin MS	13.6 c	27.0 bc	32.5 abc	34.8 a
Wheat MS	8.8 ab	23.4 ab	30.2 ab	33.3 a
Canola MR	12.3 bc	31.2 c	38.9 cd	43.4 bc

YS, MS and MR indicate Young Shoot, Mature Shoot and Mature Root respectively. Within columns, means followed by the same letter are not significantly different ($P < 0.001$) by Tukey's multiple range test.

5.3.3 Available P (P_{resin}) concentration in soil

Available P concentration (P_{resin}) in crop residue and TSP-amended soils varied with the age of residues and over time. In general, P_{resin} concentration in crop residue and TSP-amended soils was in the order of: TSP > young shoots $\geq$ mature root > control > mature shoot (Table 5.6) during 3 consecutive crop periods. The average P_{resin} concentration in TSP-amended soil was 1.5 and 1.7 fold lower in crop period 3 than 1 and 2, respectively.

In crop period 1 (D0-D28), the average P_{resin} concentration was highest in TSP-amended soil (4.2 mg P kg^{-1} soil) and lowest in canola MS-amended soil (0.2 mg P kg^{-1} soil). The average P_{resin} concentration in young shoot and canola MR-amended soils ranged from 1.3 to 2.5 mg P kg^{-1} soil and was twice as high as in the control, in pea YS, lucerne YS and canola MR-amended soil. The average P_{resin} concentration in mature shoot-amended soils was 56% to 83% lower than the control with no significant differences among the residue types. In the crop period 1, on day 7, P_{resin} concentration was highest in TSP-amended soil while on day 28, it was highest in pea YS-amended soil. In young shoot-amended soils (except lentil YS), P_{resin} concentration increased from day 7 to 28. However, in mature shoot-amended soils, P_{resin} concentration was strongly increased only in pea MS-amended soil from day 7 to 28, while in other mature shoot-amended soils the changes were less pronounced.

During the crop period 2 (D28-D56), the average P_{resin} concentration was highest in TSP and lowest in canola MS residue-amended soils, 4.5 and 0.3 mg P kg^{-1} soil, respectively. Compared to crop period 1, the average P_{resin} concentration increased in all mature shoot-amended soils and the differences among residue types were greater in crop period 2, but still the average P_{resin} concentration remained 25% to 83% lower than the control. The average P_{resin} concentration in young shoot-amended soils did not differ significantly but was lower than in

canola MR-amended soil. In crop period 2, the P_{resin} concentration in mature shoot-amended soils was higher on day 56 than days 35 and 45, while in young shoot (except lupin YS and lentil YS), canola MR and TSP-amended soils it was higher on days 35 and 45 than day 56.

In crop period 3 (D56-84), compared to the control, the average P_{resin} concentration was 2.5 fold higher in pea YS residue and TSP-amended soils and 4.2 fold lower in canola MS-amended soil. In the young shoot-amended soils, the differences in average P_{resin} concentration were more pronounced than in the crop period 2. The average P_{resin} concentration in canola MR amended soil did not significantly differ from TSP-amended soil, however it was twice as high compared to the control. The average P_{resin} concentration in the mature shoot-amended soils increased in crop period 3 compared to the crop period 1. In crop period 3, P_{resin} concentration generally decreased in young shoot, canola MR and TSP-amended soils, but increased in mature shoot-amended soils. On day 84, the differences in P_{resin} concentration between treatments were less pronounced than on days 63 and 73.

5.3.4 Microbial P (P_{mic}) concentration in soil

The trend of microbial P (P_{mic}) concentration in soils amended with crop residues and TSP was inversely related to P_{resin}. Soil P_{mic} concentration decreased in the following order: mature shoot > young shoot $\geq$ mature root > TSP $\geq$ control (Table 5.7). The average P_{mic} concentration was always higher than the average P_{resin} concentration by a factor 1.2 to 82.1. The difference between average P_{mic} concentration and average P_{resin} concentration were greatest in canola MS-amended soil and smallest in TSP-amended soil and the difference were more pronounced in crop period 1 than the crop periods 2 and 3.

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In crop period 1, the average P_{mic} concentration was highest in canola MS-amended soils (15.6 mg P kg^{-1} soil) and lowest in the control (4.5 mg P kg^{-1} soil). The differences in average P_{mic} concentration between treatments were more pronounced than in average P_{resin} concentration. Compared to the control, the average P_{mic} concentration was higher in mature shoot-amended soils (3.0 to 3.5 fold) than in young shoot and canola MR-amended soils (1.6 to 2.1 fold). In young shoot-amended soils, the average P_{mic} concentration ranged from 7.3 mg P kg^{-1} soil in lucerne YS-amended soil to 9.3 mg P kg^{-1} soil in lupin YS-amended soil. On the other hand, the average P_{mic} concentration in the mature shoot-amended soil ranged from 13.6 mg P kg^{-1} soil (pea MS) to 15.6 mg P kg^{-1} soil (Canola MS). In crop period 1, the P_{mic} concentration was generally higher on day 17 than day 7 and 28 and ranged from 5.1 mg P kg^{-1} soil (pea MS) to 25.0 mg P kg^{-1} soil (Canola MS). The P_{mic} concentration in TSP-amended soil was 1.1 to 1.5 fold higher than the control during crop period 1.

In crop period 2, in general the average P_{mic} concentration decreased by between 0.1 mg P kg^{-1} soil (control) to 2.6 mg P kg^{-1} soil (pea MS) compared to crop period 1. The average P_{mic} concentration ranged from 4.4 mg P kg^{-1} soil (control) to 13.2 mg P kg^{-1} soil (canola MS) and the average P_{mic} concentration was 1.2 to 46.6 fold higher than the average P_{resin} concentration. The average P_{mic} concentration in mature shoot-amended soils was 130%-208% higher than the control while in young shoot and canola MR-amended soils it was 54%-98% higher than the control. The average P_{mic} concentration in the young shoot-amended soils was highest in canola YS-amended soils (8.1 mg P kg^{-1} soil) and lowest in lentil YS-amended soil (6.7 mg P kg^{-1} soil). In the mature shoot-amended soils, it was highest in canola MS-amended soils (13.2 mg P kg^{-1} soil) and lowest in pea MS-amended soil (11.0 mg P kg^{-1} soil). The average P_{mic} concentration in TSP-amended soil was 1.2 fold higher than the control. During

the crop period 2, the P_{mic} concentration in mature shoot-amended soils was highest on day 35 whereas in young shoot and canola MR-amended soils it was highest on day 45.

In crop period 3 compared to crop period 1, the average P_{mic} concentration in residue-amended soils had decreased by between 1.3 mg P kg^{-1} soil (lupin YS) and 5.6 mg P kg^{-1} soil (wheat MS) but compared to crop period 2, it had decreased by 0.9 mg P kg^{-1} soil (lucerne YS) to 3.2 mg P kg^{-1} soil (wheat MS). The average P_{mic} concentration was highest in canola MS-amended soil (12.3 mg P kg^{-1} soil) and lowest in the control (4.5 mg P kg^{-1} soil). Compared to the control, the average P_{mic} concentration was higher in mature shoot-amended soils (1.9 to 2.8 fold) than in young shoot and canola MR-amended soils (1.2 to 1.5 fold). The differences in the average P_{mic} concentration among mature shoot-amended soils were more pronounced than the young shoot-amended soils. During crop period 3, P_{mic} concentration was higher on day 63 or 73 than day 84. The P_{mic} concentration among young shoot-amended soils differed only on day 63, whereas among mature shoot-amended soils it differed during the entire crop period 3. There were no significant differences in P_{mic} concentration between young shoot and TSP-amended soils on days 73 and 84.

Table 5.6 Available P (P$_{resin}$) in soils amended with different crop residues, TSP and an unamended control over time

Treatments	D7	D17	D28	Average D0-D28	D35	D45	D56	Average D28–D56	D63	D73	D84	Average D56-D84
							mg P kg^{-1} soil					
Canola YS	1.4 c	1.3 b	1.6 bcd	**1.4 b**	2.4 ef	2.2 de	2.0 cde	**2.2 de**	2.1 de	1.7 abcde	1.0 abc	**1.6 c**
Pea YS	1.9 d	2.7 d	3.0 f	**2.5 d**	3.0 fg	2.1 de	2.1 cde	**2.4 ef**	3.5 fg	3.1 e	1.6 c	**2.7 e**
Lupin YS	0.8 b	1.3 b	1.8 cd	**1.3 b**	2.0 de	2.3 def	2.6 ef	**2.3 def**	1.7 cd	2.1 bcde	1.1 abc	**1.6 c**
Lucerne YS	2.0 d	2.3 cd	2.8 e	**2.4 d**	2.4 ef	2.5 ef	1.7 bcde	**2.2 de**	2.5 e	2.5 bcde	1.1 bc	**2.1 d**
Lentil YS	2.0 d	2.1 c	1.8 d	**2.0 c**	2.2 e	2.3 def	2.6 ef	**2.3 ef**	2.8 ef	2.9 de	1.1 abc	**2.2 de**
Canola MS	0.1 a	0.1 a	0.4 a	**0.2 a**	0.1 a	0.3 a	0.4 a	**0.3 a**	0.2 a	0.3 a	0.3 a	**0.3 a**
Pea MS	0.1 a	0.2 a	1.1 abc	**0.5 a**	1.0 bc	1.1 bc	1.5 bcd	**1.2 bc**	0.9 abc	1.4 abcd	1.2 bc	**1.2 bc**
Lupin MS	0.2 a	0.2 a	1.0 ab	**0.5 a**	0.9 bc	1.0 bc	1.1 abc	**1.0 bc**	0.5 ab	1.2 ab	1.2 bc	**1.0 b**
Wheat MS	0.1 a	0.2 a	0.4 a	**0.2 a**	0.6 ab	0.6 a	0.9 ab	**0.7 ab**	1.1 bc	1.3 abc	1.3 bc	**1.2 bc**
Canola MR	3.2 e	2.3 cd	1.7 bcd	**2.4 d**	3.4 g	3.1 f	2.3 de	**3.0 f**	2.9 ef	2.8 cde	1.4 bc	**2.4 de**
TSP	6.2 f	4.5 e	1.8 cd	**4.2 e**	5.1 h	5.1 g	3.5 g	**4.5 g**	4.2 g	3.1 e	0.8 abc	**2.7 de**
Control	1.3 c	1.4 b	1.0 ab	**1.3 b**	1.4 cd	1.7 cd	1.8 bcde	**1.6 cd**	1.4 cd	1.2 ab	0.7 ab	**1.1 b**

YS, MS and MR indicated Young Shoot, Mature Shoot and Mature Root respectively. Within columns, means followed by the same letter are not significantly different ($P < 0.001$) by Tukey's multiple range test.

Table 5.7 Microbial P (P$_{mic}$) in soils amended with different crop residues, TSP and an unamended control over time

Treatments	D7	D17	D28	Average D0-D28	D35	D45	D56	Average D28–D56	D63	D73	D84	Average D56-D84
							mg P kg^{-1} soil					
Canola YS	9.0 b	10.2 e	5.1 b	**8.1 d**	8.5 c	9.0 c	7.0 bc	**8.1 d**	5.9 bcd	6.4 ab	6.3 bc	**6.2 bc**
Pea YS	8.7 b	8.9 d	6.0 bc	**7.8 d**	6.8 b	7.5 bc	6.8 bc	**7.1 c**	5.2 b	6.4 ab	5.6 ab	**5.7 bc**
Lupin YS	10.0 c	10.6 e	7.3 d	**9.3 e**	7.5 bc	13.1 e	6.4 b	**7.0 c**	7.2 e	6.4 ab	6.1 bc	**6.5 c**
Lucerne YS	8.0 b	7.6 c	6.2 bcd	**7.3 c**	6.6 b	7.1 b	6.6 bc	**6.8 c**	6.6 de	5.6 ab	5.7 bc	**6.0 bc**
Lentil YS	8.7 b	8.7 d	6.6 cd	**8.0 d**	6.7 b	7.0 b	6.3 b	**6.7 c**	5.7 bcd	5.4 ab	5.4 ab	**5.5 b**
Canola MS	10.1 c	25.0 h	11.5 f	**15.6 g**	16.0 f	12.8 e	10.9 ef	**13.2 h**	13.8 h	12.4 e	10.8 f	**12.3 f**
Pea MS	11.8 d	19.2 f	9.8 e	**13.6 f**	12.6 d	11.0 d	9.2 d	**11.0 e**	9.2 f	9.3 c	7.8 de	**8.8 d**
Lupin MS	12.0 d	18.5 f	10.6 ef	**13.7 f**	14.4 e	13.1 e	11.2 f	**12.9 g**	10.6 g	10.6 d	8.6 e	**9.9 e**
Wheat MS	10.4 c	20.9 g	11.4 f	**14.2 f**	12.7 d	12.6 de	10.1 de	**11.8 f**	9.0 f	9.2 c	7.6 de	**8.6 d**
Canola MR	8.7 b	9.2 d	6.9 cd	**8.3 d**	7.4 bc	7.8 bc	7.3 c	**7.5 c**	6.2 cde	6.9 b	6.8 cd	**6.7 c**
TSP	5.1 a	6.4 b	5.2 b	**5.6 b**	5.1 a	5.0 a	6.5 bc	**5.5 b**	5.3 bc	5.8 ab	5.6 abc	**5.6 b**
Control	4.7 a	5.1 a	3.6 a	**4.5 a**	4.4 a	4.5 a	4.3 a	**4.4 a**	4.1 a	4.8 a	4.4 a	**4.5 a**

YS, MS and MR indicated Young Shoot, Mature Shoot and Mature Root respectively. Within columns, means followed by the same letter are not significantly different ($P < 0.001$) by Tukey's multiple range test.

5.3.5 Microbial C (C_{mic}) in soil

Microbial C (C_{mic}) was determined only on days 7, 35 and 63. In all treatments, C_{mic} showed similar temporal changes to the P_{mic} concentration (Table 5.8). On day 7, compared to the control C_{mic} was 83% to 117% higher in mature shoot-amended soils while it was 24% to 65% higher in young shoot and canola MR-amended soil. On day 7, C_{mic} was highest in the pea MS-amended soil (311 mg C kg^{-1} soil) and lowest in TSP-amended soil (141 mg C kg^{-1} soil). The differences in C_{mic} were more obvious among young shoot and canola MR-amended soils than among mature shoot-amended soils. The C_{mic} in young shoot-amended soils ranged 237 mg C kg^{-1} soil in lupin YS-amended soil to 177 mg C kg^{-1} soil in lentil YS-amended soil, whereas in mature shoot-amended soils, C_{mic} was highest in pea MS-amended soil (311 mg C kg^{-1} soil) and lowest in lupin MS-amended soil (262 mg C kg^{-1} soil). Compared to the control, C_{mic} was 1.4 fold higher in canola MR-amended soil but 1.4 fold lower in TSP-amended soil. Microbial C (C_{mic}) increased in all treatments from day 7 to day 35. On day 35 compared to the control, the highest C_{mic}, in lupin MS-amended soil (416 mg C kg^{-1} soil), was only 44% higher than in the control. On day 35, the differences in C_{mic} between treatments were less pronounced than on day 7. Among the young shoot-amended soils, C_{mic} in lupin YS and lentil YS-amended soils was higher than the control. On the other hand, C_{mic} in all mature shoot-amended soils on day 35 was significantly higher (16-44%) than in the control. On day 63, C_{mic} was 14%-31% lower compared to day 35. On day 63, C_{mic} ranged from 202 mg C kg^{-1} soil in pea YS-amended soil to 304 mg C kg^{-1} soil in canola MS-amended soil. Microbial C (C_{mic}) was 1.1 to 1.3 fold higher in mature shoot-amended soils than the control but there was no significant difference between control and other residues and TSP-amended soils.

The microbial C: P (C_{mic}: P_{mic}) ratio varied over time and in general, it was smaller on day 7 than on days 35 and 63 except in canola MS and pea MS-amended soils. On day 7, the C_{mic}:

P_{mic} ratio ranged from 20:1 (lentil YS) to 32:1 (control). On day 7, the C_{mic}: P_{mic} ratio in mature shoot and canola MR-amended soils did not differ significantly, but it was significantly lower than in the TSP-amended soil and the control (Table 5.8). On the other hand, the C_{mic}: P_{mic} ratio was significantly different among young shoot-amended soils. On day 35, the differences in C_{mic}: P_{mic} ratio between treatments was more pronounced than on day 7. The C_{mic}: P_{mic} ratio was lowest in canola MS-amended soil (22:1) and highest in the control (63:1). It decreased in the order of control > TSP > young shoot ≥ mature root > mature shoot. On day 63, the differences in C_{mic}: P_{mic} ratio between treatments was less pronounced than on day 35. The C_{mic}: P_{mic} ratio in the control was significantly greater than in all other treatments. Among the residue-amended soils, the C_{mic}: P_{mic} ratio was lowest in soil amended with canola MS (22:1) and highest in soil amended with lentil YS (44:1).

Table 5.8 Microbial C (C_{mic}) and microbial C: P (C_{mic}: P_{mic}) in soils amended with different crop residues, TSP and unamended control at day 7 (D7), day 35 (D35) and day 63 (D63). Values are means of 4 replicates.

Treatments	Microbial C (mg C kg^{-1} soil)			Microbial C: P		
	D7	D35	D63	D7	D35	D63
Canola YS	200 cde	290 a	226 ab	23 ab	34 c	38 cd
Pea YS	231 ef	286 a	202 a	27 b	42 de	39 cd
Lupin YS	237 fg	356 b	246 ab	24 ab	47 ef	32 bc
Lucerne YS	190 cd	289 a	224 ab	24 ab	46 ef	34 bc
Lentil YS	177 bc	334 b	237 ab	20 a	49 f	44 d
Canola MS	277 h	353 b	304 c	26 b	22 a	22 a
Pea MS	311 i	330 b	254 bc	26 b	26 ab	28 ab
Lupin MS	262 gh	416 c	300 c	25 b	28 b	28 ab
Wheat MS	278 h	353 b	301 c	26 b	28 b	34 bc
Canola MR	210 def	282 a	209 a	25 b	38 cd	34 bc
TSP	142 a	291 a	213 ab	31 c	56 g	38 cd
Control	153 ab	282 a	226 ab	32 c	63 h	55 e

YS, MS and MR indicated Young Shoot, Mature Shoot and Mature Root respectively. Within columns, means followed by the same letter are not significantly different ($P < 0.001$) by Tukey's multiple range test.

5.3.6 Plant shoot and root dry matter

Plant shoot and root dry matter (DM) differed significantly (P < 0.001) between the residue and TSP-amended soils and the control (Figure 5.4). During the three crop periods, shoot and root DM were highest in TSP-amended soil and lowest in lupin MS-amended soil. In general, plant DM was lower in crop period 2 than crop periods 1 and 3. The lower DM in crop period 2 occurred in nearly all treatments and may be due to the cooler conditions and lower light intensity during this period. Therefore, plant DM is also expressed in percentage of the control (Figure 5.4b).

In the residue-amended soils, shoot and root DM decreased in the following order: mature root ≥ young shoot > mature shoot-amended soil. There were no significant differences among the mature shoot-amended soils in the 3 crop periods. Plant DM reduction compared to the control in soils amended with mature shoot residues was strongest in crop period 1 and least in crop period 3. Among young shoot-amended soils, shoot and root DM was highest in lentil YS amended soil. Shoot and root DM in young shoot-amended soils increased by 39%-110% compared to the control while in canola MR and TSP-amended soils they decreased by 4%-372% compared to the control across the 3 crop periods. Comapred to the control, plant shoot and root DM in canola MR-amended soil was significantly higher during the crop periods 1 and 3, while it was similar in crop period 2. Shoot and root DM were always highest in the TSP-amended soil. The increase in the TSP-amended soil compared to the control was greater in crop period 1 (4.7 fold) than in crop periods 2 and 3 (3.0 to 3.4 fold). Shoot DM was always greater than root DM and in general the shoot: root ratio in young shoot-amended soils and the control was wider in crop period 1, while in crop period 2 and 3 it was highest in canola MR-amended and in mature shoot and TSP-amended soils, respectively.
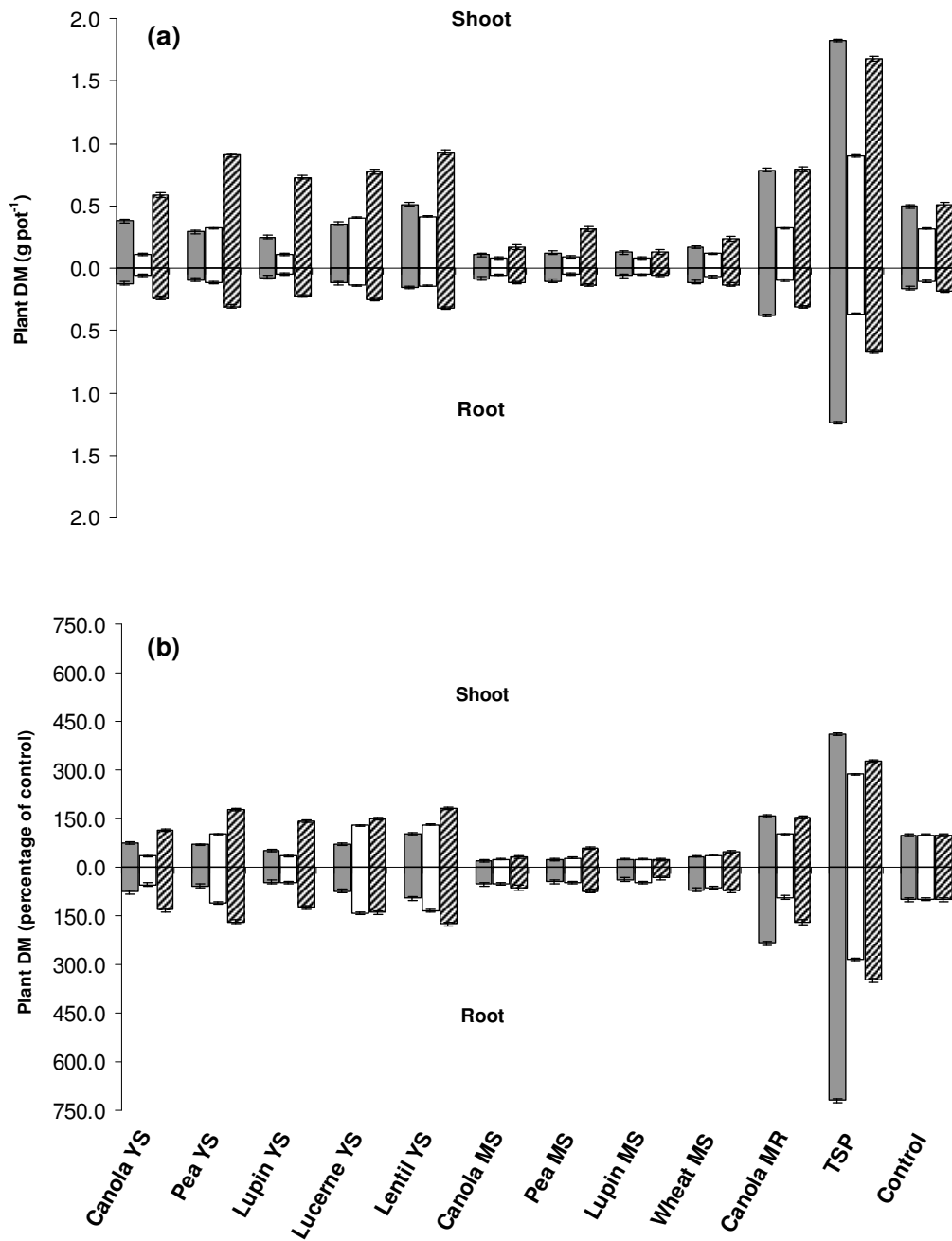
Figure 5.4 Shoot and root dry matter (DM, a) and percentage of control DM (b) of wheat plants grown in soils amended with different crop residues, TSP and unamended control in crop period 1 (gray bars), crop period 2 (white bars) and crop period 3 (diagonally hatched bars). Means of 4 replicates, vertical bars indicate standard error of means (SEM).

5.3.7 Plant shoot P concentration

Plant shoot P concentration (mg P kg^{-1} DM) in residue and TSP-amended soils and unamended control differed significantly and the differences changed over time (Table 5.9). In each of the 3 crop periods, plant shoot P concentration was generally lower in mature shoot-amended soils than in the other treatments; but over time shoot P concentration increased in mature shoot-amended soils while it decreased in crop period 3 compared to crop periods 1 and 2 in other treatments. Plant shoot P concentration followed a different trend compared to plant shoot and root DM (Figure 5.4). Plant shoot P concentration is also expressed as percentage of the control for better comparison between crop periods.

In crop period 1, plant shoot P concentration in the residue-amended soils was highest in pea YS-amended soil (1.8 mg P kg^{-1} DM) and lowest in canola MS-amended soil (0.7 mg P kg^{-1} DM) which also had lowest shoot DM. Although the shoot P concentration in young shoot, canola MR and TSP-amended soils did not differ significantly in crop period 1, it was significantly higher than in mature shoot-amended soils. Compared to the control, shoot P concentration in mature shoot-amended soils was 55%-64% lower whereas in young shoot, canola MR and TSP-amended soils it was only 7%-27% lower. In crop period 2, the differences in plant shoot P concentration between treatments were more pronounced than in crop period 1. Compared to crop period 1, plant shoot P concentration either decreased or remained unchanged in most young shoot, canola MR-amended soils and the control; but in lucerne YS, TSP and mature shoot-amended soils it slightly increased. In crop period 2, plant shoot P concentration was highest in TSP-amended soil (1.8 mg P kg^{-1} DM) and lowest in the canola MS-amended soil (0.7 mg P kg^{-1} DM). Compared to the control, plant shoot P concentration in all mature shoot-amended soils was 36%-46% lower, while in pea YS, lucerne YS and TSP-amended soils it was 1%-12% higher. In crop period 3 compared to crop period 2, plant shoot

P concentration decreased by 0.03 to 0.2 mg P kg^{-1} DM in all treatments except mature shoot-amended soils where shoot P concentration increased by 0.1 to 0.3 mg P kg^{-1} DM). The shoot P concentration was highest in canola MR-amended soil (1.5 mg P kg^{-1} DM) and lowest in canola MS-amended soil (0.8 mg P kg^{-1} DM). The differences in shoot P concentration between treatments were more pronounced in crop period 3 than in crop period 2. Compared to the control, shoot P concentration in mature shoot and lupin YS-amended soils was 1%-41% lower whereas in young shoot (except lupin YS), canola MR and TSP-amended soils it was 4%-16% higher.

Table 5.9 Shoot P concentration in mg P kg^{-1} DM and percentage of control of plant grown in soils amended with different crop residues, TSP and unamended control for 3 crop periods, crop period 1 (D0-D28), crop period 2 (D28-D56) and crop period 3 (D56-D84). Values are means of 4 replicates.

Treatments	Shoot P (mg P kg^{-1} DM)			Shoot P (% of control)		
	Crop period 1 (D0-28)	Crop period 2 (D28-56)	Crop period 3 (D56-84)	Crop period 1 (D0-28)	Crop period 2 (D28-56)	Crop period 3 (D56-84)
Canola YS	1.5 b	1.4 cd	1.4 cd	78.4 b	89.9 cd	103.5 de
Pea YS	1.8 bc	1.6 de	1.5 e	93.0 bc	101.3 de	112.6 e
Lupin YS	1.5 b	1.3 c	1.3 cd	76.7 b	83.8 c	97.9 cd
Lucerne YS	1.4 b	1.6 de	1.5 de	73.5 b	103.5 de	109.1 de
Lentil YS	1.6 bc	1.6 cde	1.4 de	84.4 bc	98.3 cde	104.9 de
Canola MS	0.7 a	0.7 a	0.8 a	37.3 a	44.2 a	59.0 a
Pea MS	0.9 a	1.0 b	1.3 cd	44.8 a	63.7 b	98.6 cd
Lupin MS	0.9 a	0.9 ab	1.0 b	45.4 a	53.5 ab	78.5 b
Wheat MS	0.7 a	0.8 ab	1.2 bc	36.1 a	51.7 ab	87.6 bc
Canola MR	1.6 bc	1.6 cde	1.5 f	83.5 bc	99.5 cde	116.0 e
TSP	1.6 bc	1.8 e	1.5 de	83.0 bc	112.0 e	109.6 de
Control	1.9 c	1.6 cde	1.4 de	100.0 c	100.0 cde	100.0 de

YS, MS and MR indicated Young Shoot, Mature Shoot and Mature Root respectively. Within columns, means followed by the same letter are not significantly different ($P < 0.001$) by Tukey's multiple range test.

5.3.8 Plant shoot P uptake

Shoot P uptake (mg P pot^{-1}) followed a similar trend to shoot DM. Shoot P uptake in all of the 3 consecutive crop growing periods was significantly ($P \leq 0.001$) different between treatments (Figure 5.5). To compare the different crop periods clearly the shoot P uptake is also expressed as percentage of the control. Shoot P uptake exceeding the amount of P in the seeds (0.3 mg P pot^{-1}) was considered as P taken up from soil. Shoot P uptake was significantly higher in TSP amended soil than all other treatments in the 3 crop periods. Shoot P uptake was lowest in canola MS-amended soils in crop period 1 and 2, but in lupin MS-amended soil in crop period 3. In crop periods 2 and 3, shoot P uptake decreased in canola MR and TSP-amended soils compared to crop period 1. On the other hand, shoot P uptake increased in young and mature shoot-amended soils in crop period 3 compared to crop periods 1 and 2. In crop period 1, in residue-amended soils shoot P uptake increased in the following order: mature shoot < young shoot < canola MR. Compared to the control in crop period 1, shoot P uptake was 15%-62% lower in the young shoot-amended soils and 82%-90% lower in the mature shoot-amended soils whereas it was 46% and 263% higher in canola MR and TSP-amended soils, respectively. In residue-amended soils, shoot P uptake was highest in lentil YS-amended soils (0.7 mg P pot^{-1}) in crop period 2. Compared to the control, shoot P uptake in crop period 2 was 7%-75% lower in young shoot-amended soils (except lentil YS) and 35%-84% lower in mature shoot and canola MR-amended soils whereas it was 25% and 123% higher in lupin YS and TSP-amended soils, respectively. In crop period 3, shoot P uptake increased from 0.1 to 1.6 mg P pot^{-1} in all treatments compared to crop period 2. Compared to the control, shoot P uptake was 0.9 to 3.8 fold higher in young shoot and TSP-amended soils but in mature shoot and canola MR-amended soils it was 1.2 to 4.5 fold lower. In mature shoot-amended soils, shoot P uptake strongly increased only in pea MS-amended soil in crop period 3.
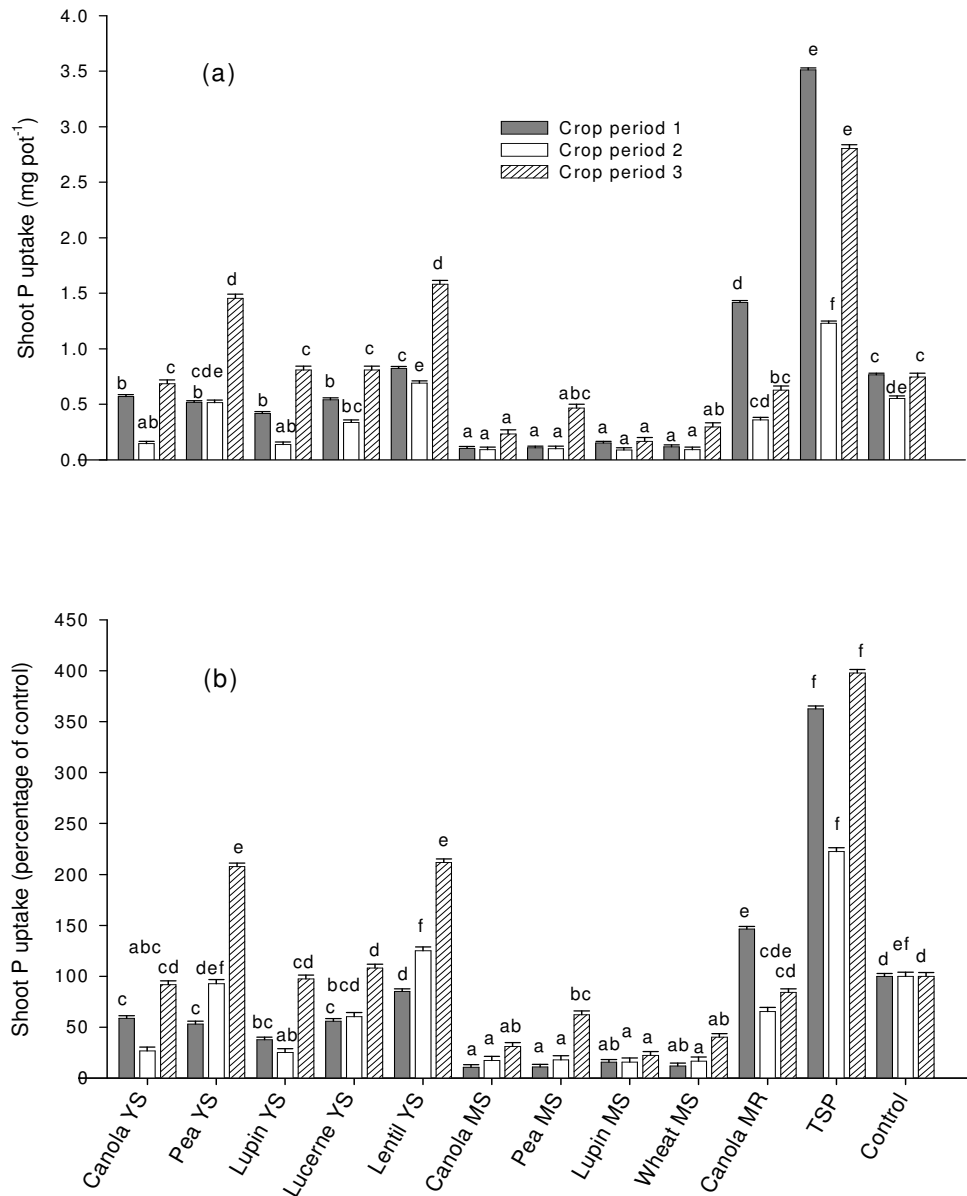
Figure 5.5 Plant shoot P uptake (a) and shoot P uptake percentage of control (b) from soils amended with different crop residues, TSP and unamended control for crop period 1, crop period 2 and crop period 3. Bars with the same letters are not significantly different. Means of 4 replicates, vertical bars indicate standard error of means (SEM).

5.3.9 Correlations between residue properties and soil and plant variables

The correlations between residue properties and soil P_{resin} or plant P uptake varied over time. Except on day 84, soil P_{resin} was positively correlated with total P and P_{H2O} of the residues. At some sampling dates, soil P_{resin} was also positively correlated with total N, N_{H2O}, O-alkyl, and amide content of residues and negatively correlated with residue C: N ratio. In contrast, soil P_{resin} had a significant negative correlation with residue C: P ratio and the amount of C added (Table 5.10). In contrast to P_{resin}, soil P_{mic} was significantly positively correlated with residue C: P ratio, C: N ratio (except on day 7) and C addition. Soil P_{mic} was significantly negatively correlated with residue total P, P_{H2O}, total N, N_{H2O}, C_{H2O}, O-alkyl and amide at some sampling dates during the incubation (Table 5.10). The strongest correlation between residue properties and soil C_{mic} was found on day 63. On day 63, soil C_{mic} had a significant positive correlation with residue C: P ratio, C: N ratio and C addition while there was a significant negative relationship with residue total P, P_{H2O}, and total N. There was a strong positive correlation between respiration rate and residue C: P ratio, C: N ratio (except day 7) and C addition during the 84 days of incubation (Table 5.10). In general, respiration rate was negatively correlated with residue total P, P_{H2O}, total N, N_{H2O}, C_{H2O}, O-alkyl and amide. Similar to respiration rate, cumulative CO_2 release was significantly positively correlated with residue C: P ratio, C: N ratio (except day 7 and 28) and C addition. On the other hand, cumulative CO_2 release was significantly negatively correlated with residue total P, P_{H2O}, total N, N_{H2O} and amide content. Residue decomposition or mineralization of added C (C_{min}/Decomp) was strongly influenced by residue properties. Crop residue decomposition was positively correlated with total P, P_{H2O}, total N, N_{H2O}, C_{H2O}, O-alkyl and amide content of the residues. In contrast, residue lignin content, C: P ratio, C: N ratio and amount of C addition were negatively correlated with residue decomposition. Residue phenolic content was negatively correlated with residue decomposition, but only in the first 28 days (data not shown, see Appendix 2). The relationship

between plant DM and residue properties was strongest at day 84. Plant DM was significantly positively correlated with residue total P, P_{H2O}, total N, N_{H2O}, O-alkyl and amide. In contrast, plant DM was negatively correlated with residue C: P ratio, C: N ratio and C addition. For each of the 3 crop periods, plant shoot P uptake was positively correlated with residue total P content and negatively correlated with residue C: P ratio and amount of C added with residues (see Appendix 3 for correlations between phenolic and soil and plant variables).

Table 5.10 Correlation coefficient (r^2) of residue properties with soil and plant variables during incubation.

Time	Parameters	P_t	N_t	P_{H2O}	C_{H2O}	N_{H2O}	Lignin	O-alkyl	Amide	C:P	C:N	C addition
Crop period 1, Day 7	P_{resin}	0.68**	ns	0.55*	ns	ns	ns	ns	ns	-0.64**	ns	-0.64**
	P_{mic}	-0.65**	ns	-0.54*	ns	ns	ns	ns	ns	0.58**	ns	0.57*
	C_{mic}	-0.58*	ns	-0.61**	ns	-0.41*	ns	ns	ns	0.56*	ns	0.56*
	Respiration rate	-0.80***	ns	-0.71**	ns	-0.43*	ns	ns	ns	0.75***	ns	0.73**
	Cumulative C	ns	ns	-0.36*	ns	ns	ns	ns	ns	ns	ns	ns
	C_{min}/Decomp	0.63**	0.85***	0.41*	0.77***	0.90***	-0.52*	0.74**	0.83***	-0.61**	-0.69**	-0.61**
Crop period 1, Day 28	P_{resin}	0.83***	0.83***	0.45*	ns	0.68**	ns	0.68**	0.78***	-0.70**	-0.71**	-0.71**
	P_{mic}	-0.74***	-0.51*	-0.83***	ns	-0.58*	ns	ns	-0.42*	0.86***	0.62**	0.86***
	Respiration rate	-0.82***	-0.64**	-0.66**	-0.40*	-0.61**	ns	-0.51*	-0.60**	0.95***	0.75***	0.96***
	Cumulative C	-0.88***	-0.45*	-0.75***	ns	-0.41*	ns	ns	ns	0.89***	ns	0.88***
	C_{min}/Decomp	0.65**	0.71**	0.58*	0.74***	0.84***	-0.48*	0.57*	0.70**	-0.75***	-0.73**	-0.76***
	Plant DM	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Plant P uptake	0.46*	ns	0.50*	ns	ns	ns	ns	ns	-0.49*	ns	-0.49*
Crop period 2, Day 35	P_{resin}	0.78***	ns	0.75***	ns	ns	ns	ns	ns	-0.83***	-0.50*	-0.84***
	P_{mic}	-0.87***	-0.57*	-0.77**	-0.41*	-0.61**	ns	-0.42*	-0.48*	0.98***	0.57*	0.98***
	C_{mic}	-0.45*	ns	ns	ns	ns	ns	ns	ns	0.54*	ns	0.53*
	Respiration rate	-0.81***	-0.63**	-0.69**	ns	-0.60**	ns	-0.74*	-0.57*	0.95***	0.75***	0.96***
	Cumulative C	-0.90***	-0.49*	-0.76***	ns	-0.45*	ns	ns	ns	0.92***	0.46*	0.91***
	C_{min}/Decomp	0.65**	0.68**	0.61**	0.74***	0.83***	-0.47*	0.53*	0.67**	-0.76***	-0.72**	-0.77***
Crop period 2, Day 56	P_{resin}	0.64**	0.39*	0.76***	0.45*	0.48*	ns	ns	ns	-0.82***	-0.62**	-0.83***
	P_{mic}	-0.79***	-0.53*	-0.81***	-0.50*	-0.65**	ns	-0.41*	-0.46*	0.95***	0.54*	0.95***
	Respiration rate	-0.76***	-0.63**	-0.71**	-0.43*	-0.65**	ns	-0.41*	-0.57*	0.89***	0.79***	0.90***
	Cumulative C	-0.90***	-0.53*	-0.77***	ns	-0.49*	ns	ns	-0.44*	0.95***	0.54*	0.94***
	C_{min}/Decomp	0.66**	0.65**	0.64**	0.74***	0.81***	-0.44*	0.50*	0.64**	-0.78***	-0.69**	-0.78***
	Plant DM	0.69**	0.54*	ns	ns	ns	ns	0.46*	ns	-0.50*	ns	-0.49*
	Plant P uptake	0.71**	0.58**	ns	ns	ns	ns	0.42*	ns	-0.47*	ns	-0.47*

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns, non-significant at 5% level of significance. P_t, residue total P, N_t, residue total N, C_{min}/Decomp, residue decomposition rate

Table 5.10 (Continued)

Time	Parameters	P_t	N_t	P_{H2O}	C_{H2O}	N_{H2O}	Lignin	O-alkyl	Amide	C:P	C:N	C addition
Crop period 3, Day 63	P_{resin}	0.93***	0.55*	0.66**	ns	0.42*	ns	ns	ns	-0.86***	-0.44*	-0.86***
	P_{mic}	-0.73**	-0.47	-0.70**	ns	-0.46*	ns	ns	ns	0.91***	0.56*	0.92***
	C_{mic}	-0.79***	-0.45*	-0.68**	ns	ns	ns	ns	ns	0.87***	0.55*	0.87***
	Respiration rate	-0.73**	-0.56*	-0.70**	ns	-0.58*	ns	ns	-0.50*	0.87***	0.74***	0.88***
	Cumulative C	-0.90***	-0.54*	-0.77***	ns	-0.50*	ns	ns	-0.44*	0.95***	0.55*	0.95***
	C_{min}/Decomp	0.67**	0.65**	0.65**	0.74***	0.81***	-0.43*	0.50*	0.63**	-0.79***	-0.69**	-0.80***
Crop period 3, Day 84	P_{resin}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	P_{mic}	-0.72**	-0.60**	-0.61**	-0.45*	-0.62**	ns	-0.47*	-0.51*	0.88***	0.63**	0.89***
	Respiration rate	-0.66**	-0.56*	-0.61**	ns	-0.57*	ns	ns	-0.53*	0.82***	0.79***	0.84***
	Cumulative C	-0.90***	-0.56*	-0.77***	ns	-0.52*	ns	ns	-0.47*	0.96***	0.59**	0.96***
	C_{min}/Decomp	0.67**	0.61**	0.67**	0.72**	0.77***	-0.41*	0.47*	0.60**	-0.81***	-0.67**	-0.81***
	Plant DM	0.94***	0.59**	0.75***	ns	0.54*	ns	0.42*	0.45*	-0.92***	-0.48*	-0.91***
	Plant P uptake	0.66**	ns	0.53*	ns	ns	ns	ns	ns	-0.86***	-0.53*	-0.87***

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns, non-significant at 5% level of significance. P_t, residue total P, N_t, residue total N, C_{min}/Decomp, residue decomposition rate

5.4 Discussion

This study showed that the decomposition dynamics of the added plant residues varied significantly, depending on the plant species, plant part (shoot or root), age and decomposition stage. The results are in agreement with several other studies (Abiven *et al.* 2005; Bertrand *et al.* 2006; Blair *et al.* 2005; Jensen *et al.* 2005; Nwoke *et al.* 2004; Wang *et al.* 2004). However, in this study, residues were added based on an equivalent amount of P from a wide range of residues hence different amounts of C were added. Moreover, P dynamics and plant P uptake were monitored during three consecutive crop periods. This design with young plants in each of the period was used to induce a similar plant P demand as opposed to having progressively older plants in the later periods. This allowed evaluating P uptake given maximum P demand, across the entire decomposition time, thus enabling better comparison of P benefit to the plants. Based on the respiration rates and consequently C mineralization, the decomposition process during the incubation can be divided into 4 distinct phases with phases 1 and 2 in crop period 1 and phase 3 and 4 in the crop period 2 and 3, respectively (Figure 5.2 and Figure 5.3).

5.4.1 Phase 1 (day 0-10)

The first 10 days of incubation are considered to be phase 1 because of higher respiration rates than during the rest of the incubation period. Compared to the later phases, the highest respiration rates were measured in soils amended with residues and differences compared to TSP and control were greatest (Figure 5.2). This confirms that C addition stimulates microbial activity which is in agreement with previous studies (Abiven *et al.* 2005; Bertrand *et al.* 2006; Jensen *et al.* 2005; Trinsoutrot *et al.* 2000b; Wang *et al.* 2004). In this initial phase mainly easily available, water-soluble low molecular weight compounds are decomposed. In this study, young residues with high content of water-soluble C, N and P and low C: P and C: N ratio produced more CO_2-C (243-388 mg CO_2-C g^{-1} C added) than mature shoot and root residues

(86-168 mg CO_2-C g^{-1} C added) which contain less of these easily degradable compounds (Table 5.4). The large differences in respiration rates were mainly due to different amounts of C added. Although similar amounts of C were added with canola YS and lupin YS, the respiration rate in lupin YS-amended soil was higher than in canola YS-amended soil due to significantly higher amount of water-soluble C and N in lupin YS. Hence, C mineralization had strong positive correlation with water-soluble C (C_{H2O}) and a negative correlation with C: P and C: N ratio (Table 5.10). Previous studies also reported similar findings (Abiven *et al.* 2005; Bertrand *et al.* 2006; Jensen *et al.* 2005; Mathers *et al.* 2007; Raiesi 2006; Trinsoutrot *et al.* 2000b; Wang *et al.* 2004; Xu *et al.* 2006). By the end of this phase, these compounds are probably depleted and therefore decomposition rates started to decline. Although the same amount of P was added, compared to the control, higher P_{mic} in mature shoot-amended soils than young shoot and canola MR-amended soils (Table 5.7 and Table 5.8) suggests that P immobilization is high with mature residues which have high C: P ratio and low P concentration because a large amount of C had to be added to achieve the same amount of P addition as young residues and this stimulated microbial activity. Hence, compared to the control and TSP-amended soil, the higher biomass C and P in residue-amended soils suggest that C addition with residues not only stimulates microbial activity but also leads to increased microbial biomass. The increase of biomass C in residue-amended soils is in agreement with other studies (Chauhan *et al.* 1981; He *et al.* 1997; Marschner *et al.* 2003) as is the increase in microbial P uptake following C addition (Bünemann *et al.* 2004; Chauhan *et al.* 1981; He *et al.* 1997). On the other hand, low respiration rates and low biomass C in the control and TSP-amended soil (respiration only from native soil organic matter) shows that microbial activity in this soil is C limited, not P limited. This is in agreement with previous studies (Chauhan *et al.* 1981; Nziguheba *et al.* 2005; Ros *et al.* 2003). However, the differences between residue types in biomass P and C were less pronounced than the differences in respiration. For example, the

respiration rate in lupin YS-amended soil was nearly twice as high compared to pea YS-amended soil but microbial P was only 15% higher and microbial C did not differ. This suggests that microbial growth responds more slowly to addition of C than microbial activity (Abiven *et al.* 2007; Oehl *et al.* 2001a). Therefore the high respiration rates in this phase are also due to an increase in activity per cell. It should be noted that the higher respiration rate in lupin YS-amended soil was probably due to the greater amount of C added since the percentage of C decomposed did not differ from that of pea YS-amended soil (Table 5.5). The increased microbial P uptake (1.5 to 2.6 fold) in residue-amended soils compared to TSP-amended soil and the control may also be related to the high P demand of microorganisms with high growth rates (Vrede *et al.* 2004). The slightly higher P_{mic} concentration in TSP-amended soil (0.4 mg P kg^{-1} soil) than the control suggests that the microbial biomass was P limited even in absence of added C. However, this increase is much smaller than in the residue-amended soil, indicating that C limitation of the soil microbial biomass was more important. On the other hand, in phase 1, P_{resin} concentration increased in young shoot (except lupin YS) and canola MR-amended soils compared to the control (Table 5.6) probably due to higher total P concentration, lower C: P ratio and lower P immobilization than the mature shoot-amended soils. Thus, P_{resin} concentration was positively correlated with residue total P concentration and negatively correlated with C: P ratio. The low P_{resin} concentration in lupin YS-amended soil can be explained by the very high initial microbial activity and therefore higher microbial P uptake than other young shoot-amended soils (Table 5.7). Hence, this study shows that residues with high total P (≥ 0.16%) and low C: P ratio (≤ 253: 1) may increase soil P_{resin} concentration. This result is in agreement with a previous study (White and Ayoub 1983) that showed residue with total P 0.34% and a C: P ratio 123: 1 increased available P in soil whereas residue with total P 0.08% and a C: P ratio 506: 1 resulted in net soil P immobilization. However, canola YS had 0.16% P and P_{resin} in canola YS-amended soil was higher than the control which disagrees with

the critical level of P (0.2%) in crop residues for net P immobilization or mineralization reported by Fuller *et al*. (1956). Hence, even though P immobilization had occurred in canola YS-amended soil (1.9 fold higher P_{mic} than the control), P mineralization was higher than P immobilization. The lower critical P level in this study suggests that microorganisms required less P for decomposition than in the study by Fuller *et al*. (1956). Compared to the control, the 4.8 fold higher P_{resin} in TSP-amended soil (Table 5.6) can be explained by the fact that the P was added in solution and that little P was taken up by the microbial biomass. This is in agreement with the previous studies (Chauhan *et al.* 1981; Hedley *et al.* 1982; Nwoke *et al.* 2004) who reported that addition of P without C increased available P while C addition decreased available P but increased P immobilization as microbial P.

5.4.2 Phase 2 (day 10 to 28)

Phase 2 consists of days 10 to 28 of incubation and is characterized by lower respiration rates than phase 1 but higher respiration rates than rest of the incubation period (Figure 5.2). During phase 2, lower respiration rates than phase 1 are likely to be due to depletion of easily degradable and water soluble compounds. This hypothesis is supported by previous studies (Mary *et al.* 1992; Trinsoutrot *et al.* 2000b; Wang *et al.* 2004) which showed that after 28 days of incubation, mainly recalcitrant compounds in the residues were decomposed. Therefore, the total amount of C respired in phase 2 was lower (78-144 mg CO_2-C g^{-1} C added) than in phase 1 (86-388 mg CO_2-C g^{-1} C added). The decline of respiration rates in phase 2 is in agreement with the previous studies (Abiven *et al.* 2005; Bertrand *et al.* 2006; Jensen *et al.* 2005; Mary *et al.* 1992; Saggar *et al.* 1998; Trinsoutrot *et al.* 2000b; Wang *et al.* 2004). However, during phase 2, compared to the control the respiration rates in mature shoot-amended soils were 1.5 to 5.0 fold higher than young shoot and canola MR-amended soils probably due to greater amount of C added with mature residues than young shoot and canola MR residues. Hence,

respiration rates in this phase were significantly and positively correlated with the amount of residue C added (Table 5.10). Due to higher respiration rates in mature shoot-amended soils in phase 2, the cumulative respiration was also higher in mature shoot-amended soils than young shoot and canola MR-amended soils (Figure 5.3). However, by the end of phase 2, the total amount of added C that was decomposed was similar in soils amended with young shoot (78-143 mg CO_2-C g^{-1} C added) and mature shoot and root (102-144 mg CO_2-C g^{-1} C added). This suggests that the microbes in the mature residue-amended soils adapted to the poorer degradability and were able to decompose them relatively quickly (Bünemann *et al.* 2004; Mary *et al.* 1992). In phase 2, the respiration rates in TSP-amended soil and the control were lower than in phase 1 and continued at a very low rate. Since only recalcitrant SOM could be mineralized in these treatments, the higher respiration rate in phase 1 than phase 2 is probably due to mixing of the soils at the start of the experiment. Bertrand *et al.* (2006) and Wang *et al.* (2004) also reported a decline in C mineralization rate in the unamended control between days 10 to 28. In agreement with phase 1, P_{mic} concentrations in phase 2 were higher in residue-amended soils than in TSP-amended soil and the control. On day 17, P_{mic} in mature shoot amended soils was 8.5 to 15 mg P kg^{-1} soil higher than the amount of P added with residues. This indicates that some of the soil P is mineralized due to production of phosphatases (Magid *et al.* 1996; Stewart and Tiessen 1987) or solubilised by carboxylates produced during decomposition (Iyamuremye *et al.* 1996a). Compared to phase 1, the P_{resin} concentration was higher in all young shoot (except lentil YS) and mature shoot-amended soils in phase 2 while it was lower in lentil YS, canola MR and TSP-amended soils which may be due to higher plant P uptake in the latter treatments (Figure 5.5). The decrease in available P due to plant P uptakes is in agreement with Dalal (1979). The increase in P_{resin} concentration in young shoot (except lentil YS) and mature shoot-amended soil in phase 2 suggests that P mobilisation exceeded microbial P demand. However, the sources of increased P_{resin} could not be identified in this

study because residues or TSP were not P-labelled. Compared to day 17, P_{mic} on day 28 decreased by 22% to 39% in mature residue-amended soils which may increase available P. This agrees with previous studies which showed that P immobilization declined after 28 days of incubation in soils amended with residues with high C: P ratio (White and Ayoub 1983) or both high and low C: P ratio (Nziguheba *et al.* 1998). In agreement with phase 1, compared to the control the P_{resin} concentration was higher in young shoot and canola MR-amended soils than in mature shoot-amended soils. Compared to the control, the lower P_{resin} concentration both in phase 1 and 2 (crop period 1) in soils amended with residues with low total P concentration and higher C: P ratio resulted in low plant DM (Figure 5.4) and plant shoot P uptake (Figure 5.5). The poor plant growth in the mature shoot-amended soils was probably due to low shoot P concentrations which are lower than the P deficiency level (0.16% of shoot DM) as suggested by Marschner (1995). On the other hand, although the average P_{resin} concentration in young shoot-amended soils was higher than the control, plant growth was lower except for lentil YS which can be explained by the low shoot P concentration (< 0.16%) in plants growing in canola YS, lupin YS and lucerne YS-amended soils. This limited plant growth, especially root growth may be due to limitation of other nutrients. The resulting poor root growth would limit the capacity of the plants to take up P and other nutrients. The low plant DM in combination with high shoot P concentrations in pea YS-amended soil indicate that there was another factor that limited the plant growth. High P_{resin} concentration and a shoot P concentration between deficiency and optimal range in lentil YS and canola MR -amended soils resulted in higher plant growth than in the control suggesting that these residues are good P sources for plants. In crop period 1, plant growth and plant P uptake were highest in TSP-amended soil. This may be explained by the high P availability because P was added with TSP as solution and only 1.3 mg P pot^{-1} was immobilized by the microbial biomass. Hence, P_{resin} was significantly positively correlated with plant DM (r^2 = 0.72, P ≤ 0.001).

In phase 2, total P[1] in the pools measured (P_{resin}, P_{mic} and plant P uptake) ranged between 3.2 to 10.7 mg P pot^{-1}. Total P was higher in mature shoot and pea YS-amended soils (7.3 to 10.7 mg P pot^{-1}) than in the soils amended with other residues or TSP (3.2 to 5.6 mg P pot^{-1}). In residue-amended soils, a large proportion of this total P (34%-95%) was microbial P. However, approximately 32%-73% of added P in young shoot residues, 7%-37% in mature shoot residues, 55% in canola MR and 63% in TSP-amended soil were not detected in the three measured pools and therefore, must have entered to other soil P pools.

5.4.3 Phase 3 (day 28 to 56)

Phase 3, between days 28 to 56 is characterised by the lower respiration rates than the earlier phases but still higher than the following periods. In phase 3, the respiration rates in residue-amended soils remained significantly higher than in TSP-amended soil and the control (Figure 5.2). However, the differences in respiration rates between residue-amended soils were smaller than in phase 2. The low respiration rates in phase 3 can be explained by the fact that only increasingly recalcitrant compounds were left to be decomposed. During phase 3, young shoot-amended soils produced 38-59 mg more CO_2-C g^{-1} C added compared to phase 2 whereas mature shoot and canola MR-amended soils produced 55-78 mg more CO_2-C g^{-1} C added. This indicates that due to the greater C addition still there is a higher C concentration remaining from mature shoot and canola MR residues than from young shoot residues. As a result, respiration rates were strongly positively correlated with residue C addition. Previous studies (Abiven *et al.* 2007; Bertrand *et al.* 2006; Mary *et al.* 1992) showed that the decomposition rate of residues with higher amounts of recalcitrant compound decreased more slowly than the

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[1] The percentage of residue P recovered was calculated by subtracting the control value (P_{resin}, P_{mic} and Plant P uptake) from the treatments value.

residues with lower amounts of recalcitrant compounds. In phase 3, microbial P decreased by 6%-24% in residues-amended soils compared to phases 1 and 2, which is probably due to the lack of C which would decrease microbial P demand. However, P_{mic} remained higher in mature shoot-amended soils than other treatments during phase 3. Although microbial activity and microbial biomass P decreased in phase 3, microbial biomass C strongly increased on day 35 compared to day 7 (Table 5.8). This is in contrast to the decreasing respiration rates and suggests that the cells were now accumulating C rather than respiring it which agrees with the increased microbial C: P ratio indicating that the cells were no longer rapidly growing. Compared to crop period 1 (phase 1 and 2), the P_{resin} concentration increased from 0.1 to 0.9 mg P pot^{-1} in all treatments other than pea YS and lucerne YS-amended soils whereas P_{mic} concentration decreased from 0.1 to 3.0 mg P pot^{-1} in crop period 2 (phase 3). The decrease of P_{resin} concentration (0.2 mg P pot^{-1}) in pea YS and lucerne YS-amended soils may be due to greater plant growth in crop period 2 than crop period 1 (Figure 5.4). However, the increase of P_{resin} concentration in phase 3 was less than the decrease of P_{mic} concentration. This indicates that P released from the microbial biomass can be taken up by plants or enter a non-resin extractable P pool (Kouno *et al.* 2002). The differences in P_{resin} concentration between residue-amended soils were more pronounced in crop period 2 than crop period 1 which may be due to differential mineralization of residue P. In agreement with phases 1 and 2, P_{resin} concentration in mature shoot-amended soils remained lower than in the control. In contrast, P_{resin} concentration in young shoot and canola MR-amended soils was higher the control during phase 3. The P_{resin} concentration was significantly positively correlated with residue total P concentration and negatively correlated with residue C: P ratio while the opposite was true for P_{mic}. In general, compared to the control, plant DM was higher only in soils amended with pea YS, lucerne YS, canola MR and TSP (Figure 5.4) although the average P_{resin} concentration in all young shoot-amended soils was higher than mature shoot-amended soils and the control.

The lower plant growth in canola YS, lupin YS and mature shoot-amended soils compared to the control can be explained by the low shoot P concentration which are in the P deficiency level (< 0.16%) as suggested by Marschner (1995). As in crop period 1, plant DM and plant P uptake were higher in soils amended with lower rates of residue C kg^{-1} soil than in soils amended with higher rates of residue C kg^{-1} soil. In this phase, total P[1] in the three measured pools (P_{resin}, P_{mic} and plant P uptake) was greater in mature shoot-amended soils (5.7 to 8.0 mg P pot^{-1}) than other treatments (2.6 to 5.7 mg P pot^{-1}), but in residue-amended soils, a large part of this total P (48%-96%) was microbial P. Compared to phase 2, more P added with residues was not detectable in the three P pools studied (P_{resin}, P_{mic}, plant) being 50%-77% of added P in young shoot residues, 30%-57% in mature shoot and canola MR residues and 57% in TSP-amended soils.

5.4.4 Phase 4 (day 56 to 84)

Phase 4, from day 56 to 84 is characterised by lower respiration rates compared to the earlier phases (Figure 5.2). By this phase it can be assumed that only very recalcitrant organic compounds were left (Wang *et al.* 2004). Nevertheless, in phase 4, the respiration rate in mature shoot-amended soils was still higher than in the young shoot and canola MR amended soils, similar to the previous phases 2 and 3. During phase 4, young shoot-amended soils produced an additional 23-26 mg CO_2-C g^{-1} C added whereas in mature shoot-amended soils it was 23-40 mg CO_2-C g^{-1} C added more compared to phase 3. This suggests that there was still more C left in soils amended with mature shoot residues due to addition of greater amount of C with mature shoot residues, but the differences between young and mature residues has decreased. Hence, respiration rate remained significantly positively correlated with C added

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[1] The percentage of residue P recovered was calculated by subtracting the control value (P_{resin}, P_{mic} and Plant P uptake) from the treatments value.

with residues (Table 5.10). Higher decomposition rate in residues high in recalcitrant compounds than in residues with low concentrations of recalcitrant compounds 2 to 3 months after residue addition was also reported by Abiven *et al.* (2007), Bertrand *et al.* (2006) and Wang *et al.* (2004). In phase 4, the average P_{mic} concentration in residue-amended soils decreased by 7%-27% compared to phase 3. As in the earlier phases, the decrease in P_{mic} can be explained by the lack of easily available C. Nevertheless, in phase 4, C_{mic} and P_{mic} concentrations remained higher in mature shoot-amended soils than in young shoot and canola MR-amended soils probably due to greater C addition. But the differences in C_{mic} and P_{mic} between residue-amended soils were not as strong as in phase 3 which is most likely due to a generally lower biomass in this later phase. Compared to phase 3, the average P_{resin} concentration decreased in all treatments in phase 4 except in pea YS and wheat MS-amended soils. As in previous phases, the P_{resin} concentration in young shoot and canola MR-amended soils was higher than the control. In contrast, in mature shoot-amended soils in phase 4, P_{resin} concentration was not different from the control except in canola MS-amended soil where it was still lower. In agreement with crop periods 2 and 3, this suggests that addition of residues with high total P concentration ($\geq$ 0.16% P) can provide higher P_{resin} concentration than the control even more than 2 months after residue addition. Over time, P_{resin} concentration increased in soils amended with mature shoot residues which had low total P concentration and high C: P ratio and this occurred probably due to decrease of net P immobilization. The lowest P_{resin} concentration in canola MS-amended soil in all phases is most likely due to the low total P concentration (0.05%) and high C: P ratio (858: 1) in canola MS residue. Hence, in order to add 10 mg P kg^{-1} soil, the greatest amounts of C were added with canola MS residues. The highest available P in soil amended with residues with high total P concentration and low C: P ratio after 2 months of residue addition is also in agreement with the previous studies (Dalal 1979; Nziguheba *et al.* 1998). Hence, P_{resin} concentration up to day 63 was significantly

positively correlated with residue total P concentration and negatively correlated with residue C: P ratio and the correlations were stronger than in the earlier phases. In phase 4, the lower P_{resin} concentration in TSP-amended soils compared to the earlier phases can be explained by plant and microbial P uptake and P fixation to a non-resin extractable pool. Compared to crop period 1 and 2, plant DM in young and mature shoot-amended soils was higher in crop period 3 probably due to decrease in microbial competition. Compared to the control, plant DM in young shoot and canola MR-amended soils was higher but in mature shoot-amended soils it was lower even 84 days after residue addition. This suggests that even after more than 2 months differences in total P concentration and/or C addition rates of the residues determine P availability. Hence, plant DM was positively correlated with residue total P concentration and the correlations were stronger than crop period 2 (Table 5.10). The increase of P_{resin} and plant P uptake in young and mature shoot-amended soils compared to the earlier crop periods suggests that net P mineralization exceeded plant P uptake. Compared to the control, higher plant growth in canola MR-amended soil with lower P uptake indicates better P utilization by the plant. Previous studies (Dalal 1979; Fuller *et al.* 1956) also reported that plant growth and plant P uptake is higher in soils amended with residues that have high total P and low C: P ratio even after 70-98 days of residue addition. But in these previous studies plants were grown continuously, thus P could have been taken up earlier, stored in the plant and then utilized for growth later. In the present study, plants were grown for 28 days in each crop period because plants generally have higher P demand in young and vegetative stage than in the older stage. Thus plant P demand was similar in the different phases of decomposition. The higher plant DM in cop period 3 than in the earlier crop periods suggests that P was still being released from crop residues and could be taken up by plants.

In phase 4, total P[1] in the three measured pools (P_{resin}, P_{mic} and plant P uptake) ranged between 1.6 mg P pot^{-1} to 7.8 mg P pot^{-1}. Total P in these pools was greater in mature shoot and canola MR-amended soils (4.0 to 7.8 mg P pot^{-1}) than young shoot and TSP-amended soils (2.1 to 4.0 mg P pot^{-1}). However, about 32%-90% of this total P in the residue-amended soils was microbial P. In this phase, of the P added initially, approximately 65%-86% in young shoot and canola MR-amended soils, 32%-61% in mature shoot-amended soils and 80% in TSP-amended soil were not detected in the measured pools and must have therefore entered to other soil P pools. Thus, it appears that a large amount of P added with residues and TSP is left in the soil as residual P even after more than 2 months of addition and the amount of residual P was lower in the earlier crop periods than the last period.

From the results of this study, it appears that the residues with high total P and N content were decomposed faster and resulted in higher plant P uptake compared to residues with low P and N content. In addition, it can be hypothesised that addition of residues may also increase solubilization of soil P, via production of phosphatase and carboxylates during the decomposition which can be taken up by the microbial biomass and plants. Decomposition of residues varied depending on the plant parts and maturity. Young shoot and canola mature root decomposed faster than the mature shoot. On the other hand, a large part of added P is immobilized from the mature residue-amended soils but immobilization decreased with time and there is potential to increase available P after turnover of microbial P. Hence, P added with young shoots and canola MR residues which have high P concentration and low C: P ratio can be mineralized quicker than mature shoot residues and therefore, may increase available P, plant P uptake and plant DM for standing crop or in the following crops. However, net available P may increase only if P mineralization/solubilization from residues and soil exceeds microbial

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[1] The percentage of residue P recovered was calculated by subtracting the control value (P_{resin}, P_{mic} and Plant P uptake) from the treatments value.

P demand which occurred earlier in high P content residues than in low P content residues. In this study, C added with residues controlled P dynamics. Addition of large amount of C with residues led to increased microbial activity and resulted in higher microbial growth and microbial P uptake. Hence, if same amount of C was added with the residue microbial P demand is similar in all residue treatments because the amount of C determines microbial activity and P demand. However, if the decomposability of the C in the residues differs, there may still be differences between residues. Hence, decomposability of residues is likely to be more important than observed in the present experiment. The amount of residue available to be added to a soil is a function of the plant species, yield, harvest index and residue management. Rarely a grower would get the opportunity to add residues based on equivalent nutrient rate mainly because residues have low bulk densities and are costly to transport. Furthermore, residue addition based on the same amount of C could be more realistic because the C concentration of the residues varies little and farmers are likely to add the same amount of residues. Therefore, a further investigation on residue decomposition rate and P availability and consequently plant P uptake is required with same amount of C addition to elucidate the value of residue addition on soil P dynamics.

Chapter 6

Effect of residue quality on decomposition rates, soil P dynamics and plant P uptake. II. Residue addition based on the same amount of C

6.1 Introduction

In the study described in Chapter 5, it was found that available P in the residue-amended soil was significantly correlated with the P concentration but the effect of C: P ratio could not be separated from the rate of C added per unit mass soil. Therefore in this chapter an experiment is described in which residues were added at the same amount of C rate per unit mass soil. Soil microbial activity, microbial P uptake and residue decomposition rates differed among residues and were related to amounts of C added with the residues. Thus different amount of C added will result in differential growth of microbial biomass and hence P immobilization potential; i.e. low P availability after addition of residues with high C addition rate kg^{-1} soil.

Few studies have been conducted on residue C mineralization with a constant amount of C added, using a limited number of substrates or crop residues. In a study, comparing the mineralization rates of three different substrates (e.g. mucilage, root residues and glucose) with two rates of C addition (200 and 400 mg C kg^{-1} soil), the mineralization of added C was not affected by C addition rate but differed among substrates (Mary *et al.* 1993). Addition of different types of residues (eg. leaves, shoot and root) of the same plant species at a constant amount of C (2.0 g C kg^{-1} soil) led to differences in C mineralization (Bertrand *et al.* 2006) which may have affected P availability as different amounts of P were added.

Bünemann *et al.* (2004) reported that P was initially immobilized in soils after addition of residues (0.06%-0.24% P) based on similar amount of C added (2.5 g C kg^{-1} soil) but in other studies it was shown that addition of residues with higher P content than the critical level of 0.2% has the potential to increase P availability (Fuller *et al.* 1956; Kwabiah *et al.* 2003b). Therefore, further studies are important to clearly understand the effect of C addition rate on P dynamics.

Roots can represent an important fraction of residue input, but are usually not included in studies with residues. Moreover, roots are a source of C and other nutrients even when shoots are removed after harvest. Therefore, it is important to understand the effect of root residues on P dynamics.

In order to better understand the role of residue chemistry (C compounds and nutrient content) it is expedient to add them at the same rate of C. Therefore, this study was conducted to (a) investigate the effect of C chemistry and P content on decomposition of residues and P dynamics in soil when residues were added based on the same amount of C, (b) evaluate growth and P uptake of wheat in different stages of the decomposition process and (c) compare the results obtained in this experiment with those of the previous experiment where residues were added based on the same amount of P.

6.2 Materials and methods

For this study, the soil was collected from the same location as the soil used in the previous experiment. The description of the soil is given in section 5.2. The soil for the present study was collected in December 2005. Soil samples were collected from 0-20 cm soil depth from two different locations within the paddock and thoroughly mixed to give a composite sample. Plant

debris and stones were removed from the soil. After air-drying, the soil was sieved to 2 mm. The properties of soil are shown in Table 6.1.

For this study, the soil was pre-incubated at 80% water holding capacity for 13 days at 18°C/24°C before the start of the actual experiment. As mentioned before, this pre-incubation was done in order that the initial flush of microbial activity and microbial P occurred before the start of the experiment (Brookes *et al.* 1982) and to ensure that the microbial activity reached a steady state after rewetting (Brookes *et al.* 1982; Oehl *et al.* 2001b).

Table 6.1 Soil properties

Soil properties			
Sand (%)	74	Total Organic Carbon (%)	0.62
Silt (%)	5	Total N (%)	0.02
Clay (%)	21	Total P (%)[a]	0.012
pH_w (1:5)	7.7	P_{resin} (mg kg^{-1})[b]	1.2
pH $_{CaCl2}$ (1: 5)	7.2	P_{mic} (mg kg^{-1})[b]	2.1
EC (dS m^{-1})	0.4	Exchangeable Ca (cmol$_c$ kg^{-1})	6.0
Water holding capacity at –10kPa (%)	14	Exchangeable Mg (cmol$_c$ kg^{-1})	0.45

soil properties without notation were estimated by MIR (Janik *et al.* 2007)

[a] soil total P concentration determined after Hanson (1950)

[b] soil P_{resin} and P_{mic} concentration measured from Bünemann *et al.* (2004)

Eight crop residues (Table 6.2) including 6 from the previous study (Table 5.2) were used: canola (*Brassica napus* L.), pea (*Pisum sativum* L.), lupin (*Lupinus albus* L.) and wheat (*Triticum aestivum* L.) with 3 young shoot, 2 mature shoot and 3 mature root residues. The use of the same residues as the study presented in Chapter 5 allowed comparison across the results of the two studies. Lupin and wheat roots were additionally included to determine if the

ability of canola roots to increase the P availability substantially as measured in Chapter 5, could be re-produced with roots of other species, i.e. if the strong increase in P availability is a property of roots in general or only canola roots. Crop residues were incorporated at a rate of 2.5 g C kg^{-1} soil. Due to a calculation error, the 3 rates of Triple Super Phosphate (1, 2, and 4 mg P kg^{-1} soil) added covered the lower range of P addition rates with residues but not those where more than 10 mg P kg^{-1} soil were added. Crop residues and TSP were mixed thoroughly with the pre-incubated soil. An unamended control treatment was also included and mixed thoroughly as per the amended treatments.

Table 6.2 Crop residues and P addition to achieve a rate of 2.5 g C kg^{-1} soil and TSP addition to cover the range of P availability in the residue treatments.

Residues and TSP	Amount of added residue and TSP (g DM kg^{-1} soil)	P added (mg kg^{-1} soil)
Canola YS	6.08	9.9
Pea YS	5.85	18.9
Lupin YS	5.82	11.7
Lupin MS	5.70	3.3
Wheat MS	5.74	4.1
Canola MR	5.82	13.0
Lupin MR	5.61	4.6
Wheat MR	6.14	3.7
TSP-1P	0.005	1.0
TSP-2P	0.01	2.0
TSP-4P	0.02	4.0

YS, Young shoot; MS, Mature shoot; MR, Mature root

Nutrients except P were added to the residue and TSP-amended soils, and the unamended control soil, at the same amounts as in the study described in Chapter 5 (Table 5.3). In this study, 1.12 kg of amended and unamended moist soil was placed into a plastic pot of 11.5 cm height and 12.0 cm diameter. The experimental design with the 3 crop periods was similar to

that described in Chapter 5, Five pre-germinated seeds of wheat were transplanted in each pot at 1-1.5 cm depth at the start of each crop. The seedlings were thinned to 3 per pot 4 days after transplanting. All pots were incubated in the glass house at 10°C/18°C, 8 h night/16 h day and a light intensity of 25 to 5000 lux. The pots were watered daily with RO (reverse osmosis) water in order to maintain 85% water holding capacity. All pots were arranged in a completely randomized block design with 4 replicates and re-randomized every 2 weeks.

Soil respiration rates in the residue and TSP amended and unamended control soils were determined in soil samples incubated separately. After mixing the soils as described above, 23.0 g of moist soil with 3 replicates per treatment were incubated in sealed glass jars at 25°C and respiration was measured using the infrared gas analyzer (see section 3.2.1.3).

During incubation, soil samples were taken from each pot with the aid of a stainless steel corer (diameter ~15 mm) on days 7, 17, 28, 35, 45, 56, 63, 73 and 84 for available P (P_{resin}) and microbial P (P_{mic}) measurement, whereas microbial C (C_{mic}) was measured only in soil samples from days 7, 35 and 63. Pots were weighed at each soil coring. The microbial biomass C data on day 35 was highly variable probably due to methodological error and is therefore not presented. On days 28, 56 and 84, soil samples were taken immediately after plant harvest.

Plants of crop period 1, crop period 2 and crop period 3 were harvested on days 28, 56 and 84, respectively (Figure 5.1) by cutting the shoot at the soil surface. Roots were carefully removed from the soil and washed in order to remove the adhering soil. Shoots and roots were dried at 60°C for 7 days, the dry weight recorded and ground for P determination (see section 3.2.2).

6.3 Results

6.3.1 Residue properties

The chemical and biochemical properties of crop residues were variable depending on age and plant part (Table 6.3). The total P concentration of the residues ranged from 0.6 mg P kg^{-1} DM in lupin MS and wheat MR to 3.2 mg P kg^{-1} DM in pea YS. The total P concentration was greater in young shoots than mature shoots. The total P concentration in canola residue was smaller in young shoots than in mature roots. In lupin on the other hand, the total P concentration of young shoots was greater than in mature roots and mature shoots. Among the young crop residues, pea YS had the highest total P (3.2 g kg^{-1} DM) while canola YS had the lowest (1.6 g kg^{-1} DM). There was no significant difference in total P concentration between lupin MS and wheat MS. Among the mature root residues, total P in canola MR (2.3 g kg^{-1} DM) was significantly higher than in the other root residues (0.6-0.8 g kg^{-1} DM). Water-soluble P (P_{H2O}) in the residues ranged from 0.1 g kg^{-1} DM (lupin MS) to 1.3 g kg^{-1} DM (canola YS). Water-soluble P in residues was positively correlated with the total P (r^2 =0.69, $P \leq$ 0.01), representing 13% (lupin MR) to 81% (canola YS) of total P.

The variation in total C concentration of the crop residues was small and ranged from 405 g kg^{-1} DM in wheat MR to 445 g kg^{-1} DM in lupin MR. Total C was significantly higher in mature shoots than the young shoots. On the other hand, water soluble C (C_{H2O}) in the residues decreased in the following order: young shoot > mature shoot ≥ mature root and ranged from 5.1 g kg^{-1} DM (wheat MR) to 140 g kg^{-1} DM (lupin YS). The C: P ratio of crop residues ranged from 133 (pea YS) to 781 (lupin MS) and was 2-6 times lower in the young shoots than the mature shoots. In lupin, the C: P ratio increased in the order: lupin YS < lupin MR < lupin MS whereas the C: P ratio in canola was in the order of canola MR < canola YS. Total N concentration was highest in pea YS (35.8 g kg^{-1} DM) and lowest in wheat MS (5.4 g kg^{-1} DM).

In lupin and canola, the total N concentration decreased in the following order: young shoot > mature root > mature shoot. The C: N ratio of the residues ranged from 12 for pea YS to 80 for wheat MS and was smaller in the young shoots than the mature shoots and roots. Water-soluble N (N_{H2O}) in the residues ranged from 0.7 g kg^{-1} DM (wheat MR) to 12.6 g kg^{-1} DM (lupin YS) and the N_{H2O} in young shoots was 2-19 times higher than the mature shoots and roots. Water-soluble N in the residues was significantly positively correlated with total N concentration of residues (r^2 =0.75, $P \leq 0.005$) and represented 11% to 57% of the total N in wheat MR and lupin YS respectively.

The biochemical properties of residues, determined by NMR spectroscopy, varied greatly between plant parts. The lignin content of residues generally increased in the following order: young shoots < mature shoots < mature roots, it was highest in wheat MR (48.7 g kg^{-1} DM) and lowest in lupin YS (27.7 g kg^{-1} DM). The O-alkyl content (mainly cellulose) of the residues ranged from 11.5 g kg^{-1} DM in wheat MR to 59.0 g kg^{-1} DM in pea YS. Aryl-C compounds representing mainly phenolics ranged from 11.4 g kg^{-1} DM to 22.3 g kg^{-1} DM in lupin YS and lupin MR, respectively. Similar to lignin content of residues, phenolic compounds increased in the following order: young shoots < mature shoots < mature roots and the content of phenolic compounds was positively correlated with lignin (r^2 =0.86, $P \leq 0.001$). The amide content ranged from 14.6 g kg^{-1} DM (wheat MR) to 46.5 g kg^{-1} DM (pea YS) and was strongly positively correlated with O-alkyl-C (r^2 =0.85, $P \leq 0.001$). There was no significant correlation between biochemical compounds and total C of the residues; but C_{H2O} was negatively correlated with lignin (r^2 =0.83, $P \leq 0.01$) and phenolic compounds (r^2 =0.74, $P \leq 0.01$), and weakly positively correlated with amide content (r^2 =0.51, $P \leq 0.05$). The O-alkyl and amide contents were strongly positively correlated with total N content of the residues (r^2 =0.92, $P \leq 0.001$ and r^2 =0.91, $P \leq 0.001$, respectively) but moderately positively correlated with N_{H2O} (r^2 =0.74, $P \leq$

0.006 and r^2 =0.81, $P \leq$ 0.002, respectively). On the other hand, lignin and phenolic compounds were negatively correlated with N_{H2O} (r^2 =0.75, $P \leq$ 0.005 and r^2 =0.65, $P \leq$ 0.015, respectively).

Table 6.3 Chemical and biochemical properties of crop residues determined by chemical analysis and NMR spectroscopy

Residues	Total P	Total C	Total N	P_{H2O}	C_{H2O}	N_{H2O}	Lignin[NMR]	O-alkyl	Phenolic	Amide	C: P	C: N
					g kg^{-1} DM							
Shoots												
Canola YS	1.6 b	411.0 b	14.6 c	1.3 e	63.4 d	8.0 d	31.5	23.0	14.9	27.2	253 c	28 c
Pea YS	3.2 d	427.7 c	35.8 f	1.1 c	69.7 e	12.1 e	33.1	59.0	12.4	46.5	133 a	12 a
Lupin YS	2.0 c	429.3 c	21.9 e	1.2 d	140.0 f	12.6 e	27.7	41.5	11.4	40.1	213 b	19 b
Lupin MS	0.6 a	438.8 d	14.4 c	0.1 a	32.4 c	3.6 bc	40.6	23.0	18.7	31.3	781 f	30 c
Wheat MS	0.7 a	435.3 d	5.4 a	0.2 b	24.5 b	1.6 a	38.9	21.9	16.3	17.0	615 e	80 f
Roots												
Canola MR	2.3 c	429.4 c	11.4 b	1.1 c	9.7 a	2.2 ab	45.5	21.1	19.8	24.7	192 b	38 d
Lupin MR	0.8 a	445.3 e	16.8 d	0.1 ab	21.1 b	5.1 c	44.4	31.8	22.3	29.3	535 d	28 c
Wheat MR	0.6 a	405.2 a	6.2 a	0.2 b	5.1 a	0.7 a	48.7	11.5	20.4	14.6	634 e	64 e

YS, MS and MR indicated Young Shoot, Mature Shoot and Mature Root respectively. Within columns, means followed by the same letter are not significantly different ($P < 0.001$) by Tukey's multiple range tests. [NMR] NMR spectroscopy was carried out on one composite sample (n=1).

6.3.2 Soil Respiration

The respiration rates in soils amended with residue and TSP or the control varied over time. During the first 4 days after treatments were applied, the respiration rates decreased in the order: young shoots > mature shoots and roots > TSP and control (Figure 6.1). Residue effects on respiration rates appeared to fit into three patterns; rapid initial peak of CO_2 production (< 4 days), followed by short exponential decay to background levels (4-40 days) in all young shoot-amended soils and in lupin MS-amended soil, medium initial CO_2 production (< 4 days) with a peak between 4 and 14 days, and then longer exponential decay to background (14-40 days) in wheat MS-amended soil and in all mature root-amended soils and stable background CO_2 production from TSP-amended soils and the control. On day 1, the respiration rates in residue-amended soils were highest in lupin YS-amended soil (310 mg C kg^{-1} soil day^{-1}) and lowest in wheat MR-amended soil (20 mg C kg^{-1} soil day^{-1}). Respiration rates decreased more rapidly in young shoot-amended soil compared to mature shoot and root-amended soil. By day 10, respiration rates had decreased by 56-89% in young shoot-amended soils with the decrease being least pronounced in canola YS-amended soil. Respiration rates in mature shoot and root-amended soils except wheat MR decreased by 12%-53% of the values at the start by day 10. In contrast to the other residues, respiration rate in wheat MR-amended soil was still 62% on day 17. On day 29, the respiration rates were in the order of mature shoots and roots ≥ young shoots > TSP and control. In the residue-amended soils, the respiration rates on day 29 were highest in wheat MS amended soil (11.1 mg C kg^{-1} soil day^{-1}) and lowest in pea YS-amended soil (6.2 mg C kg^{-1} soil day^{-1}). Compared to day 1, the respiration rates in young shoot-amended soil decreased by 92%-98% by day 29, while in mature shoot and root-amended soils except wheat MR, the respiration rates had decreased by 81%-89% of the initial rates. In wheat MR-amended soil, the decrease in respiration rates compared to the initial rates was 49%. After day 29, the differences of respiration rates in residue-amended soils were smaller and

often not significant. By day 57, the respiration rates had decreased in residue-amended soils by 91%-99% of initial respiration rates with exception of wheat MR where the decrease was only 68%. Between day 57 and the end of the incubation, the respiration rates fluctuated but there were no consistent significant differences between the treatments.

The cumulative CO_2 release from residue and TSP-amended soils and the unamended control can be divided into 3 groups (Figure 6.2): young shoots > mature shoots and roots > TSP and unamended control. The cumulative CO_2 release in residue-amended soils up to day 10 was highest in lupin YS -amended soil (1091 mg C kg^{-1} soil) and lowest in wheat MR-amended soil (289 mg C kg^{-1} soil). The cumulative CO_2 release up to day 17 in the mature shoot-amended soils was significantly higher in lupin MS-amended soil (808 mg C kg^{-1} soil) than in wheat MS-amended soil (734 mg C kg^{-1} soil). But the cumulative CO_2 release in the mature root-amended soils from day 17 onwards was in the order: canola MR > lupin MR > wheat MR. There were no significant differences between the mature shoot treatments until day 84. From day 51 to 84, the cumulative CO_2 release in lupin YS and canola YS-amended soils was higher than in pea YS-amended soil. In general, the cumulative CO_2 release from the lupin residue-amended soils ranked in the order lupin YS > lupin MS > lupin MR. There were no significant differences between TSP-amended soils and the unamended control.

The decomposition of C added with young shoot residues was faster than the mature shoots and roots (Table 6.4). By day 10, 31%-41% of added C was decomposed from young shoot-amended soils, while only 9%-21% of added C was decomposed from mature shoot and root-amended soils. By day 29, 50% of added C with lupin YS residue was decomposed, while only 22% of added C with wheat MR was decomposed. After that the rate of decomposition was slower. On day 57, 44%-52% and 29%-36% of C added with young shoots and mature roots

respectively had decomposed. On day 84, the decomposition of C added with young shoot, mature shoot and root residues was 46%-54%, 41% and 32%-39% respectively. Thus, at the end of incubation, the decomposition of C added with young shoots was about 1.3 to 1.7 times higher than mature shoot and root residues. As mentioned in section 3.3, it was assumed in this calculation that there was no priming effect.
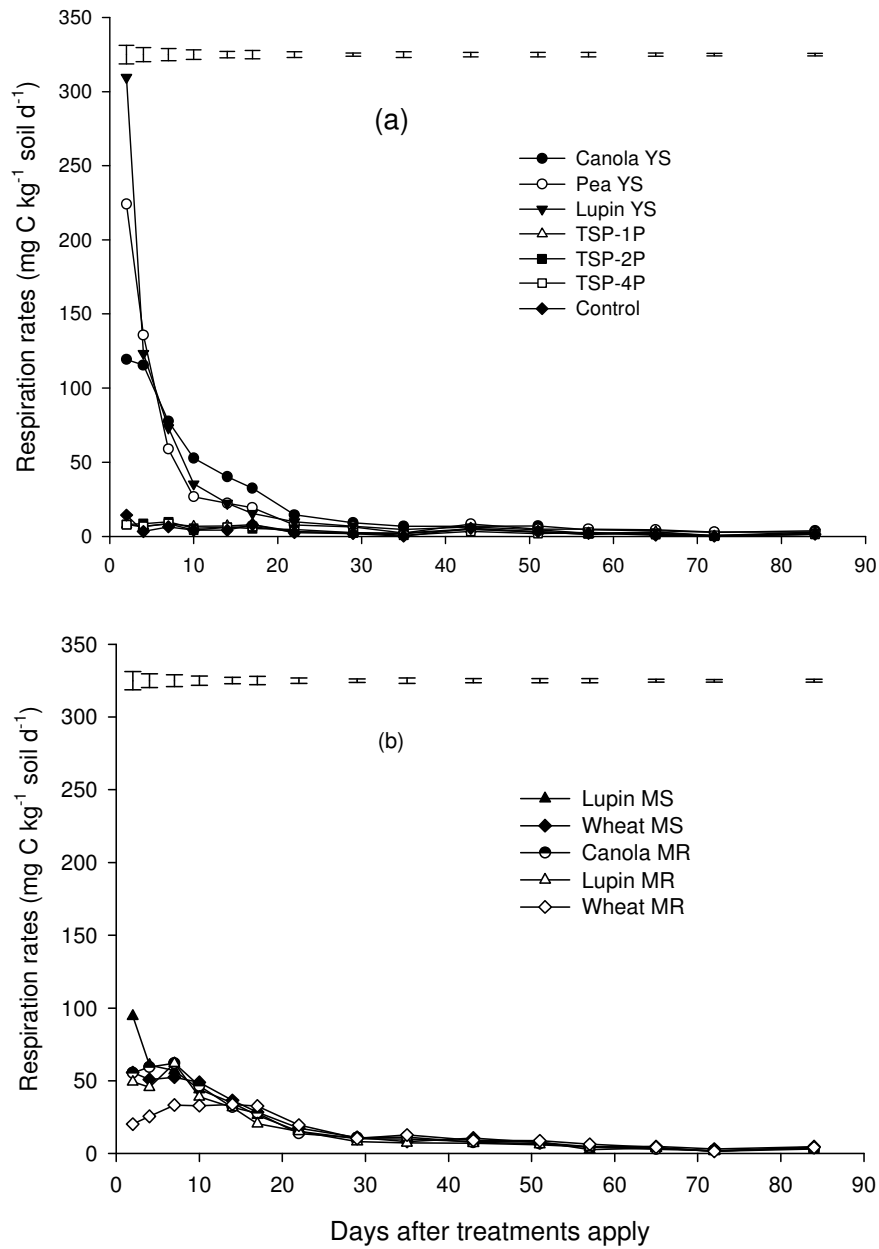
Figure 6.1 Respiration rates in soils amended with different crop residues; young shoot (a), mature shoot and root (b), TSP and unamended control over time. Means of 4 replicates, vertical bars indicate least significant difference.
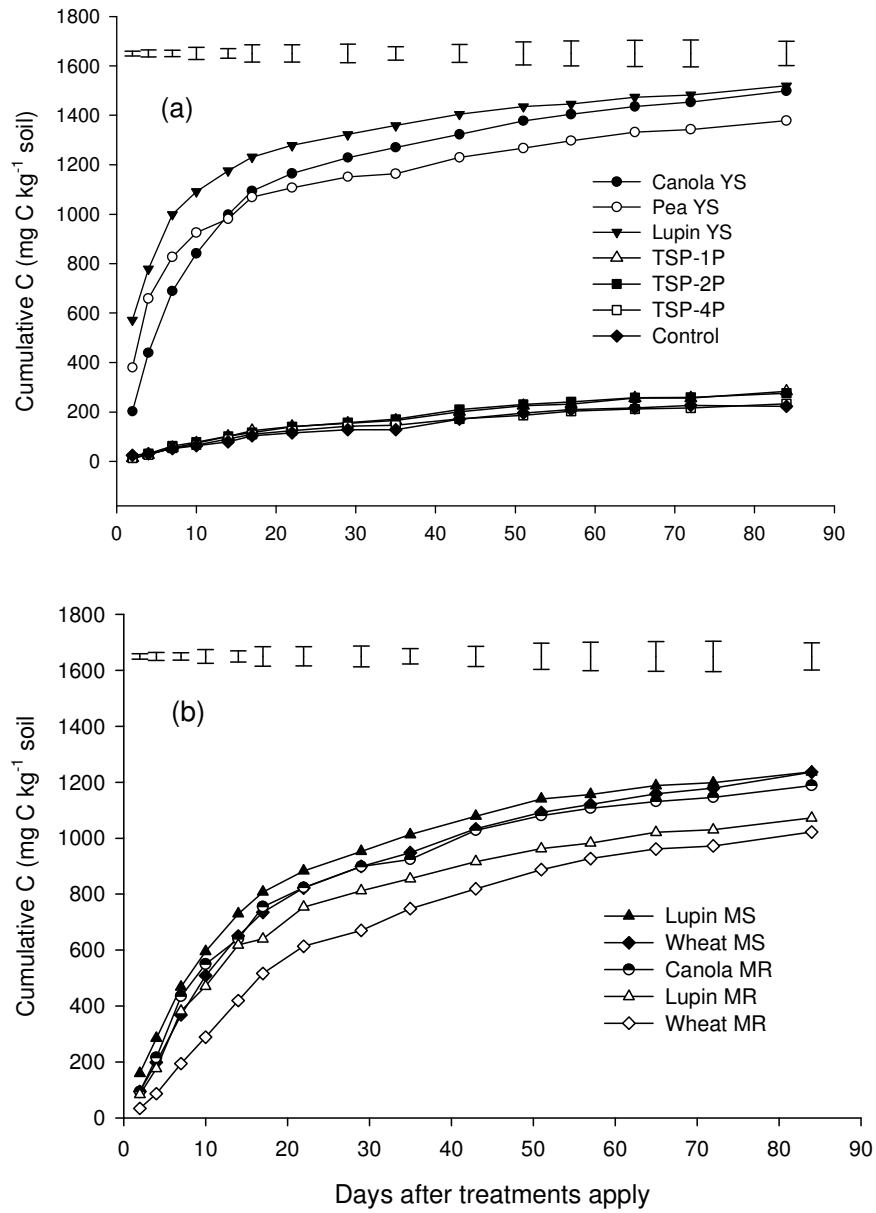
Figure 6.2 Cumulative C released from soils amended with different crop residues; young shoot (a), mature shoot and root (b), TSP and unamended control over time. Means of 4 replicates, vertical bars indicate least significant difference (LSD$_{0.05}$).

Table 6.4 Percentage of added C decomposed

Treatments	Day 7	Day 29	Day 57	Day 84
Canola YS	25.5 d	44.0 cd	47.8 c	51.0 c
Pea YS	31.9 e	40.1 c	43.5 bc	46.2 bc
Lupin YS	38.1 f	50.3 d	52.0 c	54.0 c
Lupin MS	16.7 c	33.0 b	38.0 ab	40.6 ab
Wheat MS	12.7 b	30.1 b	36.5 ab	40.5 ab
Canola MR	14.6 bc	30.1 b	36.0 ab	38.6 ab
Lupin MR	12.3 b	25.2 ab	29.0 a	32.1 a
Wheat MR	5.7 a	21.7 a	28.7 a	32.0 a

YS, MS and MR indicate Young Shoot, Mature Shoot and Mature Root respectively. Within columns, means followed by the same letter are not significantly different (P < 0.001) by Tukey's multiple range test.

6.3.3 Available P (P_{resin}) concentration in soil

The concentration of available P (P_{resin}) in the residue and TSP-amended soil and unamended control was variable over the three crop growing periods (Table 6.5). The average P_{resin} concentration during 3 consecutive crop growing periods was significantly higher in canola YS, pea YS and canola MR-amended soils than the other residue-amended soils and the control. On the other hand, the average P_{resin} concentration in mature shoot-amended soils was always lower than the control.

During crop period 1 (D0-D28), the average P_{resin} concentration was highest in pea YS-amended soils (3.1 mg P kg^{-1} soil) and lowest in wheat MS-amended soil (0.2 mg P kg^{-1} soil). The average P_{resin} concentration in young shoot and canola MR-amended soils was 0.8-3.1 mg P kg^{-1} soil, which was 1.5 to 5.6 fold higher than the control. Compared to the control, the average P_{resin} concentration in lupin and wheat mature shoot and root-amended soils were 56%-66% and 14%-44% lower, respectively. In the root residue-amended soils, the average P_{resin} concentration was 4-5-fold higher in the canola MR-amended soil compared to lupin and wheat mature root-amended soils. In TSP-amended soils, the average P_{resin} concentration was highest in TSP-4P-amended soil (1.3 mg P kg^{-1} soil) which was 139% higher than the control but 36% to 59% lower than canola MR and pea YS-amended soils, respectively.

During crop period 2 (D28-D56), the average P_{resin} concentration was highest in pea YS-amended soil (2.5 mg P kg^{-1} soil) and lowest in wheat MS-amended soil (0.5 mg P kg^{-1} soil) although the average P_{resin} concentration in wheat MS-amended soil was significantly greater (2.4 fold) than in crop period 1. Compared to the control, the average P_{resin} concentration was 1.4 to 3.8 fold higher in the young shoot-amended soil and 1.3-1.8 fold higher in the TSP-amended soils whereas it was 30% lower in the mature shoot-amended soils. The average

P_{resin} concentration in the root-amended soils was greater in canola MR-amended soil than in wheat and lupin MR-amended soils (2.7 to 3.1 fold) and the control (2.8 fold). In crop period 2, the average P_{resin} concentration in the TSP-4P amended soil was 35% to 51% lower than in canola MR and pea YS-amended soils, respectively.

In crop period 3, the ranking among the treatments was similar to in crop period 2. Compared to the control, the average P_{resin} concentration was significantly higher only in canola YS, pea YS and canola MR-amended soils (2.1 to 4.1 fold). The average P_{resin} concentration was 11%-21% lower in wheat and lupin mature shoot-amended soils than the control, respectively. In root-amended soils, the average P_{resin} concentration in canola MR-amended soils was 2.8-3.2 fold higher than in lupin and wheat MR-amended soils. The average P_{resin} concentration in the TSP-amended soils was 64%-69% and 51%-57% lower than in pea YS and canola MR-amended soils, respectively. In crop period 3, the P_{resin} concentration decreased over time only in canola YS, pea YS, canola MR and TSP-4P-amended soils.

6.3.4 Microbial P (P_{mic}) concentration in soil

During the 3 crop periods, the average microbial P (P_{mic}) concentration in residue and TSP-amended soil and unamended control was higher than the P_{resin} concentration by a factor of 2 to 30 (Table 6.6). In general, the difference between the average P_{mic} concentration and P_{resin} concentration were greatest in lupin MS-amended soils (6.0 mg P kg^{-1} soil) and smallest in the TSP-amended soils and control (2.0-2.5 mg P kg^{-1} soil). The differences in average P_{mic} concentration between the residue-amended soils were less pronounced than average P_{resin} concentration.

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During crop period 1, the average P_{mic} concentration was highest in the pea YS-amended soil (7.2 mg P kg^{-1} soil) and lowest in the control (2.7 mg P kg^{-1} soil). Compared to the control, the average P_{mic} concentration was greater in the residue-amended soils (2.0 to 2.7 fold) than in the TSP-amended soils (1.1 to 1.7 fold). The average P_{mic} concentration in lupin and wheat MS-amended soils was 15%-27% greater than in the lupin and wheat MR-amended soils but 8%-14% lower than in canola MR-amended soil. In crop period 1, P_{mic} concentration in all residue and TSP-amended soils was higher at day 7 than at days 17 and 28.

The average P_{mic} concentration in crop period 2 ranged from 2.7mg P kg^{-1} soil (control) to 6.7 mg P kg^{-1} soil (pea YS) and was 3 to 12 fold higher than the P_{resin} concentration. Compared to crop period 1, the average P_{mic} concentration in the residue-amended soils in crop period 2 decreased by 3%-13% except in wheat MR-amended soil. Compared to the control, the average P_{mic} concentration was 1.7 to 2.2 fold higher in residue-amended soil while only 1.2 fold higher in the TSP-amended soils. The average P_{mic} concentration in lupin and wheat mature shoot-amended soils was 1.1 fold higher than in mature root-amended soils.

In crop period 3, the average P_{mic} concentration decreased by 2%-19% compared to crop period 2 and ranged from 2.4 mg P kg^{-1} soil in the control to 6.2 mg P kg^{-1} soil in the canola MR-amended soil. The average P_{mic} concentration was 2.4 to 10.2 times higher than the average P_{resin} concentration during the crop period 3. Compared to the control, the average P_{mic} concentration was higher in the residue-amended soils (1.8 to 2.6 fold) than in the TSP-amended soils (1.2 to 1.4 fold).

Table 6.5 Available P (P_{resin}) in soils amended with different crop residues, TSP and an unamended control over time

Treatments	D7	D17	D28	Average D0-D28	D35	D45	D56	Average D28–D56	D63	D73	D84	Average D56-D84
							mg P kg^{-1} soil					
Canola YS	1.2 ef	0.9 d	1.3 de	**1.1 ef**	1.6 de	1.6 c	0.8 de	**1.3 c**	1.4 c	1.3 cd	1.2 d	**1.3 b**
Pea YS	3.4 h	3.1 g	2.8 f	**3.1 h**	3.6 g	2.6 d	1.3 g	**2.5 e**	2.5 d	2.6 e	2.3 e	**2.5 d**
Lupin YS	0.5 bc	0.9 d	1.1 de	**0.8 cde**	1.1 bc	0.9 abc	0.7 bcd	**0.9 abc**	0.9 abc	1.0 abcd	0.9 cd	**0.9 ab**
Lupin MS	0.2 a	0.2 ab	0.3 a	**0.2 ab**	0.6 a	0.4 a	0.4 ab	**0.5 a**	0.6 a	0.4 a	0.4 a	**0.5 a**
Wheat MS	0.1 a	0.2 a	0.3 a	**0.2 a**	0.5 a	0.5 a	0.4 a	**0.5 a**	0.5 a	0.6 ab	0.5 ab	**0.5 a**
Canola MR	2.5 g	2.1 f	1.5 e	**2.0 g**	2.2 f	2.5 d	1.1 f	**1.9 d**	2.5 d	2.1 e	0.8 bc	**1.8 c**
Lupin MR	0.3 ab	0.3 ab	0.6 ab	**0.4 ab**	0.9 abc	0.7 ab	0.5 abcd	**0.7 abc**	0.7 ab	0.6 ab	0.7 abc	**0.6 a**
Wheat MR	0.7 c	0.3 abc	0.5 a	**0.5 abc**	0.8 ab	0.5 a	0.5 abcd	**0.6 ab**	0.7 ab	0.6 abcd	0.4 a	**0.6 a**
TSP-1P	0.7 cd	0.6 c	1.0 bcd	**0.8 cd**	1.1 bc	0.9 abc	0.7 bcd	**0.9 abc**	0.8 ab	1.3 d	0.5 abc	**0.9 ab**
TSP-2P	1.0 de	1.0 d	0.9 bcd	**0.9 de**	1.2 cd	0.9 abc	0.5 abc	**0.9 abc**	0.8 ab	0.8 abcd	0.7 abc	**0.8 a**
TSP-4P	1.3 f	1.6 e	1.0 cd	**1.3 f**	1.7 e	1.3 bc	0.8 cde	**1.2 bc**	1.1 bc	1.1 bcd	0.5 ab	**0.9 ab**
Control	0.6 c	0.5 bc	0.6 abc	**0.6 bc**	0.9 abc	0.6 ab	0.5 abcd	**0.7 ab**	0.6 ab	0.6 abc	0.6 abc	**0.6 a**

YS, MS and MR indicated Young Shoot, Mature Shoot and Mature Root respectively. Within columns, means followed by the same letter are not significantly different ($P < 0.001$) by Tukey's multiple range tests.

Table 6.6 Microbial P (P$_{mic}$) in soils amended with different crop residues, TSP and an unamended control over time

Treatments	D7	D17	D28	Average D0-D28	D35	D45	D56	Average D28–D56	D63	D73	D84	Average D56-D84
							mg P kg^{-1} soil					
Canola YS	7.4 de	6.8 cd	6.5 cd	**6.9 ef**	6.5 cd	6.5 d	5.1 d	**6.1 efg**	5.8 d	5.3 cd	4.8 e	**5.3 ef**
Pea YS	7.7 e	6.8 d	7.0 d	**7.2 f**	7.0 de	7.0 d	5.9 e	**6.7 g**	6.7e	6.2 de	4.8 e	**5.9 fg**
Lupin YS	8.0 e	6.0 cd	5.5 b	**6.5 de**	6.4 cd	6.1 cd	5.1 d	**5.9 def**	5.5 cd	5.1 cd	3.8 cd	**4.8 cde**
Lupin MS	7.0 cd	6.1 cd	5.6 bc	**6.2 de**	5.9 bc	6.1 cd	4.9 cd	**5.6 cde**	4.6 b	5.3 cd	4.8 e	**4.9 de**
Wheat MS	6.4 cd	5.7 c	5.4 b	**5.8 cd**	5.4 b	5.1 b	4.4 c	**5.0 bc**	4.9 bc	4.8 cd	4.1 de	**4.6 cd**
Canola MR	7.3 de	6.3 cd	6.6 d	**6.7 ef**	7.4 e	6.2 cd	6.0 e	**6.6 fg**	5.8 d	6.9 e	5.9 f	**6.2 g**
Lupin MR	6.9 cd	4.2 b	5.1 b	**5.4 bc**	5.4 b	5.6 bc	4.7 cd	**5.2 bcd**	4.8 bc	4.3 bc	3.7 cd	**4.3 c**
Wheat MR	4.8 b	4.3 b	4.6 b	**4.6 b**	5.0 b	4.7 b	4.2 c	**4.7 b**	4.3 b	4.3 bc	4.0 d	**4.2 c**
TSP-1P	3.6 a	2.9 a	3.2 a	**3.3 a**	3.2 a	3.4 a	2.9 ab	**3.1 a**	3.1 a	2.9 ab	2.7 ab	**2.9 ab**
TSP-2P	3.2 a	2.3 a	3.0 a	**2.8 a**	3.2 a	3.6 a	3.1 ab	**3.3 a**	2.9 a	3.1 ab	2.2 ab	**2.7 ab**
TSP-4P	3.6 a	2.6 a	3.3 a	**3.1 a**	3.2 a	3.5 a	3.2 b	**3.3 a**	3.3 a	3.2 ab	3.1 bc	**3.2 b**
Control	3.0 a	2.5 a	2.7 a	**2.7 a**	2.7 a	2.9 a	2.5 a	**2.7 a**	2.5 a	2.6 a	2.0 ab	**2.4 a**

YS, MS and MR indicated Young Shoot, Mature Shoot and Mature Root respectively. Within columns, means followed by the same letter are not significantly different ($P < 0.001$) by Tukey's multiple range tests.

6.3.5 Microbial biomass C (C_{mic}) in soil

The microbial biomass C (C_{mic}) was only determined on days 7, 35 and 63 in this study. The temporal changes of C_{mic} did not follow the same trend as P_{mic} concentration (Table 6.7). On day 7, the highest C_{mic} was measured in lupin MR-amended soil (99.4 mg C kg^{-1} soil) and lowest in the control (41.9 mg C kg^{-1} soil). On day 7, compared to the control, C_{mic} was 51%-121% higher in young shoot-amended soils, 49%-96% higher in mature shoot-amended soils and 72%-137% higher in mature root-amended soils while it was only 13%-73% higher in the TSP-amended soils. However, the high C_{mic} in the TSP-4P-amended soils may be due to methodological error. In the young shoot-amended soils, C_{mic} was highest in pea YS-amended soil (92.4 mg C kg^{-1} soil) and lowest in lupin YS-amended soils (63.1 mg C kg^{-1} soil). Within the mature root-amended soils, C_{mic} decreased in the order lupin MR ≥ wheat MR ≥ canola MR. The C_{mic} results on day 35 were highly variable but the C_{mic} in residue-amended soils was comparatively higher than in TSP-amended soil and the control (data not shown). On day 63, in general, C_{mic} was higher in residue-amended soils than the TSP-amended soils and the control. On day 63 compared to day 7, C_{mic} had decreased by 4.0 to 44.2 mg C kg^{-1} soil in nearly all treatments except lupin MS, canola MR-amended soils and the control, where C_{mic} increased by 1%, 23% and 18% respectively. On day 63, C_{mic} was highest in canola MR-amended soil (88.7 mg C kg^{-1} soil) and lowest in TSP-2P-amended soils (27.3 mg C kg^{-1} soil). Compared to the control, C_{mic} was 0.2%-55% higher in the young shoot-amended soils, 2%-27% higher in mature shoot-amended soils and 38%-79% higher in mature root-amended soils, whereas C_{mic} was 13%-45% lower in the TSP-amended soils. C_{mic} in the mature root-amended soils decreased in the following order of canola MR ≥ lupin MR ≥ wheat MR.

On day 7, the C_{mic}: P_{mic} ratio ranged from 8: 1 in the lupin YS-amended soil to 24: 1 in the TSP-4P-amended soil. In general, the C_{mic}: P_{mic} ratio in the residue-amended soils was similar or

lower than in the TSP-amended soils and the control. The C_{mic}: P_{mic} ratio in the young shoot-amended soils was higher in pea YS-amended soil (12: 1) than canola YS (9: 1) and lupin YS-amended soils (8: 1). The C_{mic}: P_{mic} ratio in the root-amended soils increased in the opposite order of P_{mic} with canola MR ≤ lupin MR ≤ wheat MR, while the C_{mic}: P_{mic} ratio in the TSP-amended soils increased in the order of TSP-1P ≤ TSP-2P < TSP-4P. On day 63, the C_{mic}: P_{mic} ratio was lower than on day 7. The C_{mic}: P_{mic} ratio was smaller in TSP-2P (9: 1) and greater in the control (20: 1).

Table 6.7 Microbial C (C_{mic}) and microbial C: P (C_{mic}: P_{mic}) in soils amended with different crop residues, TSP and unamended control at day 7 (D7) and day 63 (D63). Values are means of 4 replicates.

Treatments	Microbial C (mg C kg^{-1} soil)		Microbial C: P	
	D7	D63	D7	D63
Canola YS	68.1 abc	49.6 ab	8.9 ab	9.7 a
Pea YS	92.4 cd	76.8 ab	12.0 abc	11.4 ab
Lupin YS	63.1 abc	53.8 ab	7.6 a	12.1 ab
Lupin MS	62.3 abc	63.0 ab	9.0 ab	13.0 ab
Wheat MS	82.1 cd	50.6 ab	13.0 abc	12.1 ab
Canola MR	71.9 abcd	88.7 b	9.9 ab	15.5 ab
Lupin MR	99.4 d	83.3 b	13.9 bc	17.6 ab
Wheat MR	76.4 bcd	68.6 ab	16.2 c	16.1 ab
TSP-1P	47.3 ab	43.3 ab	13.1 abc	10.8 ab
TSP-2P	47.4 ab	27.3 a	16.6 c	9.5 a
TSP-4P	72.8 abcd	28.6 a	24.5 d	13.9 ab
Control	41.9 a	49.5 ab	16.1 c	19.7 b

YS, MS and MR indicated Young Shoot, Mature Shoot and Mature Root respectively. Within columns, means followed by the same letter are not significantly different ($P < 0.001$) by Tukey's multiple range tests.

6.3.6 Plant shoot and root dry matter

During the three crop periods, shoot and root dry matter (DM) differed significantly ($P < 0.001$) between the residue and TSP-amended soils and the control (Figure 6.3a). Generally, plant DM was lower in crop period 1 than in crop periods 2 and 3. In order to better compare the three crop periods, plant DM is also expressed as percentage of the control (Figure 6.3b).

In crop period 1, plant shoot and root DM was highest in canola MR-amended soil and lowest in lupin MS-amended soil. In general, plant shoot and root DM in young shoot-amended soils was greater than in mature shoot-amended soils. In crop period 1, plant shoot and root DM in TSP-amended soils increased in the order: TSP-1P < TSP-2P < TSP-4P. Compared to the control, plant shoot and root DM were significantly higher in canola YS, pea YS, canola MR and TSP-4P-amended soils with 56%-209% for shoot DM and with 56%-223% for root DM. Plant shoot and root DM were greater in crop period 2 than in crop period 1. Moreover, plant shoot: root DM ratio was wider in crop period 2 than in crop period 1. In crop period 2, plant shoot and root DM were highest in pea YS-amended soil followed by canola MR-amended soil and lowest in wheat MS-amended soil. In crop period 2, plant shoot and root DM in TSP-amended soils increased in a similar order as in crop period 1. In crop period 3, plant shoot: root DM ratio was greater than in crop period 2 and it was 1-2 times greater than in crop period 1. Root DM decreased compared to the previous crops. In crop period 3, plant shoot and root DM were significantly higher in pea YS, canola MR and TSP-4P-amended soil than the control. During the three crop periods, plant shoot and root DM in lupin residue-amended soils were generally in the order of lupin YS > lupin MR > lupin MS while in canola residue-amended soils plant shoot and root DM was greater with canola MR than with canola YS. There were no differences in plant shoot and root DM between mature wheat shoot and root residue-amended soils.
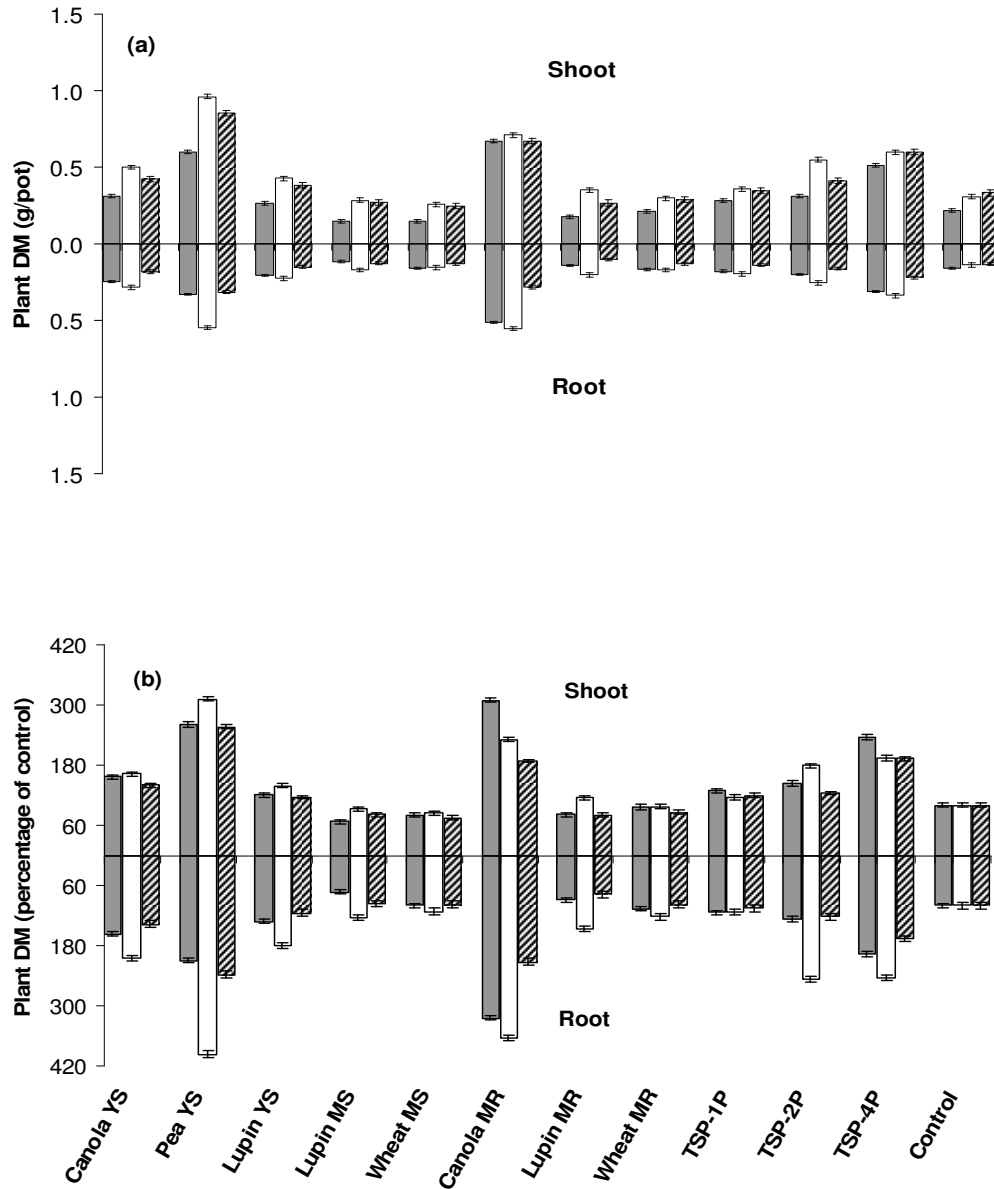
Figure 6.3 Shoot and root dry matter (a) and percentage of control (b) of wheat plants grown in soils amended with crop residues, TSP and unamended control during crop period 1 (gray bars), crop period 2 (white bars) and crop period 3 (diagonally hatched bars). Means of 4 replicates, vertical bars indicate standard error of means (SEM).

6.3.7 Plant shoot and root P concentration

Plant shoot and root P concentration (mg P kg^{-1} DM) differed between soils amended with residues or TSP and unamended control and changed over time (Table 6.8). During the three crop periods, plant shoot and root P concentrations in root residue-amended soils generally decreased in the order of canola MR > lupin MR ≥ wheat MR. Plant shoot and root P concentration followed a different trend than plant shoot and root DM (Figure 6.3). In crop period 1, plant shoot and root P concentrations were highest in pea YS-amended soil, while plant shoot and root DM were highest in canola MR-amended soils. Plant shoot and root P concentrations were lowest in lupin MS-amended soil which also had the lowest plant shoot and root DM. Compared to the control, plant P concentrations in young shoot, canola MR and TSP-amended soils were 10%-166% higher in shoots and 17%-91% higher in roots. On the other hand, plant shoot P and root P concentrations in lupin and wheat mature shoot and root-amended soils were 6%-23% and 7%-33% lower than the control. Compared to TSP-amended soils, plant shoot and root P concentrations in pea YS and canola MR-amended soils were 1.3 to 2.2 fold higher in plant shoots and 1.1-1.6 fold higher in plant roots. In crop period 2, plant shoot P concentration was highest in canola MR-amended soil while plant shoot DM was highest in pea YS-amended soil. On the other hand, plant root P concentration was highest in pea YS-amended soil while plant root DM was similar in pea YS and canola MR-amended soil. In crop period 2, plant shoot and root P concentrations were lowest in wheat MS-amended soil. Compared to the control, plant shoot P concentration in young shoot, canola MR, lupin MR and TSP-amended soils was 5%-85% higher. On the other hand, plant root P concentration was higher than the control only in pea YS, canola MR and TSP-amended soils. Compared to crop period 1, plant shoot P concentration in crop period 2 generally increased 1.1 to 1.4 fold except in pea YS and TSP-4P-amended soils, whereas root P concentration increased 1.1 to 1.2 fold in wheat and lupin mature shoot and root-amended soils and the control. In crop period 3, plant

shoot P concentration was highest in canola MR-amended soil, while plant shoot DM was highest in pea YS-amended soil. On the other hand, both plant root P concentration and root DM were highest in pea YS-amended soil. Plant shoot and root P concentrations were lowest in wheat MS-amended soil. Compared to the control, plant shoot P concentration was 6%-73% higher in residue and TSP-amended soils, except in wheat MS and wheat MR-amended soils. However, root P concentration was higher than the control only in pea YS, lupin YS, canola MR, TSP-2P and TSP-4P-amended soils. In general, plant shoot and root P concentrations in crop period 3 were lower than crop period 2.

Table 6.8 Shoot and root P concentration in g kg^{-1} DM of plant grown in soils amended with different crop residues, TSP and unamended control for 3 crop periods, crop period 1 (D0-D28), crop period 2 (D28-D56) and crop period 3 (D56-D84). Values are means of 4 replicates.

Treatments	Shoot P (g P kg^{-1} DM)			Root P (g P kg^{-1} DM)		
	Crop period 1 (D0-28)	Crop period 2 (D28-56)	Crop period 3 (D56-84)	Crop period 1 (D0-28)	Crop period 2 (D28-56)	Crop period 3 (D56-84)
Canola YS	1.7 ef	2.0 bc	1.5 ab	2.0 fg	1.6 abc	1.3 abc
Pea YS	3.4 h	2.2 c	1.4 ab	2.8 h	1.8 c	1.8 e
Lupin YS	1.4 abcde	1.7 ab	1.3 ab	1.8 ef	1.5 abc	1.4 cd
Lupin MS	1.0 ab	1.3 a	1.1 a	1.0 ab	1.1 a	1.0 ab
Wheat MS	1.0 a	1.3 a	1.0 a	1.0 a	1.1 a	1.0 a
Canola MR	2.4 g	2.8 d	1.8 b	2.3 g	1.7 bc	1.4 cd
Lupin MR	1.2 abcd	1.6 ab	1.1 a	1.3 bc	1.4 abc	1.0 ab
Wheat MR	1.1 abc	1.3 a	1.0 a	1.2 abc	1.3 ab	1.0 ab
TSP-1P	1.5 cdef	1.8 bc	1.3 ab	1.9 ef	1.7 bc	1.2 abc
TSP-2P	1.6 def	1.7 abc	1.4 ab	1.7 de	1.6 bc	1.4 cd
TSP-4P	1.9 f	1.7 ab	1.2 a	2.1 fg	1.7 bc	1.6 de
Control	1.3 abcde	1.5 ab	1.0 a	1.4 cd	1.6 abc	1.3 bcd

YS, MS and MR indicated Young Shoot, Mature Shoot and Mature Root respectively. Within columns, means followed by the same letter are not significantly different (P < 0.001) by Tukey's multiple range tests.

6.3.8 Plant shoot and root P uptake

During the three consecutive crop growing periods, plant shoot and root P uptake was significantly different ($P < 0.001$) between the control and the amended treatments (Figure 6.4). Plant shoot and root P uptake (mg P pot^{-1}) did not follow the same trend as plant shoot and root DM. Plant shoot and root P uptake is also expressed as percentage of the control to allow better comparison between different crop periods. Plant P uptake exceeding the amount of P added with the seeds (0.3 mg P pot^{-1}) was regarded as P taken up from soil (net P uptake). Except for wheat MS and lupin MS in crop period 1, net P uptake occurred in all other treatments and crop periods. In general, plant shoot and root P uptake in root residue-amended soils were in the order of canola MR > lupin MR ≥ wheat MR during the 3 crop periods. Plant shoot and root P uptake in TSP-amended soils increased in the order of TSP-1P ≤ TSP-2P < TSP-4P. In crop period 1, plant shoot P uptake was highest in pea YS-amended soil (2.2 mg P pot^{-1}) while root P uptake was highest in canola MR-amended soil (1.2 mg P pot^{-1}). Plant shoot and root P uptake were higher in young shoot-amended soils than in mature shoot and root-amended soils (except canola MR). Plant shoot and root P uptake in canola MR-amended soil were 2.5-2.7 fold higher than in canola YS-amended soil. Compared to the control, plant shoot P uptake was 34%-711% higher and root P uptake was 49-447% higher in young shoot, canola MR, and TSP-amended soils. In crop period 2, plant shoot and root P uptake were highest in pea YS-amended soils and lowest in lupin MS–amended soil and shoot P uptake ranged from 0.2 to 2.1 mg P pot^{-1} while root P uptake ranged from 0.2 to 0.9 mg P pot^{-1}. In crop period 2, plant shoot and root P uptake in canola MR-amended soil was 1.8 fold higher than canola YS-amended soil. Compared to crop period 1, shoot P uptake in crop period 2, was higher in all treatments except in pea YS-amended soil by 6%-175%. In crop period 3, shoot P uptake was highest in pea YS-amended soils (1.4 mg P pot^{-1}) and lowest in wheat MS-amended soil (0.2 mg P pot^{-1}), while root P uptake was highest in pea YS-amended soil (0.6 mg P pot^{-1}) and

lowest in lupin MR-amended soil (0.1 mg P pot^{-1}). In crop period 3, compared to the control, shoot P uptake was 34%-244% higher in young shoot, canola MR and TSP-amended soils, whilst root P uptake was 16%-250% higher in young shoot, canola MR, TSP-2P and TSP-4P-amended soils. On the other hand, shoot and root P uptake in lupin and wheat mature shoot and root-amended soils were 9%-29% and 24%-40% lower than the control. Plant shoot and root P uptake in canola MR-amended soil were higher (1.8 and 2.0 fold respectively) than in canola YS-amended soils. Compared to crop period 2, shoot and root P uptake in crop period 3 decreased by 22%-46% and 21%-64%, respectively.
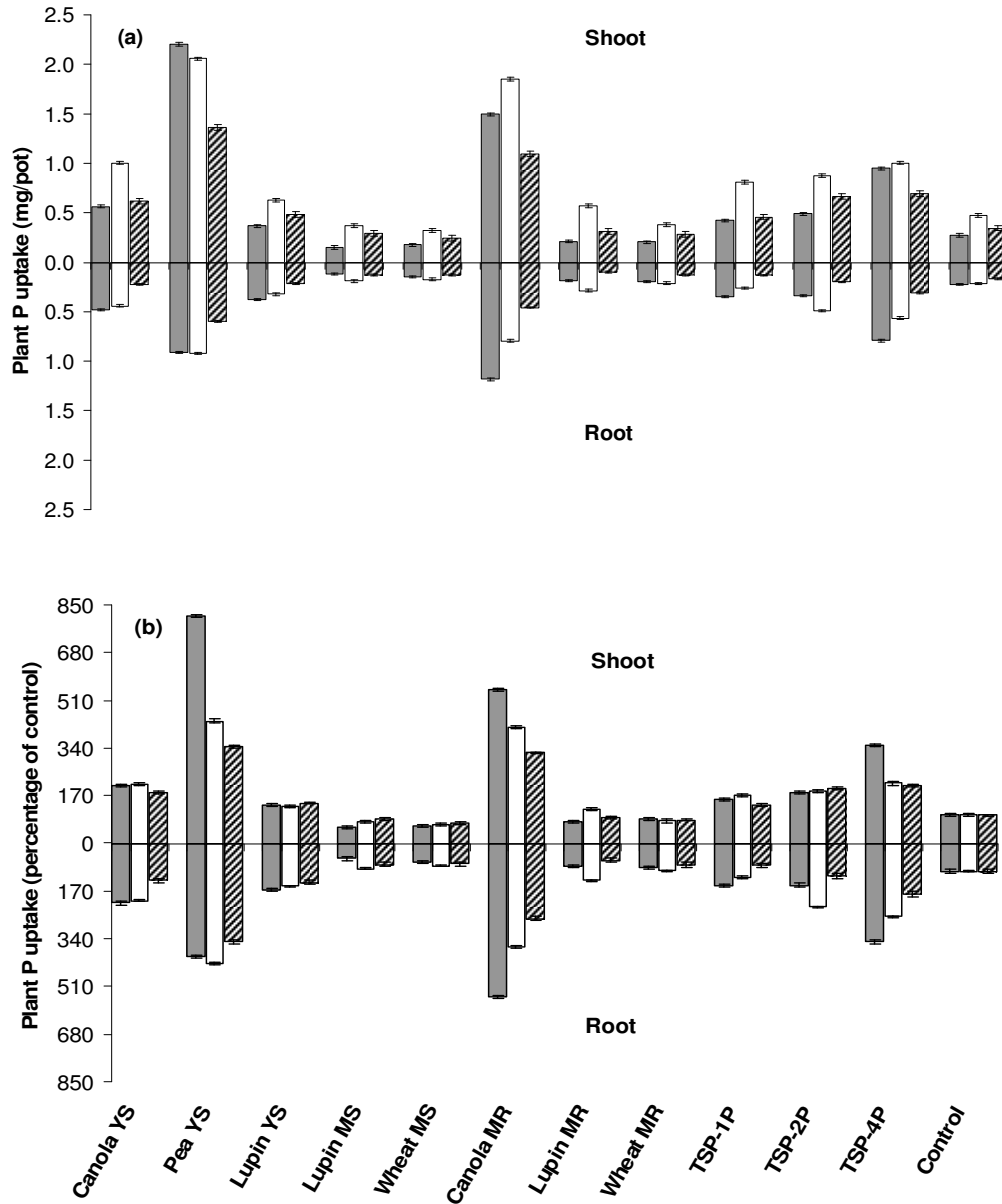
Figure 6.4 Plant P uptake (a) and P uptake percentage of control (b) from soils amended with different crop residues, TSP and unamended control during crop period 1 (gray bars), crop period 2 (white bars) and crop period 3 (diagonally hatched bars). Means of 4 replicates, vertical bars indicate standard error of means (SEM).

6.3.9 Correlations between residue properties and soil and plant variables

Many of the residue chemical and biochemical properties shown in Table 6.3 were correlated with soil and plant parameters. The correlations between residue properties and soil or plant parameters varied over time (Table 6.9). In this experiment, the effect of residue C: P ratio on P_{resin}, P_{mic} and plant P uptake can be assessed. Both correlations of C: P ratio with P_{mic} or with plant P uptake were significant and negative. Even in the first 28 days of incubation, residue C: P ratio had a slight negative influence on P_{mic}. Hence, P_{mic} and plant P were influenced by the amount of P added which is a function of the C: P ratio of the residues. Throughout the experiment, soil P_{resin} concentration was positively correlated with total P concentration of residues and the amount of P added, while it was negatively correlated with the C: P ratio of the residues. At some sampling dates, P_{resin} concentration was positively correlated with P_{H2O}, total N, N_{H2O} and amide content of residues. However, there was no significant correlation between P_{resin} concentration and C: N ratio, C_{H2O}, lignin and phenolic content of the residues. Except on days 7 and 84, soil P_{mic} concentration was significantly positively correlated with total P, P_{H2O} of residues and amount of P added while it was significantly negatively correlated with residue C: P ratio. The microbial P concentration was also significantly positively correlated with total N, N_{H2O}, lignin and amide content and the C: N ratio of residue at some sampling days. On day 7, microbial C and P were significantly correlated with soil respiration rate (r^2 = 0.33, $P \leq 0.05$ and r^2 = 0.96, $P \leq 0.001$, respectively). Soil respiration rates were significantly correlated with different parameters of residues at different sampling dates. In the first 4 days of incubation, soil respiration rates were significantly positively correlated with residue C_{H2O} and N_{H2O} (data not shown) while on days 28 and 35, respiration rates were significantly negatively correlated with total N, C_{H2O}, N_{H2O} and amide content of residues. There was a significant negative correlation between soil respiration rate and lignin content of residues on day 7. Cumulative CO_2 release and residue decomposition rate were influenced by the residue C: N

ratio more strongly initially than in the later phases. On day 7, cumulative CO_2 release and residue decomposition rate were significantly positively correlated with total N, P_{H2O}, C_{H2O}, N_{H2O}, and amide content of residues and amount of P added with the residues. On the other hand, cumulative CO_2 release and residue decomposition rate on day 7 were significantly negatively correlated with lignin, phenolic compounds and C: N ratio of residues. From day 28 to 84, cumulative CO_2 release and residue decomposition rate were positively correlated only with P_{H2O}, C_{H2O} and N_{H2O} of the residues, while they were negatively correlated with concentrations of lignin and phenolic compounds in the residues. Throughout the experiment, plant DM and plant P uptake were positively correlated with total P concentration in the residues and amount of P added; this correlation was stronger in crop period 3 than in crop periods 1 and 2. In contrast, plant DM and plant P uptake were negatively correlated with residue C: P ratio during all three crop periods (see Appendix 6 for correlations between O-alkyl and soil and plant variables).

Table 6.9 The correlation coefficient (r^2) of residue quality factors with soil and plant variables during incubation

Time	Parameters	P_t	N_t	P_{H2O}	C_{H2O}	N_{H2O}	Lignin	Phenolics	Amide	C:P	C:N	P addition
Crop period 1, Day 7	P_{resin}	0.80**	ns	ns	Ns	ns	ns	ns	ns	-0.57**	ns	0.79**
	P_{mic}	ns	ns	ns	Ns	0.58*	0.53*	ns	0.65*	ns	0.59*	ns
	C_{mic}	ns	ns	ns	Ns	ns	ns	ns	ns	ns	ns	ns
	Respiration rate	ns	ns	ns	Ns	ns	-0.54*	ns	ns	ns	ns	ns
	Cumulative C	ns	0.58*	0.58*	0.89***	0.91***	-0.86***	-0.78**	0.72**	ns	-0.53*	0.51*
	C_{min}/Decomp	ns	0.59*	0.57*	0.89***	0.91***	-0.87***	-0.81**	0.72**	ns	-0.52*	0.51*
Crop period 1, Day 28	P_{resin}	0.92***	0.67*	0.54*	Ns	ns	ns	ns	ns	-0.70**	ns	0.93***
	P_{mic}	0.71**	ns	0.56*	Ns	ns	ns	ns	ns	-0.56*	ns	0.72**
	Respiration rate	ns	-0.83**	ns	-0.55*	-0.86***	ns	ns	-0.80**	ns	0.63*	ns
	Cumulative C	ns	ns	0.61*	0.81**	0.77**	-0.94***	-0.78**	0.52*	ns	ns	ns
	C_{min}/Decomp	ns	ns	0.61*	0.84***	0.74**	-0.93***	-0.82**	ns	ns	ns	ns
	Plant DM	0.70*	ns	ns	Ns	ns	ns	ns	ns	-0.61*	ns	0.67*
	P uptake	0.84***	ns	ns	Ns	ns	ns	ns	ns	-0.61*	ns	0.82**
Crop period 2, Day 35	P_{resin}	0.86***	0.58*	ns	Ns	ns	ns	ns	ns	-0.61*	ns	0.86***
	P_{mic}	0.78**	ns	0.7*	Ns	ns	ns	ns	ns	-0.69*	ns	0.76**
	C_{mic}	ns	ns	0.60*	Ns	ns	ns	ns	ns	ns	ns	ns
	Respiration rate	ns	-0.72**	ns	-0.54*	-0.82**	0.67*	0.58*	-0.69**	ns	ns	ns
	Cumulative C	ns	ns	0.57*	0.83**	0.74**	-0.95***	-0.78**	ns	ns	ns	ns
	C_{min}/Decomp	ns	ns	0.62*	0.82**	0.69*	-0.91***	-0.80	ns	ns	ns	ns
Crop period 2, Day 56	P_{resin}	0.93***	ns	0.57*	Ns	ns	ns	ns	ns	-0.73**	ns	0.93***
	P_{mic}	0.82**	ns	0.57*	Ns	ns	ns	ns	ns	-0.65*	ns	0.79**
	Respiration rate	ns	ns	ns	Ns	ns	ns	ns	ns	ns	ns	ns
	Cumulative C	ns	ns	0.62*	0.76**	0.67*	-0.93***	-0.80**	ns	ns	ns	ns
	C_{min}/Decomp	ns	ns	0.62*	0.80**	0.64*	-0.91***	-0.83**	ns	ns	ns	ns
	Plant DM	0.88***	ns	ns	Ns	ns	ns	ns	ns	-0.66*	ns	0.86***
	P uptake	0.84**	ns	ns	Ns	ns	ns	ns	ns	-0.65*	ns	0.82**

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns, non-significant at 5% level of significance. P_t, residue total P, N_t, residue total N, C_{min}/Decomp, residue decomposition rate

Table 6.9 (Continued)

Time	Parameters	P_t	N_t	P_{H2O}	C_{H2O}	N_{H2O}	Lignin	Phenolics	Amide	C:P	C:N	P addition
Crop period 3, Day 63	P_{resin}	0.75**	ns	0.50*	ns	ns	ns	ns	ns	-0.64*	ns	0.79**
	P_{mic}	0.90***	0.56*	0.71**	ns	ns	ns	ns	ns	-0.81**	ns	0.92***
	C_{mic}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Respiration rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Cumulative C	ns	ns	0.61*	0.76**	0.68*	-0.94***	-0.81**	ns	ns	ns	ns
	C_{min}/Decomp	ns	ns	0.61*	0.79**	0.64*	-0.92***	-0.84***	ns	ns	ns	ns
Crop period 3, Day 84	P_{resin}	0.75*	0.78**	ns	ns	0.54*	ns	ns	0.56*	-0.53*	ns	0.78**
	P_{mic}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Respiration rate	ns	-0.51*	ns	ns	ns	ns	ns	-0.64*	ns	0.58*	ns
	Cumulative C	ns	ns	0.60*	0.74**	0.64*	-0.93***	-0.81**	ns	ns	ns	ns
	C_{min}/Decomp	ns	ns	0.60*	0.77**	0.62*	-0.92***	-0.84**	ns	ns	ns	ns
	Plant DM	0.88***	ns	ns	ns	ns	ns	ns	ns	-0.63*	ns	0.87***
	P uptake	0.89***	ns	ns	ns	ns	ns	ns	ns	-0.64*	ns	0.87***

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns, non-significant at 5% level of significance. P_t, residue total P, N_t, residue total N, C_{min}/Decomp, residue decomposition rate

6.4 Discussion

This study showed that the decomposition dynamics of added crop residue, plant available P and plant P uptake are controlled by numerous factors such as plant species, plant part (shoot or root), age of the residues and duration of decomposition. The results are generally in agreement with previous studies (Abiven *et al.* 2005; Bertrand *et al.* 2006; Blair *et al.* 2005; Jensen *et al.* 2005; Nwoke *et al.* 2004; Wang *et al.* 2004) and also in agreement with the previous study described in Chapter 5. Based on the respiration rates and consequently C mineralization during the incubation period, the decomposition process can be divided into 4 distinct phases such as phase 1 and 2 in crop period 1 (day 0-28), phase 3 in crop period 2 (day 28-56) and phase 4 in crop period 3 (day 56-84) respectively (Figure 6.1 and Figure 6.2).

6.4.1 Phase 1 (day 0-10)

Phase 1 consists of the first 10 days of incubation and is characterised by the highest respiration rates and C mineralization compared to rest of the incubation period. The respiration rates in residue-amended soils were higher than in TSP-amended soils and the control (Figure 6.1), showing that addition of C with residues stimulates soil microbial activity. Respiration rates differ between residue types. This is in agreement with previous studies (Bertrand *et al.* 2006; Jensen *et al.* 2005; Trinsoutrot *et al.* 2000b; Wang *et al.* 2004). It is assumed that in this initial phase, mainly easily degradable and water-soluble low molecular weight compounds are decomposed. In phase 1, soils amended with young residues produced more CO_2 C (311-413 mg CO_2-C g^{-1} C added) than soils with mature shoot and root residues (90-212 mg CO_2-C g^{-1} C added). This is in agreement with the study described in Chapter 5 (Section 5.4.1) and may be due to higher content of water soluble C, N and P and, on the other hand, low lignin content and low C: P ratio of the young residues (Table 6.3). The effect of

these residue factors is supported by their significant positive correlations with respiration rate (Table 6.9) Similar findings have been reported before in soils with different texture and pH (Bertrand *et al.* 2006; Jensen *et al.* 2005; Mathers *et al.* 2007; Raiesi 2006; Trinsoutrot *et al.* 2000b; Xu *et al.* 2006). On the other hand, the low respiration rates in TSP-amended soils (respiration of native soil organic matter only) indicate that microbial activity in soils may be C limited, not P limited (Chauhan *et al.* 1981; Nziguheba *et al.* 2005). Compared to the control, the higher microbial biomass C and P in residue-amended soils indicate that C addition with residues not only stimulates microbial activity but also microbial growth and P uptake. The increased microbial P demand is probably also due to the higher microbial activity because highly active microorganisms have a high P demand (Vrede *et al.* 2004). Chauhan *et al.* (1981) and He *et al.* (1997) also showed that microbial growth and microbial P uptake increased following C addition. The difference in respiration rates between residue types was stronger than those in biomass C in phase 1. For example, respiration rate in lupin YS-amended soil was 1.3 fold higher than lupin MS-amended soil but microbial C did not differ between these two treatments. This suggests that the respiration rate per unit biomass is increased after addition of certain residues but that there was no net growth of microbial biomass. It should be noted that this does not rule out microbial growth since net growth is a function of growth and death/predation. Abiven *et al*. (2007) also showed that the initial CO_2 release increased more rapidly than microbial growth when cauliflower and wheat straw was added at 4 g C kg^{-1} soil in a French soil. In this initial phase, the increase in P_{mic} concentration was higher than the amount of P added with residues. For example, lupin MS-amended soil had a 4.0 mg P kg^{-1} soil higher P_{mic} concentration than the control although only 3.3 mg P kg^{-1} soil was added with this residue. This suggests that some soil P may be mineralized or solubilised if microbial activity is stimulated by residue addition. Gressel and McColl (1997) suggested that decomposition of organic residues can affect P_o mineralization through enhanced production of phosphatase

enzymes. Hence, P added with residues and also P released from soil P_o through mineralization was taken up by microbial biomass. Magid *et al*. (1996) also suggested that microbial activity stimulated by the addition of residues may increase the solubilisation and mineralization of soil P. Compared to the control, the P_{mic} concentration was 9%-23% higher in TSP-amended soils, which suggests that some of the P added with TSP was also taken up by the microbial biomass. However, the source of the increased P_{mic} could not be identified in this study because the residues or TSP were not P-labelled. Compared to the control, the P_{resin} concentration was higher in soils amended with residues with high total P and therefore higher P addition to achieve the same amount of C except in lupin YS-amended soil. The low P_{resin} concentration in lupin YS-amended soil may be due to very high initial microbial activity which can be explained by the high concentrations of water soluble C, N and P (Table 6.3). Soils amended with residues with low total P concentration and greater C: P ratio and therefore small P addition had lower P_{resin} concentration than the control. This suggests that addition of crop residues with high total P ($\geq 0.16\%$) and low C: P ratio ($\leq 253: 1$) can increase soil P_{resin} concentration compared to the control even if P is partly immobilized by the microbial biomass whereas immobilisation dominates after addition of residue with low P content and high C:P ratio. In a study in low P soil (5.3 mg P kg^{-1} soil Olsen-P), White and Ayoub (1983) showed that residues with high total P (0.34%) and low C: P ratio (123:1) increased available P by 1.9 to 10. 6 mg P kg^{-1} soil Olsen-P and inversely, residues with low total P (0.08%) and high C: P ratio (506) led to net immobilization by 1.7 to 3.6 mg P kg^{-1} soil Olsen-P from day 3 to 42. The results of this study are in agreement with the study described in Chapter 5 for the critical level of P (0.16%) in residues for net P immobilization or net mineralization. Compared to the control, the P_{resin} concentration in TSP-2P and TSP-4P-amended soils was significantly higher because soluble P was added and which was therefore immediately available, but microbial P uptake was low because of the absence of freshly added carbon. This is in agreement with the

previous study in Chapter 5. Chauhan *et al*. (1981) also suggested that labile P increased after addition of P fertilizer without C but the increase was less pronounced when C was added with the P fertilizer.

In phase 1, compared to the previous study in Chapter 5, respiration rates were higher in young shoot-amended soil but lower in mature shoot and root-amended soils due to the lower amount of C added with mature shoot and root residues in this study. Concentrations of P_{resin} and P_{mic} in all residue-amended soils and the control were lower than in the previous study in Chapter 5 which may be due to slight differences in soil properties since soil used in this study was collected from a different location in the same paddock, and at a different time of the year. The differences in P_{mic} concentrations between residue-amended soils were not as pronounced as in the previous study in Chapter 5 because the same amount of C was added with all residues in this study. On the other hand, the differences in P_{resin} concentration between residue-amended soils were more pronounced in this study than the previous study as different amounts of P were added.

6.4.2 Phase 2 (day 10-28)

Phase 2, between days 10 to 28 of the decomposition process, is characterised by lower respiration rates than phase 1, but higher respiration rates than the remaining incubation period. During phase 2, the differences in respiration rates between residue treatments were less pronounced than in phase 1 and the ranking of respiration rates in the residue-amended soils changed with time of decomposition (Figure 6.1). The decomposition rates were lower than in phase 1 probably due to depletion of most of the easily degradable and water soluble compounds. By the end of phase 2, soils amended with young residues and mature shoot and root residues had produced 64-129 and 89-130 mg more CO_2-C g^{-1} C added, respectively than

in phase 1. This indicates that the easily decomposable compounds were depleted in both young and mature residue types. It is assumed that only more recalcitrant compounds were remaining, hence the decomposition rates were similar in young and mature residues. But due to the lower respiration rates of the mature residues in phase 1, the cumulative respiration at the end of phase 2 was still lower than in young residues. This is in agreement with Abiven *et al.* (2007) who showed a stronger decrease of decomposition rate of cauliflower than wheat straw between days 10 and 30, the latter having higher concentrations of recalcitrant compounds. The respiration rates in TSP-amended soils and the control declined and then remained at very low rates. This decline from phase 1 to phase 2 indicates that only recalcitrant soil organic matter (SOM) was left in phase 2. Bertrand *et al.* (2006) also showed that the rate of C mineralization in the control soil was much lower than residue-amended soils and decreased over a period of 20-30 days. The P_{mic} concentration in residue-amended soils was still higher than in TSP-amended soils and the control. However, P_{mic} in phase 2 was approximately 2%-31% lower than phase 1. Since microbial C did not seem to change over the course of the experiment, the microbial C: P ratio increased. This may be explained by a lower P demand of the less active microbial biomass (Vrede *et al.* 2004). The differences between residue types in microbial P and respiration rates were less pronounced than in phase 1. On the other hand, compared to phase 1, the P_{resin} concentration increased in canola YS, lupin YS, lupin MS, lupin MR and wheat MR and TSP-1P-amended soils but P_{resin} concentration was still higher in pea YS-amended soil than in the other treatments. The increase in P_{resin} concentration in those treatments may be due to the decrease of P_{mic} concentration, mineralization of residue P or solubilisation of soil P. Bünemann *et al.* (2004) also showed that the P_{resin} concentration increased in crotalaria-amended soil by 56 days, but the increase in P_{resin} was approximately 4 times smaller than the decrease in P_{mic}. In phase 2, both the P_{resin} and P_{mic} concentration in pea YS, canola MR, wheat MR, TSP-2P and TSP-4P-amended soils

decreased by 3%-41% and 7%-10%, respectively compared to phase 1, probably due to plant P uptake. This is in agreement with Dalal (1979) who showed that available P decreased due to plant P uptake. In phase 2, P_{resin} concentration showed a strong positive correlation with residue total P concentration and amount of P added with residue, but a moderately negative correlation with residue C: P ratio. This suggests that the higher amount of P added with young shoot and canola MR residues is still affecting soil P availability probably due to P being slowly released from residues or turnover of microbial P. Previous studies (Dalal 1979; Kwabiah *et al.* 2003b; Nziguheba *et al.* 1998; White and Ayoub 1983) also showed that addition of residues with high total P concentration and low C: P ratio increased available P four weeks after residue amendment both in presence or absence of plants. Compared to the control, the lower P_{resin} concentration both in phase 1 and 2 in soils amended with residues with low total P concentration and greater C: P ratio resulted in low plant DM (Figure 6.3) and plant P uptake. The poor plant growth may be explained by the low shoot P concentrations which are in the P deficiency range (<0.16% of shoot DM) as suggested by Marschner (1995). On the other hand, the higher plant DM in young shoot, canola MR and TSP-amended soils than the control were associated with shoot P concentrations sufficient for optimal plant growth (Marschner 1995). This suggests that if residue amendment is based on the same amount of C, residues with low C: P ratio and high total P concentration and therefore higher P addition provide sufficient P for optimal plant growth during 4 weeks although P is partly immobilized by the microbial biomass. Previous studies (Blair and Boland 1978; Dalal 1979; Fuller *et al.* 1956) also showed that plant P uptake was greater in soils amended with residues which had high total P concentration and low C: P ratio. The high plant DM and plant P uptake in TSP-amended soils can be explained by the higher available P than the control because TSP is soluble and very little P (0.2-0.6 mg P pot^{-1}) was immobilized by the microbial biomass due to absence of freshly added carbon.

In phase 2, total P[1] in the pools measured (P_{resin}, P_{mic} and plant P uptake) was highest in pea YS-amended soil (10.0 mg pot^{-1}) followed by canola MR-amended soils (7.6 mg pot^{-1}) while in other treatments it ranged from 1.2 to 5.7 mg pot^{-1}. In residue-amended soils, a large proportion of this total P (48%-99%) was microbial P. However, this represents only a fraction of the P amount added. Hence, approximately 48%-69%, 21%-48% and 42%-44% of P added with young shoot, mature shoot and root and TSP (except TSP-1P), respectively must have entered other soil P pools. On the other hand, in TSP-1P-amended soil, the sum of resin, microbial and plant P was 0.4 mg P pot^{-1} higher than the amount of added P which indicates that soil P was mineralized or solubilised.

6.4.3 Phase 3 (day 28-56)

Phase 3, between days 28 to 56 of the incubation is characterized by lower respiration rates than the earlier phases but higher rates than the following periods. In phase 3, the differences in respiration rates among the residue-amended soils were smaller than in phase 2 and by the end of phase 3 there were no significant differences between residue-amended soils (Figure 6.1). The low respiration rates in residue-amended soils were probably due to decomposition of recalcitrant compounds. During phase 3, soils amended with young residues produced 17-37 mg more CO_2-C g^{-1} C added than in phase 2, whereas soils amended with mature shoot and root residues produced 37-70 mg more CO_2-C g^{-1} C added than phase 2. This suggests that mature shoot and root residues still have a higher amount of recalcitrant compounds left than young shoot residues (Figure 6.2). This is in agreement with previous findings (Abiven *et al.* 2007; Bertrand *et al.* 2006; Mary *et al.* 1992). They showed that decomposition rate decreased more slowly in residues with a higher percentage of recalcitrant compounds than those with a

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[1] The percentage of residue P recovered was calculated by subtracting the control value (P_{resin}, P_{mic} and Plant P uptake) from the treatments value.

lower percentage of recalcitrant compounds in the period from 4 to 8 weeks after residue addition. In residue-amended soils, the decrease in microbial activity in phase 3 was accompanied by a 3%-17% lower microbial P compared to phases 1 and 2. Hence, P_{mic} concentration was positively and weakly correlated with respiration rate (r^2 = 0.35, $P \leq 0.04$). Compared to phase 2, the decrease of P_{mic} in phase 3 in residue-amended soils may have contributed to the increase of P_{resin} concentration (except pea YS and canola MR residue), but the increase of P_{resin} concentration was lower than the decrease of P_{mic} concentration. This suggests that P released from the microbial biomass was either taken up by the plant or entered a non-resin-extractable P pool. This is in agreement with Kouno *et al.* (2002) who suggested that P turned over from microbial biomass can be fixed immediately to soil colloids, some can be re-immobilized by the microbial biomass or a small fraction can be taken up by the plant. Bünemann (2003) also reported that plant P derived from residue remained stable over 42 days although the P_{mic} concentration decreased. The increase in P_{resin} concentration in lupin and wheat mature shoot and root-amended soils by 29%-140% could be due to mineralisation of residue P and/or mobilisation of soil P by carboxylates produced during decomposition (Magid *et al.* 1996; Stewart and Tiessen 1987). In agreement with phase 2, the P_{resin} concentration in phase 3 remained lower in lupin MS, wheat MS and wheat MR-amended soils than the control. Hence, P immobilization was greater than P mineralization due to the low total P concentration and high C: P ratio of the residues. As in the previous phases, the P_{resin} concentration was strongly and positively correlated with residue total P concentration and P addition and negatively correlated with residue C: P ratio (Table 6.9). Compared to crop period 1, plant DM in crop period 2 increased 1.1 to 1.7 fold (Figure 6.3), which may be explained by the increase of P_{resin} concentration and decrease of P_{mic} concentration during phase 3 (Figure 6.4) except in pea YS, canola MR and TSP-4P-amended soils. In crop period 2, in all treatments other than lupin MS, wheat MS and wheat MR, shoot P concentrations were in the

range for optimal plant growth (0.3%-0.5% of DM) (Marschner 1995). On the other hand, shoot P concentrations in lupin MS, wheat MS and wheat MR-amended soils indicated P deficiency (<0.16% of shoot DM; Table 6.8) (Marschner 1995). Hence, plant DM strongly and positively correlated with soil P_{resin} concentration (r^2 = 0.95, $P \leq 0.001$) and also residue total P concentration and amount of P added (Table 6.9). In agreement with phase 2, in phase 3, total P^1 in the three measured P pools (P_{resin}, P_{mic} and plant P) was higher in young shoot, canola MR-amended soils (3.8-7.4 mg pot^{-1}) than the other treatments (1.3-3.0 mg pot^{-1}). It was the highest in pea YS-mended soil and the lowest in TSP-2P-amended soil. In the residue-amended soils, a large percentage of this total P (17%-91%) was microbial P. In this phase, approximately 60%-70%, 22%-52% and 27%-47% of P added with young shoot, mature shoot and root and TSP (except TSP-1P) respectively entered other soil P pools. On the other hand, in TSP-1P-amended soil 0.3 mg P pot^{-1} more P was detected in the three measured P pools than the amount of P added. This additional P in TSP-1P-amended soil indicates that soil P was mineralized or solubilised in this treatment.

Compared to the previous study in Chapter 5, in phase 3, the respiration rates were similar in young shoot-amended soils but lower in mature residue-amended soils probably due to lower amount of C added in this study with mature residues. In mature shoot-amended soils, P_{mic} concentration was 2 fold higher in the previous study in Chapter 5 than in this study. This suggests that addition of large amount of C leads to prolonged P immobilization, however there is also slow P mineralization and therefore, P_{resin} concentration increased in mature shoot-amended soils in both studies in this phase. In general, P_{resin} concentration was lower in this study than the previous study in Chapter 5 probably due to higher shoot P uptake (3 to 7 fold).

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[1] The percentage of residue P recovered was calculated by subtracting the control value (P_{resin}, P_{mic} and Plant P uptake) from the treatments value.

6.4.4 Phase 4 (day 56-84)

Phase 4, from day 56 to day 84 was characterized by lower respiration rates than in the earlier phases. In phase 4, the respiration rates in residue-amended soils did not differ significantly. This suggests that in all treatments, only recalcitrant C compounds were left (Figure 6.1). As in the phase 3, the sum of CO_2-C g^{-1} C added during phase 4 was lower in young shoot-amended soils (20-32 mg CO_2-C g^{-1} C added) than in mature shoot and root-amended soils (27-41 mg CO_2-C g^{-1} C added). Similar findings have reported in the previous studies (Abiven *et al.* 2007; Bertrand *et al.* 2006; Jensen *et al.* 2005). They showed that in the period of 2 to 3 months after residue addition, decomposition rate were higher in residues with higher concentrations of recalcitrant compounds than in those with low concentrations of recalcitrant compounds. In phase 4, P_{mic} concentration decreased by 2%-19% compared to phase 3, indicating a further decrease in microbial P demand as a result of C limitation (Makino *et al.* 2003; Vrede *et al.* 2004). As in phase 3, the decrease in P_{mic} concentration was not accompanied by a similar increase in P_{resin}. In phase 4, the P_{resin} concentration was similar as in phase 3, however, plant P uptake in pea YS and canola MR-amended soils was higher (0.8-0.9 mg P pot^{-1}) than the decrease of P_{mic} in these treatments. This suggests that P added with residue was mineralized or P was solubilised from soil P which was then taken up by the plants. Although P is taken up by plant, the average P_{resin} concentration in young shoot, canola MR-amended soils was still higher compared to the control in phase 4. Plant DM in crop period 3 was correlated with P_{resin} concentration ($r^2 = 0.86$, $P \leq 0.001$). The higher plant DM in pea YS and canola MR residue-amended soil compared to TSP-4P suggests that these residues could be more efficient in producing P to grow plants than inorganic fertilizers. The low plant DM and P concentration in lupin and wheat mature shoot and root-amended soils suggests that even after more than 2 months decomposition, residues with low total P concentration and higher C: P ratio result in net P immobilisation. Similarly, Nziguheba *et al.* (1998) showed that P is immobilized in low P

content maize residue-amended soil up to 16 weeks of incubation. In phase 4, total P[1] in the three measured P pools (P_{resin}, P_{mic} and plant P) was the highest in pea YS-amended soil (6.7 mg pot^{-1}) followed by canola MR-amended soil (5.8 mg pot^{-1}) and was lowest in TSP-2P-amended soil (0.9 mg pot^{-1}). In lupin and wheat mature shoot and root-amended soils it ranged from 2.1 to 3.1 mg pot^{-1}. However, in residue-amended soils a large part of this total P (47%-95%) was microbial P. Also in this phase, approximately 60%-80%, 16%-48%, 49%-59% and 10%-60% of the initially added P with young shoot, mature shoot, mature root and TSP, respectively, were not detected in the three P pools. Compared to the earlier phases, a larger percentage of P added with residues and TSP remained in the soil as non-resin-extractable P in this phase.

In phase 4, the respiration rates in this study were lower than in the previous study in Chapter 5 and did not differ between treatments. As a result of the smaller amount of C was added in this study, the average P_{mic} concentration in mature residue-amended soils was 2 fold lower than in the previous study in Chapter 5 in phase 4, while there was no difference in young shoot-amended soils between the two studies since the amount of C added with young residues was similar (≤ 2.5 g C kg^{-1} soil). Moreover, the average P_{resin} concentration was higher in phase 4 in the study in Chapter 5 which may be because of higher P mineralization or P release from microbial biomass compared to this study. In crop period 3, plant DM was generally lower than the study in Chapter 5, which is probably due to lower concentration of available P and therefore lower plant P availability.

In the field, residue additions occur naturally after crop harvest or physiological maturity is reached and are therefore related to addition of C with no intent to manipulate P. Previous

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[1] The percentage of residue P recovered was calculated by subtracting the control value (P_{resin}, P_{mic} and Plant P uptake) from the treatments value.

studies have based residue addition on C (Bünemann *et al.* 2004), P (McLaughlin and Alston 1986a; Nwoke *et al.* 2004; Nziguheba *et al.* 1998) or dry matter (Dalal 1979; Kwabiah *et al.* 2003b; White and Ayoub 1983). In all cases residues with high total P concentration and low C: P or C: N ratio improved soil P availability and plant growth. However there are no studies in which the same residues are added based on C as well as P. The comparison of the results of lupin YS, canola MR and wheat MS in Chapters 5 and 6 (Table 6.10 and Table 6.11) can be used to illustrate the consequences of the different approaches.

Table 6.10 Amount of P and C added with different crop residues in the approaches based on same amount of P and C.

Treatments	Based on same amount of			
	P		C	
	Amount of P added	Amount of C added	Amount of P added	Amount of C added
	mg kg^{-1} soil			
Lupin YS	10.0	2.1	11.7	2.5
Wheat MS	10.0	6.2	4.1	2.5
Canola MR	10.0	1.9	13.0	2.5

YS, MS and MR indicated young shoot, mature shoot and mature root respectively

Table 6.11 Average P_{resin}, P_{mic}, plant P uptake (percentage of added P) and sum of P[1] in the three measured pools (P_{resin}, P_{mic}, plant P uptake) during 3 crop periods

Treatments	Parameters (Percentage of added P)	Based on same amount of P added			Based on same amount of C added		
		Crop period 1	Crop period 2	Crop period 3	Crop period 1	Crop period 2	Crop period 3
Lupin YS	P_{resin}	12.3	22.1	15.4	7.1	7.8	7.9
	P_{mic}	89.2	67.4	62.6	55.4	50.3	40.8
	Plant P uptake	3.6	1.2	7.0	2.8	4.9	3.8
	Sum of measured P	105.1	90.7	85.0	65.3	63.0	52.5
	P not recovered	-	9.3	15.0	34.7	37.0	47.5
	Additional P from soil	5.1	-	-	-	-	-
Wheat MS	P_{resin}	2.2	6.6	11.7	4.7	11.3	13.1
	P_{mic}	136.1	112.6	82.2	141.8	120.7	112.7
	Plant P uptake	1.0	0.8	2.6	3.8	7.0	5.4
	Sum of measured P	139.3	120.0	96.5	150.3	139.0	131.2
	P not recovered	-	-	3.5	-	-	-
	Additional P from soil	39.3	20.0	-	50.3	39.0	31.2
Canola MR	P_{resin}	23.1	28.2	22.5	15.5	14.7	13.9
	P_{mic}	79.2	71.8	63.6	51.7	50.5	47.7
	Plant P uptake	12.3	3.1	5.5	10.4	13.0	7.7
	Sum of measured P	114.6	103.2	91.6	77.6	78.2	69.3
	P not recovered	-	-	8.4	22.4	21.8	30.7
	Additional P from soil	14.6	3.2	-	-	-	-

YS, MS and MR indicated young shoot, mature shoot and mature root respectively

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[1] The percentage of residue P recovered was calculated by subtracting the control value (P_{resin}, P_{mic} and Plant P uptake) from the treatments value.

In the study in Chapter 5, different amounts of C were added to achieve the same amount of P and therefore, more C was added with high C: P residues than with low C: P residues. The greater C addition resulted in higher microbial activity and long-term P immobilization. On the other hand, different amounts of P are added when residues are added based on same amount of C as in the study described in this chapter. Therefore, the differences between treatments in microbial activity, microbial growth and P_{mic} concentration were less pronounced than in the P-based residue addition. The P_{resin} concentration and plant shoot P uptake differed over time in soils depending on the approaches used for residue addition. In lupin YS and canola MR-amended soils, 20% to 30% more C and P were added in the C-based residue addition experiment compared to the P-based residue addition experiment (Table 6.10), but there was a lower percentage of total P in the three measured pools (P_{resin}, P_{mic} and plants), i.e. a greater percentage was not recovered (Table 6.11). The similar values of the total P (P_{resin}, P_{mic} and plants) for lupin YS and canola MR of the two experiments suggest that (i) microbial and plant P uptake were limited by another factor (not C) or were in optimal threshold and (ii) the P_{resin} concentration represents an equilibrium value. Therefore, if more P and C are added, a smaller percentage of added P enters these pools and more remains in non-recovered pools. In wheat MS-amended soils, 50% less P and 2.5 times more C were added in P-based residues addition experiment compared to C-based residue addition experiment. The greater amount of C addition resulted in a greater proportion of P in the microbial biomass, but the proportion was not 2 fold higher which suggests that microbial P uptake was limited by something else. Surprisingly, the percentage of total P in plants also increased despite greater competition by soil microbes. This can be explained by mobilization of soil P. It appears that greater C addition stimulates microbial activity (solubilisation/mineralization) and both plants and microbes can benefit from the greater mobilization.

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Hence, in terms of P recovery from residues, addition of residue based on P is better for low C: P ratio residues although slightly more P was added in the C-based residue addition experiment. Addition of residues with high C: P ratio based on P rather than C, maximizes P mobilization from soil.

Chapter 7

Phosphorus availability as affected by the addition of organic and inorganic P and their combination

7.1 Introduction

Many Australian soils are deficient in available phosphorus (P), an essential element for plant growth, despite a long history of P fertilizer addition. Phosphorus deficiency is due to naturally low total P content (Brady and Weil 2000) or fixation of applied P fertilizer (Bertrand *et al.* 2003). To overcome P deficiency, recycling of crop residues or organic manures is advocated (Kwabiah *et al.* 2003b; Nwoke *et al.* 2004; Nziguheba *et al.* 1998; Reddy *et al.* 2005). The studies in Chapters 5 and 6 on crop residue addition have shown that young residues or residues with high total P (> 0.16% P) and low C: P ratio (< 253:1) can increase P availability and plant P uptake. In contrast, addition of mature shoot residue (< 0.16% P, C: P ratio > 253:1) resulted in long-term P immobilization.

In wheat-growing areas of South Australia, crop residues are added to the soil after grain harvest, at a mature growth stage, and hence with a high C: P ratio. Thus, every year large amounts of crop residues are added which could be a source of P and also costly P fertilizers are applied. It is hypothesised that inorganic P addition may offset P immobilization during the early stages of decomposition enabling residues to provide a slow release P source. This combination of treatments may enable farmers to reduce P fertilizer application rates, and consequently costs. In a study in P deficient African soil, Nziguheba *et al.* (1998) found that a combined application of maize stover and TSP resulted in higher available P than maize stover alone and microbial P did not increase as compared to either maize stover or TSP alone. In

other studies, it was found that addition of residues with inorganic P fertilizer increased available P, plant P uptake compared to residue alone and microbial P (Bah *et al.* 2006; McLaughlin and Alston 1986a; Reddy *et al.* 2005).

Organic and inorganic fertilizer amendments affect the activity of soil microorganisms. Organic amendments increase the soil organic C content which leads to an increase in microbial biomass (Marschner *et al.* 2003). Addition of organic residues with inorganic fertilizer can change the distribution of P between different soil P pools or P fractions (Chauhan *et al.* 1979; Hedley *et al.* 1982; Reddy *et al.* 2005). Various studies showed an increase in P concentration in the soil solution following the combined application of organic residue and inorganic fertilizer. The common perception is that organic anions formed by decomposition of added organic residues compete with P for adsorption sites and consequently enhance P availability in soil (Iyamuremye *et al.* 1996a; Sanyal and Datta 1991). Additionally, microorganisms decomposing the added residues can excrete phosphatase which mineralizes soil P_o increasing available P concentration (Dalal 1977; Stewart and Tiessen 1987). The effectiveness of residues to increase P availability in the combined application of residue and inorganic fertilizer will also depend on the residue quality. The combined application of fertilizer and residue (0.08% P and C: P ratio 558:1) can offset initial P immobilization (Reddy *et al.* 2005). However, the effect of wheat residue in combination with inorganic fertilizer on available P at different application rates has not been investigated in Australian soils.

The objective of this study was to evaluate the effect of varying amounts of low quality (low P, high C: P and C: N ratio) wheat residues and inorganic P fertilizer separately or in combination on soil P availability and P immobilization.

7.2 Materials and methods

This study was conducted using the Monarto soil described in Chapter 5. The field moist soil was stored in buckets for 14 months. Plant debris and stones were removed from the soil and after air-drying the soil was sieved to < 2 mm. The properties of the soil are shown in Table 5.1. Before starting the incubation experiment, the soil was pre-incubated at 85% water holding capacity for 12 days at 18°C/24°C, 8 h night/16 h day. This was undertaken to allow the flush of microbial activity prior to imposing the treatments that may occur when air-dried soil is moistened (Brookes *et al.* 1982; Oehl *et al.* 2001b). It has been shown that after 10 days of incubation, microbial activity becomes stable (Oehl *et al.* 2001a).

Mature shoot residue of wheat (*Triticum aestivum* L.) and inorganic P as TSP (triple super phosphate) were added at rates of 5.0, 7.5 and 10 mg P kg^{-1} soil, and for TSP also 20 mg P kg^{-1} soil. Additional treatments were based on a P addition rate of 20 mg P kg^{-1} soil, with wheat mature shoot, P applied to provide 5.0, 7.5 and 10 mg P kg^{-1} soil and the remaining P added as TSP. The rates of residue addition were much higher than that expected in dry land cropping. However, these addition rates were used to achieve P amounts that had resulted in significantly increased available P and wheat seedling growth in the previous experiments. As in the previous studies described in this thesis, TSP was added as solution. Wheat residue treatments are referred to as W5, W7.5, W10 and TSP treatments are T5, T7.5, T10 and T20 and the combined wheat residue and TSP treatments are termed TW25, TW37.5 and TW50 with 25, 37.5 and 50 referring to the percentage of P added as wheat residue. The amounts of residue and TSP as well as C added in soils are shown in Table 7.1. Wheat residue and TSP were mixed thoroughly into the pre-incubated soil. A thoroughly mixed unamended control treatment was also included.

In this study, 400 g moist amended or unamended soil was weighed into plastic pots of 10.5 cm height and 9.5 cm diameter. The pots were incubated in the dark at 25°C in the first 27 days and then at 25°C to 30°C up to day 42 due to problems with the temperature control in the incubation room. Soil moisture content was adjusted every 2 days to maintain 85% water holding capacity. Pots were weighed at each soil coring. All pots were arranged in a completely randomized block design with 4 replicates and re-randomized at every sampling date.

Table 7.1 Addition of wheat residue (0.07% Pt, 43.5% Ct) and TSP (20% P)

Amendments	Treatments			
TSP	**T5**	**T7.5**	**T10**	**T20**
P (mg P kg^{-1} soil)	5.0	7.5	10.0	20.0
Amount TSP added (g kg^{-1} soil)	0.025	0.038	0.05	0.10
Wheat	**W5**	**W7.5**	**W10**	
P (mg P kg^{-1} soil)	5.0	7.5	10	
C (g C kg^{-1} soil)	3.1	4.6	6.2	
Amount DM added (g kg^{-1} soil)	7.1	10.6	14.1	
Wheat + TSP	**TW25**	**TW37.5**	**TW50**	
(at 20 mg P kg^{-1} soil)				
P as TSP (mg P kg^{-1} soil)	15.0	12.5	10.0	
P as Wheat (mg P kg^{-1} soil)	5.0	7.5	10.0	

Pt, total phosphorus, Ct, total carbon

Soil respiration rates of each treatment were measured by incubation of a sub-sample of 23.0 g of moist soil with three replicates of each treatment. The incubation was carried out in a sealed glass jar in the dark under similar conditions as the pot incubation (25°C in the first 27 days and then at 25°C to 30°C up to day 42). Respiration was measured twice a week until day 21 and then weekly using the infrared gas analyzer (see section 3.2.13 for details).

During incubation, soil samples were taken from each pot using a stainless steel corer (diameter ~10 mm) on days 3, 7, 14, 28 and 42 for available P (P_{resin}) and microbial P (P_{mic}) and microbial C (C_{mic}) was measured only on days 7 and 35.

7.3 Results

7.3.1 Residue properties

Wheat MS residue was selected for this study because wheat is the main cereal crop grown in South Australia and strong and long-term P immobilization was found with wheat residue addition in previous studies (Chapters 5 and 6). The chemical and biochemical properties of Wheat MS are shown in Table 5.4. Wheat MS contained very little P and N (0.07% P and 0.54% N) and therefore had very high C: P (615: 1) and C: N (80:1) ratios. Water-soluble C, N and P in wheat MS were 2.45%, 0.16% and 0.02%, respectively. The C fractions in wheat MS, determined by NMR spectroscopy were 3.9% lignin, 2.2% O-alkyl, 1.63% phenolic and 1.7% amide.

7.3.2 Soil respiration

Soil respiration rates in residue-amended soils, residue+TSP-amended soils were significantly higher than in the soils amended with TSP alone and the control throughout the incubation (Figure 7.1). In the first 4 days, respiration rates in residue-amended soils and residue+TSP-amended soils decreased in the order of W10 > W7.5 > W5 and TW50 > TW37.5 > TW25. On day 1, the respiration rate in residue-amended soils and residue+TSP-amended soils was highest in TW50 treatment (121 mg C kg^{-1} soil day^{-1}) and lowest in W5 treatment (59 mg C kg^{-1} soil day^{-1}). After 10 days of incubation, the respiration rates in residue-amended soils and residue+TSP-amended soils had declined by 34%-68% compared to day 1. On day 10, the

ranking of respiration rate changed and respiration rate in residue-amended soils and residue+TSP-amended soils was highest in W5 and TW10 (39 mg C kg^{-1} soil day^{-1}) while it was lowest in TW25 (31 mg C kg^{-1} soil day^{-1}) but there was no difference in respiration between other treatments. By day 10, the respiration rate in residue-amended soils and residue+TSP-amended soils were 7.4 to 9.2 fold higher than the control (Figure 7.1). From day 10 to 28, the respiration rates in residue+TSP-amended soils decreased by 34%-46% while in residue-amended soils it decreased by 27%-57%. On day 28, there was no difference in respiration rates between treatments in residue+TSP-amended soils but in soils amended with residue alone, the respiration differed significantly, decreasing in the order of W10 ≥ W7.5 ≥ W5. On day 28, the respiration rates in residue-amended soils and residue+TSP-amended soils were still higher than the control (18.4 to 28.2 fold) (Figure 7.1). After day 28, the respiration rates in all treatments increased unexpectedly probably due to the increase in temperature in the incubation room (see section 7.2). Throughout the incubation, the respiration rates in TSP-amended soils and the control did not differ significantly (Figure 7.1).

The cumulative respiration in soils amended with residues alone or residue+TSP was higher than the TSP-amended soils and the control (Figure 7.2). Among the residue-amended soils, the cumulative respiration decreased in the order of W10 > W7.5 ≥ W5 while in residue+TSP-amended soils it was decreased in the order of TW50 > TW37.5 ≥ TW25. On day 10, the cumulative respiration was highest in TW50 (695 mg C kg^{-1} soil) and lowest in T5 (46 mg C kg^{-1} soil) while the cumulative respiration on day 42 was highest in W10 (1812 mg C kg^{-1} soil) and lowest in T7.5 (228 mg C kg^{-1} soil). There were no significant differences in cumulative respiration between TSP-amended soils and the control at any time during the incubation.

On day 6, the decomposition of C added with residue alone or with TSP was highest in TW25 (11.7%) and lowest in W7.5 (7.3%). From day 10 onwards, the decomposition of C added with residues decreased in the order of W5 > W7.5 ≥ W10 while in residue+TSP-amended soils, the decrease of decomposition was in the order of TW25 > TW37.5 ≥ TW50. On day 21, the decomposition of added C was 1.5 to 1.6 fold higher than on day 10. On day 42, the decomposition of added C was highest in W5 (37%) and lowest in W10 (24%) and the decomposition had only increased by 9%-13% compared to day 21.
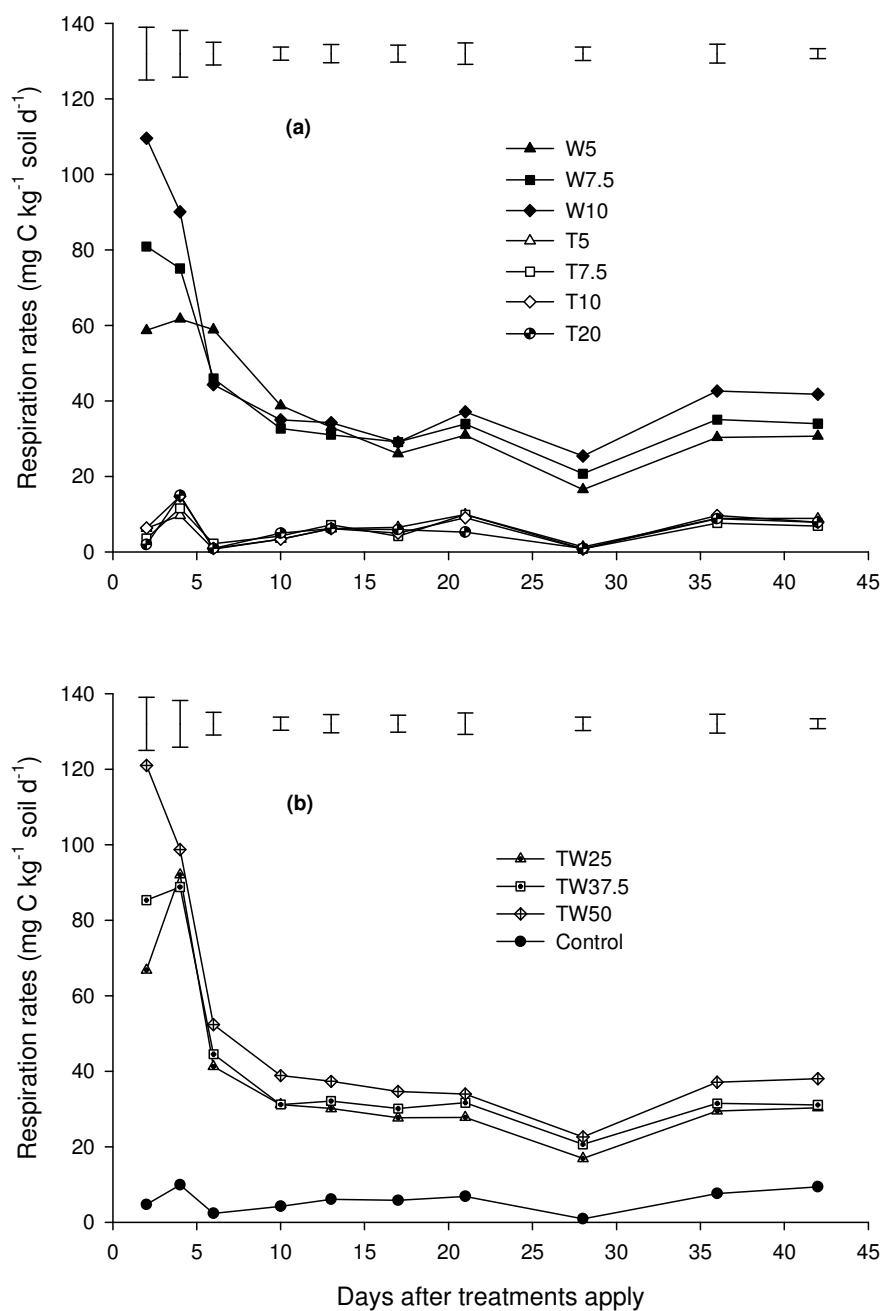
Figure 7.1 Respiration rates in soils amended with wheat MS and TSP alone (a), wheat MS+TSP-amended soils and the control (b) over time. Means of 4 replicates, vertical bars indicate least significant difference (LSD$_{0.05}$).
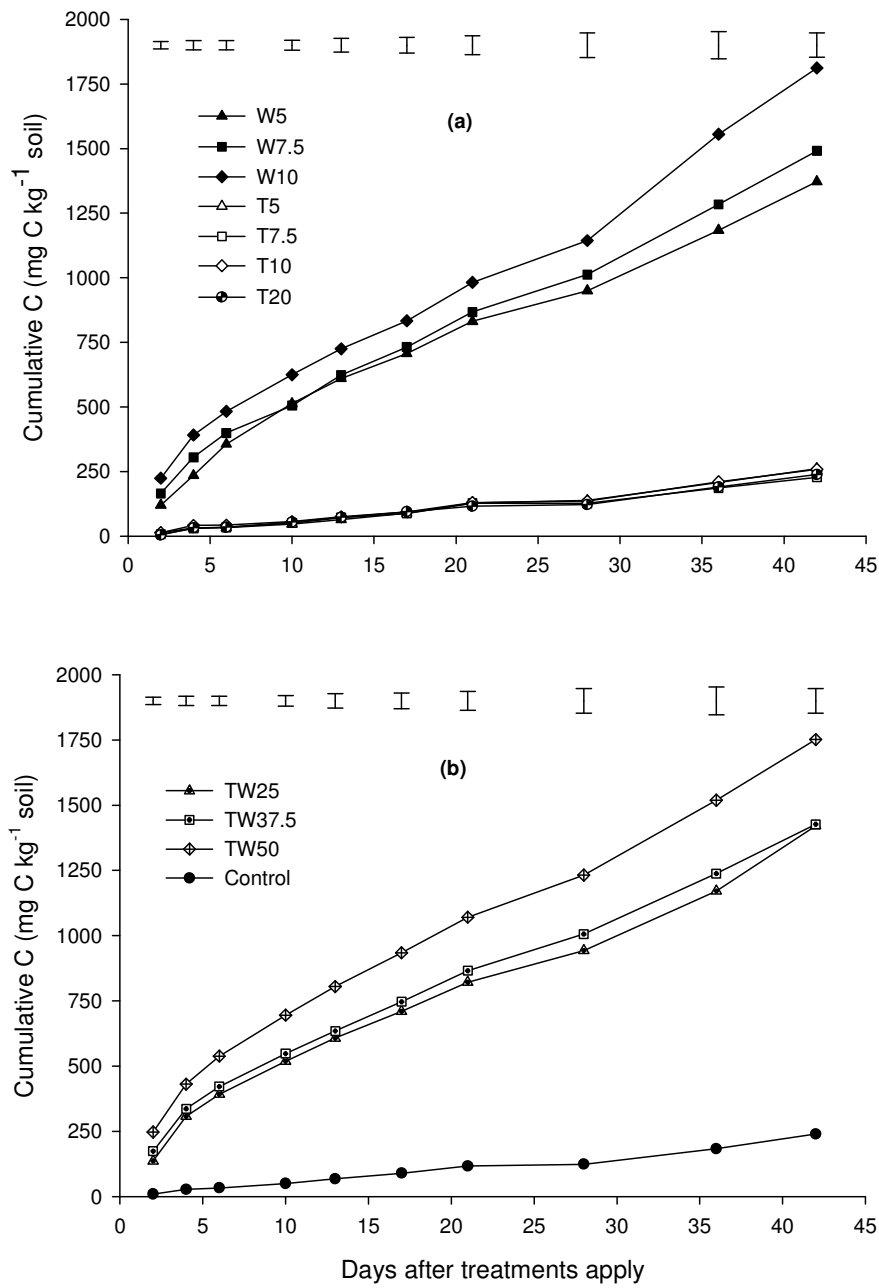
Figure 7.2 Cumulative C released from soils amended with wheat MS and TSP alone (a) and wheat MS+TSP-amended soils and the control over time. Means of 4 replicates, vertical bars indicate least significant difference (LSD$_{0.05}$).

7.3.3 Available P (P_{resin}) concentration

The available P concentration (P_{resin}) differed significantly between soils amended with residue alone, TSP applied with residue and TSP alone. In general, the P_{resin} concentration was in the order of: TSP-amended soils ≥ residue+TSP-amended soils > control > residue-amended soils (Figure 7.3a). The P_{resin} concentration was highest in T20 (13.2 mg P kg^{-1} soil) while it was lowest in W5 (0.6 mg P kg^{-1} soil). On day 7, the P_{resin} concentration in the residue-amended soils was in the order of W5 > W7.5 > W10, on the other hand, in the TSP-amended soils, the P_{resin} concentration increased in the order of T5 < T7.5 < T10 < T20. The P_{resin} concentration in residue+TSP-amended soils followed a similar trend as in residue-amended soils with the lowest P availability in the treatment with the greatest amount of residue (TW50). From day 14 to 42, the P_{resin} concentration in TSP-amended soils and residue+TSP-amended soils decreased (0.4-3.2 mg P kg^{-1} soil) compared to day 7. The P_{resin} concentration in the soils amended with wheat residue alone and the control treatment did not change significantly over time.

7.3.4 Microbial P (P_{mic}) concentration

The microbial P (P_{mic}) concentration generally followed an opposite trend to that of P_{resin} concentration (Figure 7.3b). The P_{mic} concentration was 1.5-2.7 times higher in residue and residue+TSP-amended soils than the soils amended with TSP alone and the control. The P_{mic} concentration was highest in TW50 (10.2 mg P kg^{-1} soil) while it was lowest in T10 (2.8 mg P kg^{-1} soil). In soils amended with residue and residue+TSP, the P_{mic} concentration increased in the order of W5 > W7.5 > W10 and TW25 > TW37.5 > TW50 respectively. With increasing P addition in TSP-amended soils, the P_{mic} concentration remained unchanged whereas the P_{resin} concentration increased. Compared to P_{resin} concentration, P_{mic} concentration was 1.1-11.9 fold

higher in residue and residue+TSP-amended soils but 1.1-4.4 fold lower in the TSP-amended soils. From day 3 to 28, the P_{mic} concentration in W10, TW37.5 and TW50 decreased while it increased in W5.
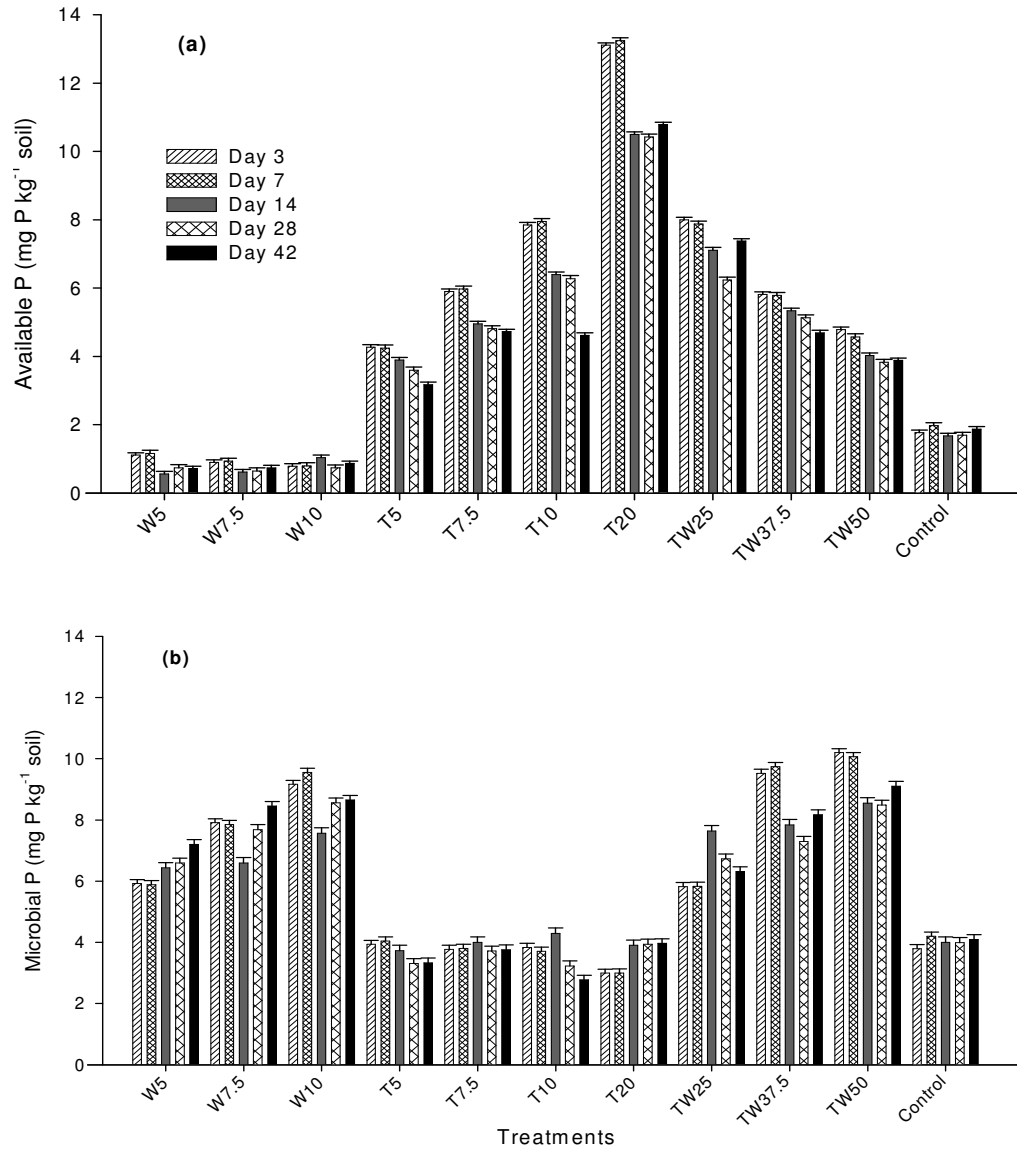
Figure 7.3 Available P (P_{resin}) concentration (a) and microbial P (P_{mic}) concentration (b) in soils amended with wheat, TSP, wheat+TSP and unamended control over time (days). Means of 4 replicates, vertical bars indicate standard error of means (SEM).

7.3.5 Microbial biomass C (C_{mic}) in soil

Microbial biomass C (C_{mic}) was determined only on days 7 and 35 (Figure 7.4). In general, C_{mic} showed a similar trend to P_{mic}. On day 7, C_{mic} was higher in residue-amended soils and residue+TSP-amended soils than the soils amended with TSP alone and the control. On day 7, C_{mic} ranged between 116 and 225 mg C kg^{-1} soil and was highest in W10 and lowest in T20. Compared to the control, C_{mic} was 31%-90% higher in residue-amended soils while it was 63%-71% higher in residue+TSP-amended soils. In TSP-amended soil, C_{mic} was only 17%-26% higher than the control. An exception was T20 where C_{mic} was similar to the control. Compared to day 7, C_{mic} increased on day 35 in all treatments from 21 to 135 mg C kg^{-1} soil. On day 35, C_{mic} was highest in TW37.5 (307 mg C kg^{-1} soil) and lowest in T20 (184 mg C kg^{-1} soil). Compared to the control, C_{mic} was 2%-25% and 19%-28% lower in residue-amended soils and TSP-amended soils, respectively, whereas in residue+TSP-amended soils C_{mic} was 4%-21% higher. In residue-amended soils C_{mic} increased in the order of W5 < W7.5 < W10 (Figure 7.4).

The microbial C: P ratio (C_{mic}: P_{mic}) was generally higher in TSP-amended soils than in the other treatments and ranged between 18:1 and 62:1 (Table 7.2) and it was lowest in TW50 on day 28 and highest in the control on day 42. The higher C addition in this study yielded double the biomass C found in Chapter 6 with respiration rates that were similar. Biomass C: P ratio was wider than in Chapter 6 despite P availability being greater in some treatments.
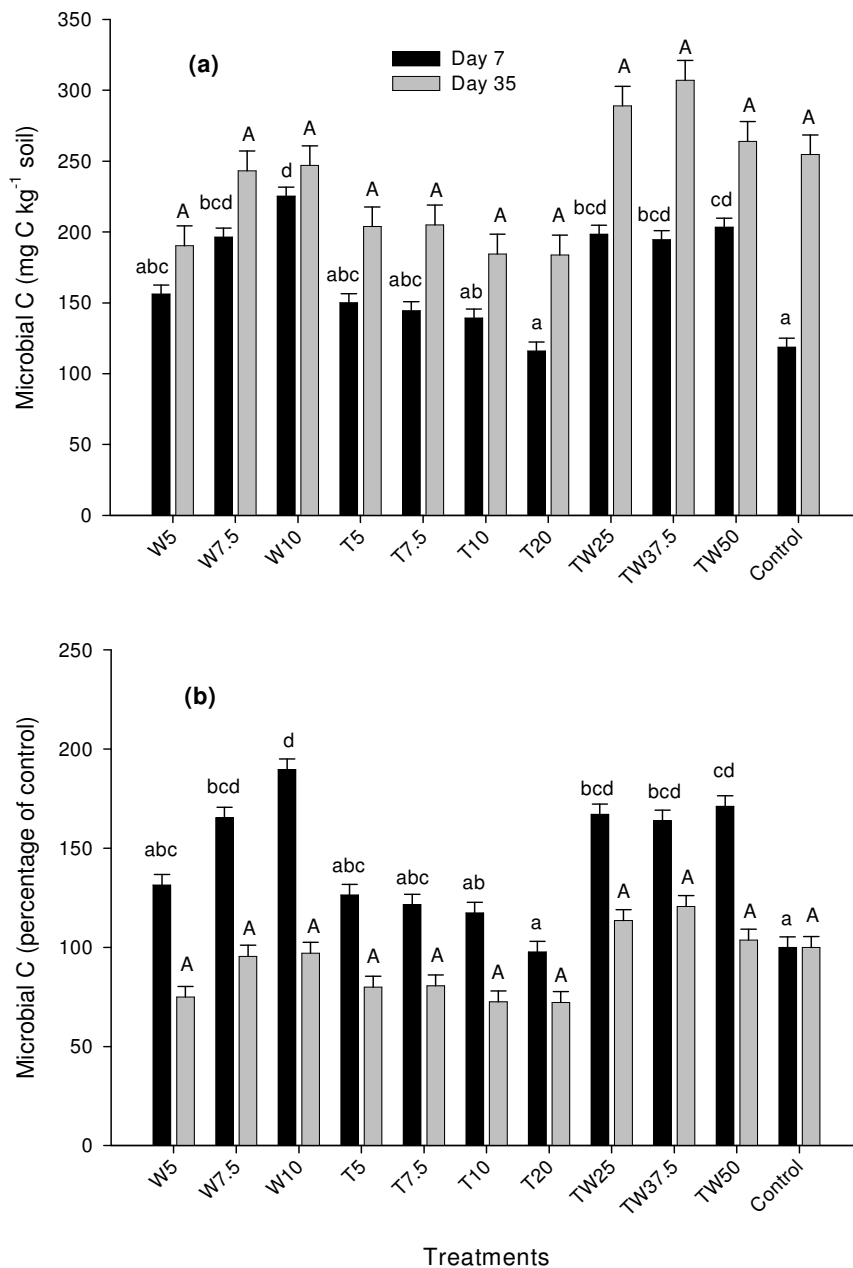
Figure 7.4 Microbial C (a) and microbial C as a percentage of the unamended control in soils amended with wheat, TSP, wheat+TSP and unamended control on days 7 and 35. Means of 4 replicates, vertical bars indicate standard error of means (SEM). Lower case letters indicate significant differences between treatments on day 7 and upper case letters indicate significant differences between treatments on day 35.

Table 7.2 Microbial C: P ratio (C_{mic}: P_{mic}) in soils amended with wheat, TSP, wheat+TSP and unamended control at different days.

Treatments	Microbial C: P ratio (C_{mic}: P_{mic})[2]				
	Day 3	Day 7	Day 14	Day 28	Day 42
W5	27 abc	27 abc	24 a	26 b	26 a
W7.5	25 abc	25 ab	30 ab	32 bc	29 a
W10	24 ab	24.a	30 ab	32 bc	29 a
T5	38 c	37 bc	40 b	45 de	35 ab
T7.5	38 c	38 c	37 ab	52 ef	49 ab
T10	37 bc	38 c	34 ab	59 g	49 ab
T20	34 abc	25 ab	31 ab	56 fg	47 ab
TW25	31 abc	31 abc	25 ab	39 cd	47 ab
TW37.5	22 a	21 a	29 ab	43 de	38 ab
TW50	20 a	20 a	24 a	18 a	35 ab
Control	31 abc	30 abc	30 ab	57 fg	62 b

[2] C_{mic}: P_{mic} ratio was calculated using C_{mic} on day 7 with P_{mic} on day 3, 7 and 14 and C_{mic} on day 35 with P_{mic} on day 35 and 42, Means followed by the same letter within a column are not significantly different ($P \leq 0.001$) by Tukey's multiple range tests.

7.4 Discussion

This study showed that the decomposition dynamics of residue with or without TSP was a function of the amount of C added (Figure 7.2; Table 7.1), but was not related to the P addition rate. Initially higher respiration rates in residue-amended soils and residue+TSP-amended soils than the soil amended with TSP alone and the control (Figure 7.1) was due to C addition resulting in the decomposition of readily available and water-soluble low molecular weight compounds. This is in agreement with the studies described in Chapters 5 and 6 and other studies (Abiven *et al.* 2005; Bertrand *et al.* 2006; Jensen *et al.* 2005; Mary *et al.* 1993; Trinsoutrot *et al.* 2000b; Wang *et al.* 2004).

In this study, the higher biomass C and microbial P and lower resin-extractable P in soils amended with wheat residue compared to the control and TSP alone-amended soils is in agreement with the previous studies (Chapters 5 and 6). However, in this short-term study (42 days), a decrease in microbial P or increase of P_{resin} concentration was not observed in residue-amended soils. In the studies described in Chapters 5 and 6, P_{mic} decreased and P_{resin} concentration increased by approximately 23% and 37% despite plant P uptake after 63 days of incubation with wheat residues. In this study, although respiration rates declined and microbial P uptake remained constant, microbial biomass C was higher on day 35 compared to day 7 and biomass C: P ratio was highest on day 28. This suggests that microbial cells were still growing even if respiration declined and microbial cells were accumulating C rather than respiring C. This is in agreement with the previous study described in Chapter 5. Moreover, the greater biomass C and wider biomass C: P ratio in this study indicates that more C was used for growth (less for respiration) which could be due to the greater amount of residue addition. The wide C: P ratio in the TSP treatment may be due to the lack of C. Since the biomass did not grow, its P requirement would be low.

The P_{mic} concentration increased with the increase of C addition rate which is in agreement with the previous study in Chapter 5, but for a given C addition rate, P_{mic} was not affected by addition of soluble P in residue+TSP-amended soils as compared to residue alone-amended soils (Figure 7.3b). This suggests that microbial P uptake is governed by the amount of C addition and not by the amount of P addition. Hence, P_{mic} concentration had a strong positive correlation with C added with residues (r^2 = 0.83, $P \leq$ 0.01). This is in agreement with the previous study by Bünemann *et al.* (2004) who reported that after addition of maize stover in a fertilized soil (50 kg ha^{-1} year^{-1}) microbial P uptake was not affected by the amount of available P in the soil.

The results of this study showed that the addition of wheat residue with a P content (0.07%) less than the critical value of 0.16% P (Chapter 5) and C: P ratio higher than the critical value of 253: 1 caused net P immobilization by the microbial biomass. In the residue-amended soils, approximately 34%-62% of added P was immobilized by the microbial biomass during 42 days of incubation. As a result of this immobilisation, P_{resin} was decreased by 37%-67% in the residue-amended soils compared to the control. Nevertheless, when TSP was added with wheat residue, the P_{resin} concentration was higher than in the control and approximately 10%-31% of added P was available. This indicates that the P immobilization effect of wheat residue can be offset when residue is applied together with P fertilizer. This study agrees with the previous studies (McLaughlin and Alston 1986a; Reddy *et al.* 2005) who demonstrated that, compared to residue added alone, the combination of residue with inorganic fertilizer increase available P offsetting the P immobilization by the microbial biomass. The present study showed that P immobilization can be offset at any of the combinations of TSP and residue (C: P ratio 155:1 to 310: 1). Moreover, compared to the control, P_{resin} in residue+TSP-amended soils

increased by 3.0 mg P kg^{-1} soil in TW50 (Figure 7.3a). This increased P_{resin} may be adequate for plant growth as the study in Chapter 6 showed that plant growth is optimal when available P increased by 0.7-2.9 mg P kg^{-1} soil above the control. Hence, this study suggests that addition of wheat residue with TSP at 1:1 residue-P: TSP-P ratio (C: P ratio 310: 1) would be useful for improving P availability to achieve optimal plant growth. Available P was highest in TW25, (6.2 mg P kg^{-1} soil) however this high P concentration may not be necessary to achieve good plant growth. Moreover, addition of crop residues may increase soil organic matter content, supplying other plant nutrients and improving soil physical, chemical and biological properties.

In TSP-amended soils, although P_{resin} concentration increased with increasing P addition rate (Figure 7.3) only 46%-61% of added P as TSP was available in the first 7 days and after that, it decreased by 26% to 45%. Hence, a large amount of added P in TSP-amended soils became non-resin extractable P. This non-resin extractable P was either fixed by the soil particles (Bah *et al.* 2006) or entered to the soil P_o pool. However, the nature of this non-resin extractable P could not be identified in this study because P was not labelled and P fractionation was not carried out. In the residue+TSP-amended soils, approximately 51%-67% of added P was non-resin extractable. Nevertheless, in the residue+TSP-amended soils there is a potential to increase available P through turnover of microbial P. The previous studies in Chapters 5 and 6 showed that despite plant P uptake, available P can increase by 23% to 37% due to turnover of the microbial P after 84 days of incubation.

The combination of residue and TSP may not supply immediately as much available P as TSP alone which may limit growth of plants with high P demand in the early stages of growth if planted immediately after residue addition. On the other hand, more than 50% of P added with TSP alone was not resin extractable. This fixed P is unlikely to become available to the crop as

the study in Chapter 5 showed that P_{resin} in the treatment with TSP alone did not increase over time. Thus, addition of residue in combination with inorganic P fertilizer would be more beneficial to the soil and plant available P than addition of TSP alone.

Although plants were not grown in this study, the presence of plants could affect the ratio between P_{resin} and P_{mic} in residue-amended soils with or without TSP. Addition of residue with or without TSP stimulates microbial activity and therefore there will be a competition for available P between plants and microorganisms. Despite plant P uptake, the previous study in Chapter 5 showed that microbial P in wheat MS and also in TSP-amended soils was higher than the control. This additional P may have been solubilized from soil P. Generally, plants and microorganisms release protons, OH^{-}, or CO_2 and organic anions which can increase solubility of inorganic P. Plants and microorganisms also release phosphatases enzymes which mineralize soil organic P (Marschner 2007). Hence, plant exudates could stimulate microbial growth and microbial P uptake by root exudates. This suggests that microbial P could be higher than shown here if plants had been present or possibly the effect of the plant would not be detectable in bulk soil measurement because the effect is limited to the rhizosphere.

The results found in this study may be dependant on soil properties such as P fixation capacity. In the soil used here, P fixation was low, but in a soil with high P fixation there may be little increase in available P with addition of TSP and P released from microbial biomass may not become available. In soil with lower P sorption capacity, addition of residue alone or with TSP may more efficiently increase P availability than soil with higher P adsorption because the former would fix less P and therefore allow a greater build up of microbial and organic P. This agrees with the previous studies by Reddy *et al.* (2001) and Nziguheba *et al.* (1998) who showed that microbial and organic P was higher in low P-adsorbing soil than in high P-

adsorbing soil after 16 weeks of incubation in fertilizer-P alone and in residue+fertilizer-P-amended soils. Soils with high organic C generally have a large microbial biomass. Thus, addition of residue with or without TSP may result in higher microbial P uptake than those found in this study.

Hence, the present study demonstrated that addition of TSP may not completely off-set immobilisation of P after addition of mature wheat residues but available P should be high enough to support plant growth if 50% of the total P addition is added as wheat residues. Phosphorus immobilisation was a function of the amount of C added; therefore microbial P uptake could be manipulated by varying the ratio of wheat residues to TSP to optimize plant P availability and the long-term effects of a build-up of microbial P.

Chapter 8

General discussion and future research

The main objective of the work described in this thesis was to investigate the potential role of crop residues in increasing P availability in P-limited soils typical of a large area of southern Australian farming systems. These soils are generally also characterised by low organic C (<1.0%) and N impoverished. Using a range of techniques described in Chapter 3 the studies reported in Chapters 4-7 provide quantitative evidence over long time periods that some residues release substantial amounts of P during decomposition whereas others result in long-term net P immobilization. In the following paragraphs the most important findings from the work will be discussed, namely the key biochemical properties governing residue decomposition and P release, the relationship between P mobilisation and immobilisation and how P immobilisation may be reduced by combined application of high C: P residues with inorganic P.

8.1 Young crop residues vs. mature crop residues in absence of fertilizer P

Overall, the results showed that crop residues play an important role in P cycling in these soils and hence are a critical component for supply of plant-available P in southern Australian farming systems. Furthermore, these studies examined a larger number of crop residues ranging in age from young to mature than reported by other experiments on these soils, and hence a wider range of residue properties was encompassed. By using a wide range of residues the work provides the opportunity to compare residues with vastly different biochemical properties as well as investigating those which differ only slightly. The correlations between residue properties and decomposition rate, P release, microbial and plant P uptake

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are based on an extensive and statistically validated data set which can be confidently and reliably applied to the quantitative description of P cycling from crop residues in these soils.

The detailed studies showed that both total P content of the residues, and the C: P ratio, affected residue decomposition rate as well as plant growth and P uptake. Additionally, decomposition rate was affected by the C chemistry of the residues. Whereas decomposition rate was positively correlated with the content of easily available C (water-soluble C), it was negatively correlated with the relative content of poorly decomposable C compounds such as lignin and polyphenols. These properties have been shown to be important for residue C and P mineralization in studies using soils from other locations around the world (Abiven *et al.* 2005; Bertrand *et al.* 2006; Dalal 1979; Palm and Rowland 1997; Trinsoutrot *et al.* 2000; Wang *et al.* 2004), and the work reported here confirms, to a large extent, that the same parameters are important for these particular soils. The work also provides some unique data for P release from root residues which are rarely included in studies on residue decomposition and nutrient release, despite the fact that they can represent a large proportion of total plant biomass. Although addition of mature canola shoot residues resulted in long-term P immobilization, canola root residues released as much P as young shoots due to having a similar C: P ratio. However, decomposition of canola roots was delayed because of high lignin and polyphenol content. Some root residues of mature crops may have a substantially lower C: P ratio than shoots. To investigate if decomposing root residues of other crops also released more P than the corresponding shoot residues, mature lupin and wheat roots were added to soil. The results showed that, compared to the control, canola root released much more P (2.8 to 5.4 fold) and increased plant growth (2.3 to 3.7 fold) more than lupin and wheat root residues which had a high C: P ratio. In addition, wheat mature shoot and root residues had similar C: P ratio but decomposition and P release was higher in wheat mature shoot residue than root residue

because of the higher water-soluble C and N, and low lignin and polyphenol content. Thus, canola root residues appear to be a special case which should be investigated further.

In residue-amended soils, P dynamics were controlled by soil microbial activity and microbial nutrient uptake which was governed by the amount of C input associated with the added residues (Chapters 5, 6 and 7). Addition of residues with high C: P ratio caused initial high microbial activity which stimulated microbial P uptake (Chapter 5). Hence, soil microbial activity as measured by respiration rates and microbial P uptake was strongly positively correlated with amount of C added. Net P immobilization of residues with high C: P ratio lasted from 28 to 84 days or more. In addition, the length of net P immobilization was related to the C: P ratio of the residues. For example, a C: P ratio of 781 (lupin mature shoot) resulted in net P immobilization for 63 days whereas at a C: P ratio of 535 (lupin mature root) the period of net P immobilization was only 45 days (Chapter 6).

Residues with high total P concentration ($\geq$ 0.16% P) and low C: P ratio ($\leq$ 253: 1) decomposed faster and released more P (1.7 to 12.8 fold) than the residues with low total P ($\leq$ 0.08% P) and high C: P ratio (> 253: 1) because the P demand of the microbial biomass was satisfied and hence P mineralization exceeded P immobilization. Thus, the average available P concentration in young shoot residue and canola mature root residue-amended soils was 1.1 to 5.6 fold higher compared to the control over 84 days. Plant P uptake in the young shoot and canola mature root-amended soils was 8%-244% higher compared to the control after 84 days. This suggests that addition of young residues and canola root residues not only supply P to plants in the initial stages of decomposition but also over longer periods of time. Compared to TSP-amended soils, available P did not increase as much in soils amended with young shoot and canola mature root residues, because TSP is water-soluble and therefore enters the soil

solution immediately whereas most of the residue P needs to be mineralised before it enters the plant available pool. Nevertheless, P uptake into plant biomass with low C: P residue was as high as with TSP. This suggests that plants take up P that is released from a non-resin extractable P pool which could be organic P in the residues or the soil organic matter, microbial biomass P or poorly available soil P that is released during residue decomposition (Dalal 1979; Kouno *et al.* 2002; Magid *et al.* 1996). However, young shoot, particularly pea young shoot and canola mature root only increased available P and plant growth to a greater extent than TSP when P added with these residues was 3.2 to 4.7 times higher than the amount added with TSP.

Rapid and sustained net P mineralization was expected with addition of residues with a higher total P concentration than the critical level of 0.2% (Fuller *et al.* 1956; Kwabiah *et al.* 2003b). However, canola young shoot had 0.16% P but still resulted in net P mineralisation (Chapters 5 and 6). Although approximately 18%-44% of added P was taken up into the microbial biomass in canola young shoot-amended soil, P mineralization was greater than P immobilization. The contrast between the results obtained here and previous studies indicates that the critical residue P concentration for P immobilization is a function of soil P availability. A reason for the lower critical P level in this study may be that there are less microorganisms in Monarto soil and therefore less P was taken up by microorganisms than in the study by Fuller *et al.* (1956). Australian soils are characterised by a relatively low microbial biomass compared to soils in temperate regions (Broos *et al.* 2007; Franzluebbers 1999; Sparling 1997). This study suggests that the critical P level (0.2% P) for net P mineralization or immobilization is not valid for all soils. It depends on the size of the microbial biomass and the availability of other nutrients that limit the capacity of microorganisms to take up P.

Several of the studies reported in this thesis used three successive crop periods, each period lasting for 28 days, to continuously assess plant P uptake from soils which had been amended with residues and TSP. This experimental design was selected so that the plants would be at a similar growth and nutrient demand stage during the different phases of residue decomposition. Most previous studies on the effect of residue quality on decomposition and P availability were conducted with plants growing from 34 up to 95 days after residue addition (Dalal 1979; Fuller *et al.* 1956; McLaughlin and Alston 1986a; McLaughlin *et al.* 1988a; Nwoke *et al.* 2004). In those studies, plant P demand will be initially high, but will decrease as the plants mature. Thus, in the later stages of decomposition, it is likely that P released from the residues will not be taken up by the plant. This type of experimental setup would then more easily identify residues that release large amounts of P early in the decomposition phase. However, residues with later release of P which are detected by the experimental design used in the present study are perhaps of importance to plant-available P supply in southern Australian cropping systems, where crops are not sown immediately after residue addition. On the other hand, the limitation of the experimental design used here is that no information is obtained concerning grain yield. Moreover, due to the short growth period, C released from the growing plant in form of exudates and dead roots was minimized. This rhizodeposition could enhance microbial P uptake and affect soil P dynamics.

As hypothesised, increasing inorganic fertilizer P addition rate from 1.0 to 20.0 mg P kg^{-1} soil increased both available P and plant P uptake. In this study, P added with TSP was water-soluble and thereby P was immediately available, but over time available P in TSP-amended soils decreased probably due to plant P uptake and/or P-fixation (Chapters 5 and 7). One week after TSP addition approximately 49% of added P as TSP was resin extractable P in the presence of plants and this decreased to 8% after 4 weeks while only 13% and 16% of added

P was taken up by the plant and microbial biomass respectively (Chapter 5). Hence, approximately 63% of P added with TSP fertilizer was not extractable from the soil when plants were grown for a short period (28 days). The percentage of P added that was non-extractable decreased with increasing TSP addition rate (Chapter 7). This suggests that the soil has only a certain capacity to fix P. Inorganic P as TSP addition alone did not increase microbial biomass P which confirms that the microbes in the soil were C not P limited. Thus, although water-soluble P fertilizers provide plants with immediately available P, a large proportion becomes unavailable over time. Addition of low C: P residues on the other hand, may not result in high amounts of immediately available P, but the P supply is more sustained due to P release from decomposing residues and turnover of microbial biomass P.

There was a significant negative relationship between soil available P and microbial P in the experiment with different amounts of C added to achieve the same amount of P (Chapter 5). However, the relationship was not significant in the study with different amounts of P added to achieve the same amount of C (Chapter 6) which can be explained by the narrower range of microbial P values in the C addition experiment. Despite plant P uptake, the decrease of microbial led to an increase of available P; microbial P decreased by 1.0 to 8.2 mg P kg^{-1} soil whereas available P increased by only 0.1 to 1.1 mg P kg^{-1} soil by 35 days. Thus only a small proportion of microbial P enters the available P pool, while the rest becomes unavailable. In mature shoot-amended soils, net P mineralization occurred after about 73 days except for canola mature shoot (C: P ratio 858: 1) however the increase in available P in mature shoot-amended soils was not sufficient for optimal plant growth. Therefore, addition of inorganic P fertilizer with mature shoot residues would be necessary for optimal plant growth.

8.2 Combining residues with fertilizer P

The studies showed that addition of young crop residues can strongly increase P availability in these soils, and indicates that situations where 'green manuring' is practised will prove valuable as an alternative to input of inorganic P sources in southern Australian farming systems. However, in the low rainfall regions (< 300 mm growing season rainfall) green manuring is often not viewed as an economically viable option. Instead, crop residues that are left in the field after grain harvest will generally have a low total P concentration and high C: P ratio and are likely to cause P immobilization as discussed above. In this case, the combination of these crop residues with addition of TSP, as is current practise, may be a reasonable approach to managing soil P availability in respect of plant demand during the growing season. The experiment in this thesis which combined application of mature wheat shoot residue with TSP showed that increasing C addition rates from 3.1 to 6.2 g C kg^{-1} soil increased microbial P uptake both in soils amended with residue alone or residue+TSP-amended soils as expected. On the other hand, available P increased only in residue+TSP-amended soils but not in soils amended with residue alone. This can be explained by the differences in C: P ratio. While the C: P ratio was 615: 1 when wheat residue was added alone, TSP addition to wheat residue decreased the C: P ratio to 155: 1 to 310: 1. This suggested that P immobilization after addition of residues which have low P content (0.08% P) and high C: P ratio (615: 1) may be offset when residue is applied together with inorganic P fertilizer if the resulting C: P ratio is 300: 1 or less. This C: P ratio is similar to that of the young residues which increased P availability immediately but slightly less than the residue+TSP treatment because P as TSP was water-soluble. Moreover, P availability was lower in the combined treatment than if TSP is applied alone over the 42 days of the experiment.

Although P cycling from additions of plant residues available in broad-acre farming cannot completely replace inorganic P fertilizer, residue addition has other beneficial effects such as the stimulation of microbial activity and subsequently nutrient cycling, and improvement of soil structure. Hence, residue addition can have positive long-term effects that go beyond providing nutrients for the current crop.

This study has highlighted the potential role that crop residues, either alone or in combination with inorganic P, can play in increasing P availability in the light textured, low organic matter, P-limited soils typical of many southern Australian farming systems. Young P-rich crop residues were as effective as inorganic P in increasing growth and P uptake of young wheat plants. It is acknowledged that the results of this study may not be directly transferable to the field situation since the incubation studies were conducted under optimal conditions for decomposition. In the field various environmental factors, especially moisture, temperature, disease, soil organism diversity, availability of other nutrients and soil properties all influence P dynamics and plant P uptake. Nevertheless, the results provide important quantitative information about the potential of a wide range of crop residues to supply plant-available P for wheat. This quantitative information will be valuable for the construction or validation of mechanistic models of residue decomposition for low organc matter light textured soils and will ultimately assist in the development of economic management strategies for minimizing P fertilizer inputs and maximizing the benefits of biollogical cycling of P in farming systems of southern Australia.

8.3 Further research

Although it was evident that addition of certain crop residues caused initial P immobilization by the microbial biomass, young residues resulted in an immediate increase of available P and plant P uptake while for some mature residues P availability increased after about 8 weeks.

Nevertheless, it is not clear if the increase in available P in the later phases is due to turnover of microbial P or release of soil P. It is also not clear how much of the P mineralized from the residues was taken up by the plants during a growth period. The use of labelled P to track P from crop residues in different P pools and plants could provide answers to these questions, particularly in combination with sequential fractionation.

Root residues are rarely used in studies on decomposition; however the strong P mineralisation of canola roots raises the question which other root residues have similar high P release rates. The two other root residues used in the present study (wheat and lupin) did not release P to a similar extent. Clearly, more studies with a range of root residues should be conducted.

The experiments in this study were conducted under controlled conditions. They would need to be verified under field conditions or at least under conditions that reflect field conditions better. Experiments with varying soil moisture, such as drying and rewetting cycles could be conducted. It would be important to observe plant P uptake in the residue-amended soils over the entire growth period of the plants to assess the long-term effect of residues on plant growth. This could be particularly interesting if plants are sown at different times after residue addition. In this study the effect of inorganic P fertilizer to offset P immobilization was investigated only with one crop residue. Further investigation with various types of residues (cereals, legumes, shoot or root) combined with inorganic fertilizer could be used to relate residue properties to the amount of inorganic P needed to offset P immobilization and for optimal plant growth.

One of the factors limiting the use of residues as alternative to inorganic P fertilizers in dryland agriculture is the amount of residue obtained. Also the amount of residue available to apply to a soil and supply nutrients depends on the plant species, yield, quality, and residue management

such as green manuring or addition of mature residue after harvest. Therefore it would be important to investigate the fertilizer required to obtain plant residues with sufficiently high P concentrations. This fertilizer requirement would have to be weighted against the benefit of using residues as P source because addition of plant residue provides additional benefits such as increase soil organic matter content, supply other plant nutrients and improve physical, chemical and biological properties of soil.

In the present studies, the residue addition rates were relatively high compared to those in dryland agriculture. Future experiments should investigate with lower rates of residue addition and test the effect of residue amount on P availability. The soils used in the experiments had a very low P availability in order to achieve the maximal effect of P added with residues. Since the range of P concentrations in Australian cropping soils is quite large, experiments should also be conducted in soils with higher P availability to investigate if residue alone could also be a P source in such soils.

Lastly, the economic studies such as fertilizer needed to produce large amounts of residues with sufficiently high P concentration and the use of green manuring versus use of mature crop residues should be conducted. A study of the cost associated with growing large amount of green manuring crops and/or mature crop residues versus transporting crop residues from other places would be useful to determine the economic benefits of adding crop residues.

Chapter 9

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Chapter 10

Appendices

Appendix 1 Chemical shift regions into which the acquired total ^{13}C NMR signal intensity were divided. The name of the major types of carbon associated with each chemical shift region and the calculation used to determine the percentage of total signal intensity.

Chemical shift region (ppm)	Proposed dominant type of C	Calculations used to quantify the amounts of signal intensity associated with each type of carbon
0 - 45	Alkyl C	(0-45 ppm)-(215-245 ppm)
45 - 65	N-alkyl C and Methoxyl	(45-65 ppm)-(245-265 ppm)
65 - 90	O-alkyl C	(65-90 ppm)-(265-290 ppm)
90 - 110	Di-O-Alkyl C	((90-110 ppm)
110 - 145	Aryl and unsaturated C (Aromatic)	(110-145 ppm)+2*(215-245 ppm)
145 - 165	O-Aryl C (phenolic and furan)	(145-165 ppm)+2*(245-265 ppm)
165 - 190	Carbonyl and Amide C	(165-190 ppm)+2*(265-290 ppm)
190 - 215	Ketone C	(190-215 ppm)
215 - 245	Aryl spinning side band	
245 - 265	O-Aryl spinning side band	
265 - 290	Carbonyl and amide spinning side band	
-50 and 300	Total signal intensity	

Adapted from Baldock and Smernik (2002)

Appendix 2 Photos of pot arrangement and plant growth in the experiment with P-based residue addition in the glasshouse.

Figure 10.1 Photos of pots arrangement (a) and comparision of plant growth between canola root and TSP to control (b) in the glasshouse in the experiment with P-based residue addition.

Appendix 3 Correlation coefficient (r^2) of residue properties with soil and plant variables during incubation in the study of P dynamics in the experiment with P based residue addition.

Time	Parameters	P_t	N_t	P_{H2O}	C_{H2O}	N_{H2O}	Lignin	O-alkyl	Phenolic	Amide	C:P	C:N	C addition
Crop period 1, Day 7	P_{resin}	0.68**	ns	0.55*	ns	ns	ns	ns	ns	ns	-0.64**	ns	-0.64**
	P_{mic}	-0.65**	ns	-0.54*	ns	ns	ns	ns	ns	ns	0.58**	ns	0.57*
	C_{mic}	-0.58*	ns	-0.61**	ns	-0.41*	ns	ns	ns	ns	0.56*	ns	0.56*
	Respiration rate	-0.80***	ns	-0.71**	ns	-0.43*	ns	ns	ns	ns	0.75***	ns	0.73**
	Cumulative C	ns	ns	-0.36*									
	C_{min}/Decomp	0.63**	0.85***	0.41*	0.77***	0.90***	-0.52*	0.74**	-0.54*	0.83***	-0.61**	-0.69**	-0.61**
Crop period 1, Day 28	P_{resin}	0.83***	0.83***	0.45*	ns	0.68**	ns	0.68**	ns	0.78***	-0.70**	-0.71**	-0.71**
	P_{mic}	-0.74***	-0.51*	-0.83***	ns	-0.58*	ns	ns	ns	-0.42*	0.86***	0.62**	0.86***
	Respiration rate	-0.82***	-0.64**	-0.66**	-0.40*	-0.61**	ns	-0.51*	ns	-0.60*8	0.95***	0.75***	0.96***
	Cumulative C												
	C_{min}/Decomp	0.65**	0.71**	0.58*	0.74***	0.84***	-0.48*	0.57*	-0.41*	0.70**	-0.75***	-0.73**	-0.76***
	Plant DM	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Plant P uptake	0.46*	ns	0.50*	ns	ns	ns	ns	ns	ns	-0.49*	ns	-0.49*
Crop period 2, Day 35	P_{resin}	0.78***	ns	0.75***	ns	ns	ns	ns	ns	ns	-0.83***	-0.50*	-0.84***
	P_{mic}	-0.87***	-0.57*	-0.77**	-0.41*	-0.61**	ns	-0.42*	ns	-0.48*	0.98***	0.57*	0.98***
	C_{mic}	-0.45*	ns	ns	ns	ns	ns	ns	ns	ns	0.54*	ns	0.53*
	Respiration rate	-0.81***	-0.63**	-0.69**	ns	-0.60**	ns	-0.74*	ns	-0.57*	0.95***	0.75***	0.96***
	Cumulative C												
	C_{min}/Decomp	0.65**	0.68**	0.61**	0.74***	0.83***	-0.47*	0.53*	ns	0.67**	-0.76***	-0.72**	-0.77***
Crop period 2, Day 56	P_{resin}	0.64**	0.39*	0.76***	0.45*	0.48*	ns	ns	ns	ns	-0.82***	-0.62**	-0.83***
	P_{mic}	-0.79***	-0.53*	-0.81***	-0.50*	-0.65**	ns	-0.41*	ns	-0.46*	0.95***	0.54*	0.95***
	Respiration rate	-0.76***	-0.63**	-0.71**	-0.43*	-0.65**	ns	-0.41*	ns	-0.57*	0.89***	0.79***	0.90***
	Cumulative C												
	C_{min}/Decomp	0.66**	0.65**	0.64**	0.74***	0.81***	-0.44*	0.50*	ns	0.64**	-0.78***	-0.69**	-0.78***
	Plant DM	0.69**	0.54*	ns	ns	ns	ns	0.46*	ns	ns	-0.50*	ns	-0.49*
	Plant P uptake	0.71**	0.58**	ns	ns	ns	ns	0.42*	ns	ns	-0.47*	ns	-0.47*
Crop period 3, Day 63	P_{resin}	0.93***	0.55*	0.66**	ns	0.42*	ns	ns	ns	ns	-0.86***	-0.44*	-0.86***
	P_{mic}	-0.73**	-0.47	-0.70**	ns	-0.46*	ns	ns	ns	ns	0.91***	0.56*	0.92***
	C_{mic}	-0.79***	-0.45*	-0.68**	ns	ns	ns	ns	ns	ns	0.87***	0.55*	0.87***
	Respiration rate	-0.73**	-0.56*	-0.70**	ns	-0.58*	ns	ns	ns	-0.50*	0.87***	0.74***	0.88***
	Cumulative C												
	C_{min}/Decomp	0.67**	0.65**	0.65**	0.74***	0.81***	-0.43*	0.50*	ns	0.63**	-0.79***	-0.69**	-0.80***

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns, non-significant at 5% level of significance. P_t, residue total P, N_t, residue total N, C_{min}/Decomp, residue decomposition rate

Appendix 3 (Continued)

Time	Parameters	P_t	N_t	P_{H2O}	C_{H2O}	N_{H2O}	Lignin	O-alkyl	Phenolic	Amide	C:P	C:N	C addition
Crop period 3, Day 84	P_{resin}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	P_{mic}	-0.72**	-0.60**	-0.61**	-0.45*	-0.62**	ns	-0.47*	ns	-0.51*	0.88***	0.63**	0.89***
	Respiration rate Cumulative C	-0.66**	-0.56*	-0.61**	ns	-0.57*	ns	ns	ns	-0.53*	0.82***	0.79***	0.84***
	C_{min}/Decomp	0.67**	0.61**	0.67**	0.72**	0.77***	-0.41*	0.47*	ns	0.60**	-0.81***	-0.67**	-0.81***
	Plant DM	0.94***	0.59**	0.75***	ns	0.54*	ns	0.42*	ns	0.45*	-0.92***	-0.48*	-0.91***
	Plant P uptake	0.66**	ns	0.53*	ns	ns	ns	ns	ns	ns	-0.86***	-0.53*	-0.87***

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns, non-significant at 5% level of significance. P_t, residue total P, N_t, residue total N, C_{min}/Decomp, residue decomposition rate

Appendix 4 Correlation coefficient (r^2) between soil variables and soil P_{resin}, P_{mic} and C_{mic} over time in the study of P dynamics in the experiment with P based residue addition.

Days of Incubation	Measured variables	P_{resin}	P_{mic}	C_{mic}	Respiration rate	Cumulative C	C_{min}/Decomp	Plant DM	Plant P uptake
Day 7	P_{resin}	1.00	-0.50**	-0.56**	-0.49*	-0.48*	ns	nd	nd
	P_{mic}		1.00	0.81***	0.83***	0.80***	ns	nd	nd
	C_{mic}			1.00	0.68***	0.49*	-0.50*	nd	nd
Day 17	P_{resin}	1.00	-0.62**	nd	-0.67***	-0.65**	0.54*	nd	nd
	P_{mic}		1.00	nd	0.93***	0.68***	-0.75***	nd	nd
	C_{mic}			1.00	nd	nd	nd	nd	nd
Day 28	P_{resin}	1.00	-0.38*	nd	-0.54**	ns	0.66**	0.72***	0.73***
	P_{mic}		1.00	nd	0.83**	0.83***	-0.72**	-0.37*	-0.36*
	C_{mic}			1.00	nd	nd	nd	nd	nd
Day 35	P_{resin}	1.00	-0.56**	-0.40*	-0.57**	-0.59**	0.49*	nd	nd
	P_{mic}		1.00	0.55**	0.95***	0.90***	-0.81***	nd	nd
	C_{mic}			1.00	0.49*	0.67***	ns	nd	nd
Day 45	P_{resin}	1.00	-0.58**	nd	-0.57**	-0.60**	0.64**	nd	nd
	P_{mic}		1.00	nd	0.87***	0.96***	-0.84***	nd	nd
	C_{mic}			1.00	nd	nd	nd	nd	nd
Day 56	P_{resin}	1.00	-0.51**	nd	-0.67***	-0.64**	0.76***	0.72***	0.66***
	P_{mic}		1.00	nd	0.83***	0.87***	-0.87***	-0.50*	-0.55**
	C_{mic}			1.00	0.49*	nd	nd	nd	nd
Day 63	P_{resin}	1.00	-0.58**	-0.71***	-0.59**	-0.62**	0.55*	nd	nd
	P_{mic}		1.00	0.76***	0.94***	0.82***	-0.67***	nd	nd
	C_{mic}			1.00	0.73***	0.83***	-0.54*	nd	nd
Day 73	P_{resin}	1.00	-0.52**	nd	-0.59**	-0.49*	0.61**	nd	nd
	P_{mic}		1.00	nd	0.91***	0.82***	-0.86**	nd	nd
	C_{mic}			1.00	nd	nd	nd	nd	nd
Day 84	P_{resin}	1.00	ns	nd	ns	ns	ns	0.78***	0.62**
	P_{mic}		1.00	nd	0.91***	0.78***	-0.78***	-0.46*	-0.82***
	C_{mic}			1.00	nd	nd	nd	nd	nd

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns, non-significant at 5% level of significance. C_{min}/Decomp, residue decomposition rate, not determined.

P_{resin}, Available P, P_{mic}, Microbial P and C_{mic}, Microbial biomass C

Appendix 5 Photos of plant growth and pot arrangement in the glasshouse at crop period 3 in the experiment with C-based residue addition.
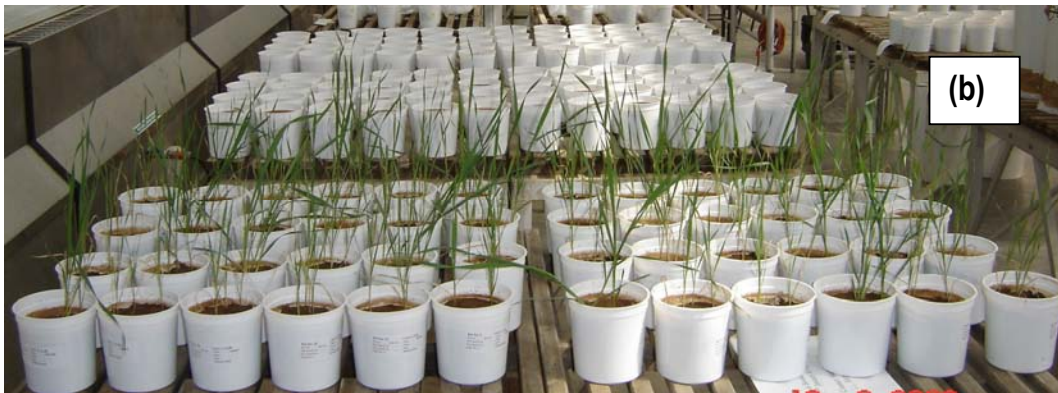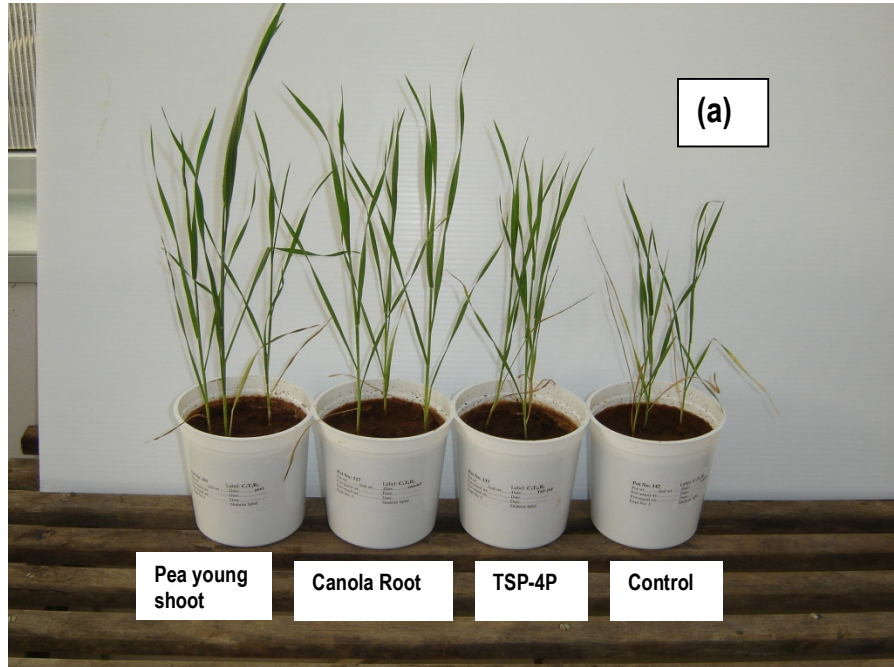

Figure 10.2 Photos of plant growth comparison (a) and pot arrangement in the glasshouse in the experiment with C-based residue addition experiment.

Appendix 6 the correlation coefficient (r^2) of residue properties with soil and plant variables during incubation in the study of P dynamics in the experiment with C-based residue addition.

Time	Parameters	P_t	N_t	P_{H2O}	C_{H2O}	N_{H2O}	Lignin	O-alkyl	Phenolics	Amide	C:P	C:N	P addition
Crop period 1, Day 7	P_{resin}	0.80**	ns	ns	ns	ns	ns	ns	ns	ns	-0.57**	ns	0.79**
	P_{mic}	ns	ns	Ns	ns	0.58*	0.53*	ns	ns	0.65*	ns	0.59*	ns
	C_{mic}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Respiration rate	ns	ns	ns	ns	ns	-0.54*	ns	ns	ns	ns	ns	ns
	Cumulative C	ns	0.58*	0.58*	0.89***	0.91***	-0.86***	0.59*	-0.78**	0.72**	ns	-0.53*	0.51*
	C_{min}/Decomp	ns	0.59*	0.57*	0.89***	0.91***	-0.87***	0.60*	-0.81**	-0.72**	ns	-0.52*	0.51*
Crop period 1, Day 28	P_{resin}	0.92***	0.67*	0.54*	ns	ns	ns	0.57*	ns	ns	-0.70**	ns	0.93***
	P_{mic}	0.71**	ns	0.56*	ns	ns	ns	ns	ns	ns	-0.56*	ns	0.72**
	Respiration rate	ns	-0.83**	ns	-0.55*	-0.86***	ns	-0.81**	ns	-0.80**	ns	0.63*	ns
	Cumulative C	ns	ns	0.61*	0.81**	0.77**	-0.94***	ns	-0.78**	0.52*	ns	ns	ns
	C_{min}/Decomp	ns	ns	0.61*	0.84***	0.74**	-0.93***	ns	-0.82**	ns	ns	ns	ns
	Plant DM	0.70*	ns	ns	ns	ns	ns	ns	ns	ns	-0.61*	ns	0.67*
	P uptake	0.84***	ns	ns	ns	ns	ns	ns	ns	ns	-0.61*	ns	0.82**
Crop period 2, Day 35	P_{resin}	0.86***	0.58*	ns	ns	ns	ns	ns	ns	ns	-0.61*	ns	0.86***
	P_{mic}	0.78**	ns	0.7*	ns	ns	ns	ns	ns	ns	-0.69*	ns	0.76**
	C_{mic}	ns	ns	0.60*	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Respiration rate	ns	-0.72**	ns	-0.54*	-0.82**	0.67*	-0.85***	0.58*	-0.69**	ns	ns	ns
	Cumulative C	ns	ns	0.57*	0.83**	0.74**	-0.95***	ns	-0.78**	ns	ns	ns	ns
	C_{min}/Decomp	ns	ns	0.62*	0.82**	0.69*	-0.91***	ns	-0.80	ns	ns	ns	ns
Crop period 2, Day 56	P_{resin}	0.93***	ns	0.57*	ns	ns	ns	ns	ns	ns	-0.73**	ns	0.93***
	P_{mic}	0.82**	ns	0.57*	ns	ns	ns	ns	ns	ns	-0.65*	ns	0.79**
	Respiration rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Cumulative C	ns	ns	0.62*	0.76**	0.67*	-0.93***	ns	-0.80**	ns	ns	ns	ns
	C_{min}/Decomp	ns	ns	0.62*	0.80**	0.64*	-0.91***	ns	-0.83**	ns	ns	ns	ns
	Plant DM	0.88***	ns	ns	ns	ns	ns	ns	ns	ns	-0.66*	ns	0.86***
	P uptake	0.84**	ns	ns	ns	ns	ns	ns	ns	ns	-0.65*	ns	0.82**

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns, non-significant at 5% level of significance. P_t, residue total P, N_t, residue total N, C_{min}/Decomp, residue decomposition rate

Appendix 6 (continued)

Time	Parameters	P_t	N_t	P_{H2O}	C_{H2O}	N_{H2O}	Lignin	O-alkyl	Phenolic	Amide	C:P	C:N	P addition
Crop period 3, Day 63	P_{resin}	0.75**	ns	0.50*	ns	ns	ns	ns	ns	ns	-0.64*	ns	0.79**
	P_{mic}	0.90***	0.56*	0.71**	ns	ns	ns	0.54*	ns	ns	-0.81**	ns	0.92***
	C_{mic}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Respiration rate	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Cumulative C	ns	ns	0.61*	0.76**	0.68*	-0.94***	ns	-0.81**	ns	ns	ns	ns
	C_{min}/Decomp	ns	ns	0.61*	0.79**	0.64*	-0.92***	ns	-0.84	ns	ns	ns	ns
Crop period 3, Day 84	P_{resin}	0.75*	0.78**	ns	ns	0.54*	ns	0.71**	ns	0.56*	-0.53*	ns	0.78**
	P_{mic}	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	Respiration rate	ns	-0.51*	ns	ns	ns	ns	ns	ns	-0.64*	ns	0.58*	ns
	Cumulative C	ns	ns	0.60*	0.74**	0.64*	-0.93***	ns	-0.81**	ns	ns	ns	ns
	C_{min}/Decomp	ns	ns	0.60*	0.77**	0.62*	-0.92***	ns	-0.84**	ns	ns	ns	ns
	Plant DM	0.88***	ns	ns	ns	ns	ns	ns	ns	ns	-0.63*	ns	0.87***
	P uptake	0.89***	ns	ns	ns	ns	ns	ns	ns	ns	-0.64*	ns	0.87***

*** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, ns, non-significant at 5% level of significance. P_t, residue total P, N_t, residue total N, C_{min}/Decomp, residue decomposition rate

Appendix 7 Correlations coefficient (r^2) between soil variables and soil P_{resin}, P_{mic} and C_{mic} over time in the study of P dynamics in the experiment with C based residue addition.

Days of Incubation	Measured variables	P_{resin}	P_{mic}	C_{mic}	Respiration rate	Cumulative C	C_{min}/Decomp	Plant DM	Plant P uptake
Day 7	P_{resin}	1.00	ns	ns	ns	ns	ns	nd	nd
	P_{mic}		1.00	0.38*	0.96***	0.82***	-0.66*	nd	nd
	C_{mic}			1.00	ns	ns	ns	nd	nd
Day 17	P_{resin}	1.00	ns	nd	ns	ns	ns	nd	nd
	P_{mic}		1.00	nd	0.58**	0.89***	0.52*	nd	nd
	C_{mic}			1.00	nd	nd	nd	nd	nd
Day 28	P_{resin}	1.00	ns	nd	ns	ns	ns	0.76***	0.94***
	P_{mic}		1.00	nd	0.60**	0.85***	ns	ns	ns
	C_{mic}			1.00	nd	nd	nd	nd	nd
Day 35	P_{resin}	1.00	ns	ns	ns	ns	ns	nd	nd
	P_{mic}		1.00	0.81***	0.36*	0.86***	ns	nd	nd
	C_{mic}			1.00	ns	0.47*	ns	nd	nd
Day 45	P_{resin}	1.00	ns	nd	ns	ns	ns	nd	nd
	P_{mic}		1.00	nd	0.35*	0.90***	ns	nd	nd
	C_{mic}			1.00	nd	nd	nd	nd	nd
Day 56	P_{resin}	1.00	ns	nd	ns	ns	ns	0.95***	0.96***
	P_{mic}		1.00	nd	ns	0.83***	ns	ns	ns
	C_{mic}			1.00	0.49*	nd	nd	nd	nd
Day 63	P_{resin}	1.00	0.41*	ns	ns	ns	ns	nd	nd
	P_{mic}		1.00	0.48*	0.49**	0.85***	Ns	nd	nd
	C_{mic}			1.00	ns	ns	ns	nd	nd
Day 73	P_{resin}	1.00	ns	nd	ns	ns	ns	nd	nd
	P_{mic}		1.00	nd	0.61**	0.73***	ns	nd	nd
	C_{mic}			1.00	nd	nd	nd	nd	nd
Day 84	P_{resin}	1.00	ns	nd	ns	ns	ns	0.86***	0.92**
	P_{mic}		1.00	nd	0.43*	0.67***	ns	ns	ns
	C_{mic}			1.00	nd	nd	nd	nd	nd

*** $P \le 0.001$, ** $P \le 0.01$, * $P \le 0.05$, ns, non-significant at 5% level of significance. C_{min}/Decomp, residue decomposition rate, DNM, not determined. P_{resin}, Available P, P_{mic}, Microbial P and C_{mic}, Microbial biomass C

Appendix 8 Average P_{resin}, P_{mic}, plant P uptake and Total P (P_{resin}, P_{mic}, plant P uptake)

(mg pot^{-1}) during 3 crop periods

Treatment	Parameters (mg pot^{-1})	Based on same amount of P addition			Based on same amount of C addition		
		Crop period 1	Crop period 2	Crop period 3	Crop period 1	Crop period 2	Crop period 3
Lupin YS	P_{resin}	**1.4**	**2.5**	**1.8**	**0.9**	**1.0**	**1.0**
	P_{mic}	**10.3**	**7.7**	**7.2**	**7.1**	**6.5**	**5.3**
	Plant P uptake	**0.42**	**0.14**	**0.81**	**0.37**	**0.63**	**0.49**
	Total P (TP)	**12.1**	**10.4**	**9.8**	**8.4**	**8.1**	**6.8**
	P_{resin} (% of TP)	12	25	18	11	12	15
	P_{mic} (% of TP)	85	74	74	85	80	78
	Plant P uptake (% of TP)	3	1	8	4	8	7
Wheat MS	P_{resin}	**0.3**	**0.8**	**1.3**	**0.2**	**0.5**	**0.6**
	P_{mic}	**15.7**	**13.0**	**9.5**	**6.4**	**5.4**	**5.1**
	Plant P uptake	**0.12**	**0.09**	**0.30**	**0.17**	**0.32**	**0.24**
	Total P (TP)	**16.0**	**13.8**	**11.1**	**6.8**	**6.3**	**5.9**
	P_{resin} (% of TP)	2	5	12	3	8	10
	P_{mic} (% of TP)	97	94	85	94	87	86
	Plant P uptake (% of TP)	1	1	3	3	5	4
Canola MR	P_{resin}	**2.7**	**3.2**	**2.6**	**2.2**	**2.1**	**2.0**
	P_{mic}	**9.1**	**8.3**	**7.3**	**7.4**	**7.2**	**6.8**
	Plant P uptake	**1.42**	**0.36**	**0.63**	**1.49**	**1.85**	**1.10**
	Total P (TP)	**13.2**	**11.9**	**10.5**	**11.1**	**11.2**	**9.9**
	P_{resin} (% of TP)	20	27	25	20	19	20
	P_{mic} (% of TP)	69	70	69	67	65	69
	Plant P uptake (% of TP)	11	3	6	13	17	11
Control	P_{resin}	**1.4**	**1.8**	**1.2**	**0.6**	**0.7**	**0.7**
	P_{mic}	**4.9**	**4.9**	**4.9**	**3.0**	**3.0**	**2.6**
	Plant P uptake	**0.76**	**0.55**	**0.75**	**0.27**	**0.47**	**0.34**
	Total P (TP)	**7.1**	**7.2**	**6.9**	**3.8**	**4.2**	**3.6**
	P_{resin} (% of TP)	19	25	18	16	18	19
	P_{mic} (% of TP)	70	67	71	77	71	72
	Plant P uptake (% of TP)	11	8	11	7	11	9