The effects of legumes on arbuscular mychorrhizal colonisation and phosphorus uptake on wheat

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Grateful to God, the Almighty

Dedicated to my beloved Father

Andi Mappelawa

you are always in my memory and my heart

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ABSTRACT

A number of studies have shown that biomass and P uptake in cereals following legumes are higher than in cereals following cereals. The positive effect of legumes on the following wheat may be due to the growth of legumes prior to wheat and/or due to nutrients released during decomposition of legume residue that are used by the subsequent wheat. The aim of this study is to assess the effects of legumes and/or their residues on AM colonisation, P uptake and the growth of the following wheat.

A series of short experiments were carried out to assess the relationship between P addition, P availability, P uptake and AM colonisation of wheat in a soil with low P availability under conditions in which N was not limiting. Young and mature faba bean shoots (FYS, FMS) and mature chickpea shoots (CP) were added to the soil at different rates. Other treatments included addition of inorganic P at different rates. As expected, inorganic P addition increased growth and P uptake, but decreased AM colonisation. AM colonisation was not correlated with available P in the soil amended with residues, whereas there was significant negative correlation between available P and AM colonisation within the treatments with inorganic P. FMS and CP addition not only decreased wheat growth and P uptake but also AM colonisation despite low P availability in the soil. It is concluded that addition of some legume residues cannot be explained solely by soil P availability.

The aim of the first experiment with legume pre-crops was to identify the effect of legumes as a pre-crop and their residues on AM colonisation and P uptake by the following wheat. Four pre-crops (chickpea, faba bean, white lupin and wheat) were grown for 10 weeks in the loamy sand. Before planting wheat as the following crop, several treatments were imposed: (1) both roots and shoots of the pre-crop were removed completely; (2) only roots (0.04 % w/w) were added back into the soil to determine; (3) only shoot residues(0.24% w/w) mixed with soil; (4) a mixture of shoot and root residues (0.24% shoots + 0.04 % w/w roots) was added. Wheat growth and P uptake were greatest in the previously unplanted soil. Among the legume pre-crops, only white lupin increased the growth and P uptake but decreased AM colonisation of the following wheat compared with wheat as a pre-crop.

The aim of last study was to investigate the effect of legume pre-crop and soil water content during the fallow period on P uptake and AM colonisation by the following wheat. The experimental design was similar as in the study described above but there was fallow period of one month. During the fallow there were two treatments: (1) soil moisture was maintained at 70% water-holding capacity, and (2) allowed to dry and maintained dry until wheat sowing and rewet to 70% water-holding capacity. Dry weight was generally similar with previous study while N and P concentrations of faba bean and white lupin were higher in this study. If compared with previous experiment without fallow time, this experiment showed a surprising results for N concentrations were about 50% lower in constant moisture treatment, while in drying-rewetting treatment resulted similar value with previous experiment.

DECLARATION

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1 REVIEW OF LITERATURE

1.1 Introduction

Wheat is a staple food and thus an important crop in Australia. Many techniques have been implemented to improve its growth and production. The availability of macronutrients and micronutrients is a crucial element in improving wheat growth, particularly nitrogen (N) and phosphorus (P). Holford (1997) contends that after N, P is a limiting nutrient for crop growth in almost all regions in the world, including Australia. In order to avoid P deficiency, farmers apply P fertiliser to the soils. However, even after application of P fertilisers, the concentration of plant available P is low because P is immobile and is mostly unavailable in soil because of precipitation and adsorption.

Because of the low P fertiliser efficiency, continuous application of P fertiliser may lead to the formation of a soil P bank which increases the risk of P leaching from soil into ground and surface water which can have detrimental impacts on the environment. High concentrations of P in surface and ground water may lead to eutrophication, i.e. oxygen deprivation and toxic algal blooms (Holford 1997; Sharpley *et al.* 2000). According to other studies (Armstrong *et al.* 1997; Asseng *et al.* 1998; Nuruzzaman *et al.* 2005a), legumes as pre-crops have positive impacts on subsequent wheat growth. Hence, maximising the role of legume in rotations has become an important technique for increasing growth and yield of the subsequent cereal crop, improving P availability of P in soil and maintaining sustainability of soil health. Several mechanisms have been proposed to explain the positive effect of legumes on growth and P uptake by the following cereal, for example P release from legume

residues (Nuruzzaman *et al.* 2005a), mobilization of soil P by legume exudates(Kamh *et al.* 2002), increased colonisation by arbuscular mycorrhizal (AM) fungi(Borie *et al.* 2002). However, the relative importance of these mechanisms is not clear. Moreover, there is scarce information about distinguishing between the effect of legumes as pre-crops alone and their residues to the following wheat and also their influence on P uptake, available P and AM colonisation.

Environmental factors also play an important role in mycorrhizal symbiosis and nutrient uptake. Soil moisture affects the formation and function of AM fungi in its symbiosis with plants. AM colonisation improves the capability of plants to cope with stress conditions, for instance drought (Entry *et al.* 2002). However, the references on this issue are limited. Furthermore, fluctuating soil moisture can influence decomposition rate of residues (Sorensen 1974). In southern Australia, soil temperature and moisture vary, because the region has a low rainfall and long dry season. Hence, soil moisture during decomposition of legume residues could affect the impact of legumes on following cereals. But there are only few studies on P release from decomposing legume residues and how this is influenced by soil moisture.

1.2 Phosphorus in soils

The total amount of P in soil is high, however, more than 80% of P in soils is unavailable and immobile due to its change to organic forms, precipitation and adsorption (Figure 1) (Holford 1997). The P concentration in the soil solution is very low, frequently less than 1 μM (Barber 1995). There are two main pools of P in soils; organic and inorganic but plants only absorb the inorganic P in the soil solution, whereas the majority of soil P is poorly soluble and only few forms available for plants (Holford 1997; Reddy *et al.* 1999). The available forms in the soil solution are the dibasic anion (HPO_4^{-2}) and the monobasic anion ($H_2PO_4^-$) (Swift *et al.* 1979), both of

which can be taken up by plants. The proportion of organic P can ranging from 20 to 80% of total P, mainly as phytate (Richardson *et al.* 1994)

Inorganic P consists of up to 170 types (Holford 1997). P solubility depends on chemical and physical properties of the soils. The main properties that influence the P solubility in soil are: aluminium (Al), calcium (Ca) and iron (Fe) concentrations and pH (Holford 1997). In alkaline and neutral soils, P exists as Ca and Mg phosphates and P bound to Ca and Mg carbonate surfaces. In soil with lower pH (acidic soils), P forms Al and Fe phosphate.

Figure 1. Phosphorus cycle (Hyland *et al.* 2005).

1.3 Mechanisms to increase P uptake by plants

Under P deficiency, plants develop various mechanisms for obtaining sufficient P for growth. The mechanisms include morphological changes (root growth rate, root length, root branching) and physiological changes (Richardson 2001). Morphological changes aim at increasing the volume of explored soil. The volume of soil explored by roots is an important aspect in nutrient acquisition (Fageria and Baligar 1997),

particularly for poorly mobile nutrients such as P. according to Lynch (1995), the morphology and geometry of roots play essential role in P acquisition, since root systems that have a greater surface area will have larger volume of soil explored. Root hairs are responsible for 63 percent of total plant P uptake (Gahoonia and Nielsen 1998). Moreover, there is a positive relationship between root hair density and the P content in plants, hence plants with low root hair density have low P content in plant shoots (Föhse *et al.* 1991; Gahoonia and Nielsen 1998). The soil volume exploited can also be increased by colonisation of the roots by AM fungi because the fungi form a extensive network of hyphae that increases the volume of soil explored (Smith and Read 1997). Some plants also have abilities to form proteoid roots (clusters) to acquire more P in rhizosphere soil under P deficiency condition (Marschner *et al.* 1987).

Physiological mechanisms improving P uptake are the mobilisation of P by lowering the pH, releasing exudates such as organic acid anions into the rhizosphere or release of phosphatases which mineralise organic P (Gerke and Meyer 1995; Tarafdar and Claassen 1988). The release of organic acid anions by the roots is thought to be related to the P concentration in the plant (Pearse *et al.* 2006).However, the effectiveness of organic acid anions and phosphatases in mobilising P can be reduced by sorption to soil particles and decomposition (George *et al.* 2005; Jones 1998).

Plant species differ in capacity to grow in soils with low P availability. Phosphorus efficiency refers to the ability of plants to reach high biomass at low P availability. This depends on the ability of the roots to take up P, the longevity of active roots and also the number of roots per unit shoot (Föhse *et al.* 1988). In soils with low available P, it has been shown that legumes have a higher ability to take up P than cereals (Kamh *et al.* 2002; Nuruzzaman *et al.* 2005a; Pypers *et al.* 2007). Moreover, Bolland *et al.*(1999) showed that legumes (chickpea, faba bean and white lupin) responded less to P fertilisation than cereals. The high P efficiency of legumes is most

likely due to their ability to mobilise sparingly soluble P in the soil (Kamh *et al.* 1999). White lupin is able to access P pool that not available to soybean (Braum and Helmke 1995). Similarly other legumes e.g. cowpea, narrow-leaf lupin, pigeon pea have been shown to have access to P pools that are not available to cereals (Hocking *et al.* 1997; Pypers *et al.* 2006).

The ability of legumes to access poorly available P pools may be due to the ability to release exudates e.g. phosphatases or organic acid anions and to change the pH in the rhizosphere (Pypers *et al.* 2006). White lupin cluster roots for example, release a large amount of citrate (Bagayoko *et al.* 2000a; Marschner *et al.* 1987). In alkaline soils, the acidification of rhizosphere leads to P solubilisation (Vu *et al.* 2008).

1.4 Agronomic measures to increase P uptake by crops

P deficiency is common in most of Australian soils (Boto 2010; Evans and Scott 2007). Therefore farmers apply P fertilisers to increase growth and yield of crops (Richardson *et al.* 2009). About 450 kt P-based fertilisers used every year in Australia(IFIA 2001). In most cases these are 'water soluble' forms e.g. superphosphate (Richardson *et al.* 2009), However, P added via fertilisers rapidly becomes unavailable because of rapid formation insoluble and immobile forms that are not accessible for many crops (Holford 1997). Of the total applied P fertilisers in a year, very small amount of P is taken up by plants, only 10-20 % can be utilised (Holford 1997; McLaughlin *et al.* 1988).

Consequently, many techniques have been applied to improve the effectiveness of P fertilisers e.g. banding of P, using triple superphosphate, novel P fertiliser formulations such as fluid P and application of rock phosphate to soils (Bordoli and Mallarino 1998; Rivaie *et al.* 2008). Banding P fertiliser is often more effective than broadcast application. Compared to broadcast application, only with banding one third

of the P rate was required to achieve the same yield in lettuce (Sanchez *et al.* 1990). It has been reported that the application of fluid P fertilisers increases yield and P uptake in wheat compared with granular forms (Holloway *et al.* 2001). However, the reserves of phosphate rocks which are the source of all P fertilisers are limited. (Holford 1997).

To reduce the reliance to chemical fertilisers, organic matter such as manures and plant residues can be used to improve and restore soil productivity (Parr *et al.* 1989; Parr and Hornick 1992). The application of organic matter to soils lead to many benefits: improving soil structure, increasing water holding capacity and cation exchange capacity (especially in sandy soils) and increasing nutrient availability by releasing inorganic nutrients through mineralisation (Bationo and Mokwunye 1991). Decomposition products can also reduce P binding via chelation and anion exchange (Buresh *et al.* 1996). For example, application of millet straw and inorganic P fertiliser can lead to a higher P uptake rate by pearl millet (*Pennisetum glaucum*) compared to unamended control (no residues) and without P fertiliser (Hafner *et al.* 1993).

1.5 Residue decomposition and P release

Crop residues are a source of the important nutrients for plant growth such as N and P (Ha *et al.* 2008). Nutrient release during decomposition of plant residues has become essential in order to improve nutrient management for maintaining nutrient availability and reducing the loss of nutrients in soils (Tian *et al.* 2007). Several factors influence the decomposition process such as the residue quality and soil moisture (Tian *et al.* 2007). Residues are utilised in crop rotation in order to improve the availability of nutrients for the subsequent crops (Salas *et al.* 2003) (Njunie *et al.* 2004). During decomposition of organic material, nutrients are initially released rapidly, within 21 days, 25% of the N and 38% of the P in the organic material is released. This is followed by a slower rate of release in the next stages (Lavelle and Spain 2002).

The C/N ratio is an important factor in controlling the decomposition process of residues or plant materials. Decomposition is slower in residues with a high C/N ratio than residues with a low C/N ratio.Therefore,plant material with high N concentration will be decompose faster and release nutrient faster (Kumar and Goh 1999). The C/P ratio is important for P mineralisation and or immobilisation with net P immobilisation above a C/P ratio of 300 (Vaughan and Malcolm 1985).

1.6 Role of legumes in agriculture

Legumes have become an important crop in the agricultural systems, including Australia. Legumes have different abilities to utilise and mobilise P nutrient from soils. Farmers grow legumes in rotation with cereals mainly to improve N uptake (Asseng *et al.* 1998) but legumes are also used to control weeds and as break crops to reduce crop diseases (Bolland *et al.* 1999; Hamblin 1987; Hungria and Vargas 2000).

It is well-established that that cereal in rotation with legumes benefit from N2 fixation by the legumes and the increase in available N (Armstrong *et al.* 1997; Bagayoko *et al.* 2000b). According to Asseng *et al.* (1998) the yield of wheat after lupin increased by 42-47% compared with other legumes (faba bean and chickpea) and was 131% higher than with wheat as a pre-crop. In another study showed that narrow leaf lupin and white lupin resulted in higher wheat grain N (20%) compared with field pea and chickpea (Armstrong *et al.* 1997). Therefore, in terms of improving N, lupins have shown superiority amongst legumes. However there are also studies that show that legumes are able to improve the growth of the following wheat when P is a limiting factor, not N, with faba bean having a greater effect than white lupin and field peas (Nuruzzaman *et al.* 2005a; b).

Intercropping legumes with wheat has a direct effect on wheat because wheat can utilise the P made available by legume roots. In pot experiments, chickpea facilitated P uptake by wheat through contact of roots between both crops (Li *et al.*

2003). This is different in rotation systems, where wheat is planted after legumes on the same field. The increased P availability to the following wheat may be related to the ability of legume roots to release carboxylates and mobilise P in soil. Moreover, previous research (Li *et al.* 2003) has demonstrated that chickpea has the ability to uptake not only inorganic P but also organic P, whereas wheat only absorbs very little organic P. However, the role of the exudates has been questioned because of the rapid decomposition of organic acids in the soil (Nuruzzaman *et al.* 2005a). The increased P uptake of wheat after legumes may also be due to P release during legume residue decomposition (Nuruzzaman *et al.* 2005a). Another beneficial effect of the application of legumes as a pre-crop in the cropping systems is improving mycorrhizal colonisation. In West African soil, the rotation between legumes and cereals lead to increased yield of cereals, improved the colonisation by AM fungi and reduced nematode density (Bagayoko *et al.* 2000b). Legume residues are source of P (Nuruzzaman *et al.* 2005a) and also have shown a positive effects on AM colonisation (Borie *et al.* 2002). Therefore, both legume residues and legume pre-crops may improve the availability of P as well as increase the mycorrhizal colonisation from the pre-crop roots for the subsequent wheat. However, it is not clear which of the two factors (pre-crops or residues) is more important.

1.7 Symbiosis between AM fungi and wheat

The association between AM fungi and plant is a mutualistic symbiosis. The plant provides carbohydrates to the fungus and in return the fungus helps the plant in nutrient and water absorption particularly P (Smith and Read 2008). It has been shown for many species in pot experiments that mycorrhizal plants grow better in P deficient conditions than non-mycorrhizal plants. Often, the host plants has a higher P concentration than their non-mycorrhizal counterparts at a given P availability (Smith

and Read 2008) because the fungal hyphae can extend further into the soil than root hairs (Li *et al.* 2008).

AM fungi form a symbiosis with a wide range of plants, including cereal crops such as wheat (Smith and Read 2008). Numerous studies have confirmed that AM fungi colonise wheat roots (Entry *et al.* 2002; Li *et al.* 2006; Li *et al.* 2008). Moreover, AM colonisation leads to a higher P content in grain and tissue of wheat (Li *et al.* 2008). Physically, the symbiosis between plant and mycorrhiza leads to the larger exploration in the soils, thereby reducing the distance for P diffusion and improving surface area to uptake more P (Tinker 1978). (Sanders and Tinker 1971).

In the AM symbiosis with plants, the plants absorb P from soil in two ways. Firstly, inorganic P is absorbed via root epidermis and root hairs. Secondly, AM absorb P by the extra-radical hyphae and transferred to the plants (Smith *et al.* 2003; Tinker 1978). Inorganic P is transferred from AM to the host through arbuscules in root cortical cells (Smith *et al.* 2003). Both processes occur simultaneously and allow AM plants to have higher P uptake if compared with non-mycorrhizal plants. Moreover, AM has been shown to have not only assisting plant to take up more P, N, (Leigh *et al.* 2009) and probably K (Marschner and Dell 1994) from soil, but increasing micronutrient uptake. For example, maize may take up Zn from 16 to 25% via hyphae in calcareous soil (Kothari *et al.* 1991) and white clover may take up up to 62% Cu via AM in a calcareous soil (Li *et al.* 1991). AM association may also improve plant tolerance to metal toxicity, (Ferrol *et al.* 2009). Moreover, fungi can suppress pathogen growth in host plants (Newsham *et al.* 1995b). For example, the Am fungus *Glomus* sp suppressed the infection of *F. oxysporum and E. chlamydospora* in *Vulpia ciliate* (Newsham *et al.* 1995a).

1.8 Effect of soil P availability and plant P concentration on AM colonisation

The main problem in utilising AM in agriculture is that the mycorrhizal colonisation tends to be suppressed by P fertilizer application (Jasper *et al.* 1979; Menge *et al.* 1978). Moreover, it has been demonstrated that P addition inhibits hyphal branching of *G. margarita* in *Daucus carota* L roots *in vitro* (Nagahashi *et al.* 1996). However, according to Grant *et al.* (2005), the inhibiting effect of available P on AM colonisation is indirect They suggest that it is the plant P concentration which affects AM colonisation more strongly than soil available P. (Grant *et al.* 2005).

The adverse effects of plant P status on AM have been shown in other studies. Increasing P tissue concentration in plants leads to decreasing AM association (Lu *et al.* 1994; Valentine *et al.* 2001). Menge *et al.* (1978), found significant amount AM colonisation in soil with high P concentration but low P content in roots. High P tissue concentration also can depress formation of spores and external hyphae (Bruce *et al.* 1994; De Miranda and Harris 1994) . One proposed mechanism by which the plant P concentration inhibits AM development is the composition of plant exudates. (Marschner 1998). The exudation of plants with low P concentration enhances the growth of AM hyphae and their branching (Nagahashi *et al.* 1996).

1.9 Effects of soil moisture on decomposition of residues, P uptake and AM fungal colonisation

Soil moisture affects residue decomposition and may also affect the capacity of AM fungi to survive in soil in absence of a host plant. In dry soil the decomposition rate of plant residues is low (Tian *et al.* 2007) thus decreasing P release from the residues. Therefore, the soil moisture during decomposition of residues may play an important role in how residues or legume pre-crops affect the growth and P uptake of the following wheat.

1.10 Objectives of this study

a. Determine the effect of legumes (pre-crop/residue application) on AM colonisation, growth and P uptake of the following wheat.
b. Assess the effect of soil moisture during residue decomposition (i.e. between legume harvest and sowing of wheat) on AM colonisation, growth and P uptake of the following wheat.

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CHAPTER 2

LEGUME RESIDUE INFLUENCE ARBUSCULAR MYCORRHIZA COLONISATION AND P UPTAKE BY WHEAT

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Legume residue influence arbuscular mycorrhiza colonisation and P uptake by wheat

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Abstract

Legumes have been shown to increase growth and P uptake of the following cereal. This could be, in part, due to nutrients released by the decomposing legume residues. To investigate the effect of P in legume residues on wheat, a number of experiments were conducted with different legume residues added to a soil with low P availability under condition in which N was not limiting. Young and mature faba bean shoots (FYS, FMS) and mature chickpea shoots (CP) were added to soil at different rates (0.5-2 % w/w) with the P concentration being greatest in the young shoot material and least in the mature CP residues. Other treatments included addition of inorganic P at different rates (0-80 mg P kg^{-1}). Available P, growth and P uptake and arbuscular mycorrhiza (AM) colonisation of wheat were measured after 6 weeks. As expected, inorganic P addition increased growth and P uptake, but decreased AM colonisation. The effect of the residues was more complex. AM colonisation was not correlated with available P in the soil amended with residues, whereas there was significant negative correlation between available P and AM colonisation within the treatments with inorganic P. Addition of FYS to soil increased subsequent wheat shoot growth and P uptake and decreased AM colonisation. However, FMS and CP addition not only decreased wheat growth and P uptake but also AM colonisation despite low P availability in the soil. It is concluded that addition of some legume residues can improve the growth of subsequent cereals but

others have a negative effect on wheat growth and AM colonisation which cannot be explained solely by soil P availability.

Keywords: legumes, P uptake, arbuscular mycorrhiza, wheat

Introduction

A number of studies have shown that biomass and P uptake in cereals following legumes are higher than in cereals following cereals (Armstrong *et al.* 1997; Asseng *et al.* 1998; Nuruzzaman *et al.* 2005a). The positive effect of legumes on the following cereal may be due to the growth of legumes prior to wheat and/or due to nutrients released during decomposition of legume residue that are utilised by the subsequent cereal. It is well-established that the positive effect of legumes on the following crop is often due to improved N availability (Armstrong *et al.* 1997). However, positive effects of legumes on the following crop have also been found in situations where N was not limiting the growth of the cereal (Asseng *et al.* 1998).

Legumes can mobilise P in soils and therefore often have higher P concentrations in roots and shoots than cereals (Nuruzzaman *et al.* 2005b). This accumulated P will become available during decomposition of legume residues. Indeed, the application of residues of faba bean (*Vicia faba* L.), field pea (*Pisum sativum* L.) and white lupin (*Lupinus albus* L.) resulted in higher P availability and growth of the following wheat, with P as the main limiting factor (Nuruzzaman *et al.* 2005a). Besides being a P source, legume residues may also promote wheat growth by improving the P acquisition of wheat by increasing AM colonisation. It has been shown that the application of lupin residues resulted in a higher mycorrhizal colonisation of wheat (Borie *et al.* 2002). However, if large amounts of P are released from decomposing legume residues AM colonisation may be reduced because high P availability in soils can decrease mycorrhizal colonisation (Smith and Read 1997).

In African soil, legume pre-crops resulted in several changes of biological properties in the following cereal. These changes include earlier colonisation of cereal roots by AM fungi as well as decreased rate of nematode infection and changes in microbial community structure (Bagayoko *et al.* 2000; Marschner *et al.* 2004). It is not known if such biological changes also occur in soils with low P availability in other parts of the world, and in particular how AM fungal colonisation of wheat responds to legume residue application and P availability in such soils. Therefore, the aim of this study was to determine the effects of added legume residues differing in quality (i.e young or mature) on P availability, wheat growth and P uptake, and mycorrhizal colonisation in a typical soil from a low P environment in Australia.

Materials and Methods

Soil

A loamy sand (0-20 cm) from Monarto (35°05′ S and 139°06′ E), South Australia was used. The soil was air dried, thoroughly mixed and sieved to 2 mm before use. The soil properties are 82.6 % sand, 10% silt, 7.5% clay, 1.26 % organic matter, 15% w/w water holding capacity, pH (1: 5 soil: water) 8.82, 229.6 mg kg^{-1} total P, 4.5 mg P kg^{-1} resin P, 0.09 % total N and 0.73 % organic C. During all experiments, soil moisture was maintained at 70% water holding capacity by adding reverse osmosis (RO) water at 2 day intervals. This soil water content was optimal for microbial activity and wheat growth in previous experiments with this soil (Mat Hassan, personal comm.,).

Plant residues

Three types of legume residues were used: faba bean young shoots (FYS), faba bean mature shoots (FMS) and mature chickpea shoots including pods (CP). These residues were chosen because they differ in P concentration and because faba bean and chickpea

are frequently used in rotations with cereals. The residues were oven-dried (70°C) for two days, ground and sieved to yield particles from 0.25 to 2 mm. Total P was the highest in FYS (6.55 g kg^{-1}), medium in FMS (2.09 g P kg^{-1}) and lowest in CP (0.7 g P kg^{-1}). Total P, N, C, C/N and C/P are given in Table 1.

Assessment of AM colonisation

At harvest, roots were removed from the soil and washed. Root subsamples (~0.2 g) were cut to approximately 1 cm length before clearing in KOH 10% for 2-3 days at room temperature and then stained using the method described in Vierheilig *et al.* (1998). Stained roots were scored for AM colonisation using the gridline intersection method (Brundrett *et al.* 1996; Giovannetti and Mosse 1980) under a dissecting microscope at 40× magnification.

Soil and plant analyses

Available P in soil was extracted using an anion exchange membrane method (Kouno *et al.* 1995) and determined colorimetrically (Murphy and Riley 1962). Total P concentration of plant shoots was measured using the phosphovanado-molybdate method (Hanson (1950). Total N concentration was determined by the Kjeldahl method (Bradstreet 1965) and measured colorimetrically at 650 nm wavelength (Bremner and Black 1965).

Experimental design

This study consisted of 4 experiments with information from the first three experiments used to design the fourth experiment.

Experiment 1 was conducted over 8 weeks in the glasshouse. The aim of this experiment was to measure the potential AM colonisation of the soil and to investigate how colonisation changes over time in order to choose the best time for determining colonisation in the following experiments. To avoid severe P deficiency but still ensure reasonably high and measurable AM colonisation rates, 15mg P kg^{-1} as KH_2PO_4 and 150 mg kg^{-1} $CaCl_2$ were added to the soil. Two days after germination, four wheat (*Triticum aestivum* L. cv Krichauff) seedlings were planted in pots with 300gsoil and after 1 week thinned to two plants per pot. A nutrient solution (µM) with NH_4NO_3, 200; K_2SO_4, 100; $MgSO_4$, 54; $CuSO_4$, 0.02; $MnSO_4$, 0.24; H_3BO_3, 2.4; $NaMO_4$, 0.03; and $ZnSO_4$, 0.1 was added at 7 ml per pot every week to ensure that these nutrients did not limit plant growth (Nuruzzaman *et al.* 2005a).

Atr 2, 4, 6 and 8 weeks after planting AM colonisation and dry weight of the plants were determined on four replicate pots. Resin P was measured at the end of the experiment to assess available P in the soil.

Experiment 2 was carried out to investigate the relationship between soil P availability and AM colonisation in wheat. Wheat (Krichauff) was grown over 6 weeks in pots containing 300 g soil at same rate of nutrients as in the first experiment, except inorganic P which was added at 11 different rates (0, 2.5, 5, 7.5, 10, 12.5, 15, 20, 25, 30, and 40 mg P kg^{-1} as KH_2PO_4) with four replicates. AM colonisation and available P were determined after 6 weeks.

Experiment 3 was conducted to investigate the relationship between type and rate of residue addition and available P. Before adding the residues, the soil was pre-incubated for 10 day at 70% WHC to activate the microorganisms. Previous experiments have shown that 10 days after rewetting of air-dry soil, microbial activity (respiration)

stabilised and remained unchanged thereafter (Chowdury, personal comm.). The three types of residues (CP, FMS and FYS) were mixed into the soil at four rates (0.5, 1, 1.5 and 2% w/w) equating to 5, 10, 15 and 20 mg residue/g soil in four replicates. Aliquots of 20 g soil were incubated in the dark at room temperature for 1 week and available P was determined.

Experiment 4 aimed to determine the relationship between residue type, P addition rate as inorganic fertiliser or in residue, and AM colonisation in wheat. The three residues from Experiment 3 were added at two rates: 0.5 and 1.5% (w/w). Inorganic P was added at four rates: 0, 15, 40 and 80 mg P kg^{-1} (P0, P15, P40 and P80). There were four replicates per treatment. Pre-germinated wheat seedlings (Krichauff) were planted and thinned to 2 plants per pot after 10 days. Nutrient solution was added as described in Experiment 1. Available P, AM colonisation, shoot and root dry weight, total N concentration and P uptake were measured after 6 weeks.

Statistical analysis

The treatments were arranged in a Complete Randomised Design (CRD) and data was analysed using Genstat 11th edition (Windows) for analysis of variance (ANOVA) and means were tested using Tukey's at 95% confidence intervals. PASW 18 (Windows) was used for regressions of data from Experiments 2 and 4.

Results

Experiment 1

After the first two weeks AM colonisation was 5% and reached the highest percentage in 4 weeks and 6 weeks with around 20% at 12 mg P kg^{-1} available P. Therefore, in the following experiments AM colonisation was determined after 6 weeks.

Experiment 2

After 6 weeks, AM colonisation was highest (20%) in soil with no inorganic P addition (Figure 1). Inorganic P addition significantly decreased the percentage of colonisation compared with the soil without added P. The addition of ≥ 12.5 mg P kg^{-1} decreased the colonisation to less than a half of the control; only around 5-7%. AM colonisation was negatively correlated with available P concentration (Figure 2).

Experiment 3

Addition of CP and FMS residues resulted in very low available P concentrations which did not differ among rates of residue addition (Figure 3). Available P increased with increasing addition rate of FYS , with the greatest increase between 0.5 and 1% addition rate. As expected, available P nearly doubled. The increase in available P between 1 and 1.5 % and 1.5 to 2% was smaller and did not match the increased amount of P added with the residues.

Experiment 4

Available P increased with increasing addition of inorganic P. The application of FYS at 1.5% resulted in highest available P which was 42.5 mg P kg^{-1}. A similar available P concentration was acchieved with addition of 80 mg P kg^{-1} (P80) as inorganic P. Addition of CP or FMS did not increase available P compared to the unamended control.

Wheat shoot dry weight was highest following addition of FYS and lowest with CP where it was even lower than in the unamended control (Table 2). In all residues, shoot dry weight did not differ significantly between addition rates. Similarly, shoot dry

weight did not differ between low (P15) and high (P80) rates of addition of inorganic P. Root dry weight was not affected by treatment except for FYS addition where wheat root dry weight at 0.5% was higher than in all other treatments, but decreased significantly at 1.5%. With FYS addition, both P uptake (Figure 6 and Table 2) and P concentration in wheat shoots increased with addition rate, whereas increasing the addition rate of FMS had no effect on P concentration of wheat. Increasing addition rate of CP decreased wheat shoot P concentrations. In the inorganic P treatments, shoot P concentration was higher with P40 and P80 than with P15 or the control. Addition of FYS at 0.5% achieved similar wheat shoot P concentrations as P40 and P80. P uptake by wheat followed a similar trend as shoot dry matter, being highest in the treatment with FYS added at 1.5% w/w and lowest in the CP addition (Figure 6). Addition of inorganic P increased shoot P uptake.

There were positive relationships between amount P added from residues and available P (r^2=0.94) as well as between available P and shoot P uptake in residue treatments (r^2=0.85). Shoot N concentrations were highest with FYS and FMS at 1.5% and lowest in the unamended control (Table 2). For FYS and FMS, shoot N concentration increased with increasing residue addition rate whereas this was not the case for CP. Inorganic P addition did not affect shoot N concentrations.

Increasing addition of inorganic P decreased AM colonisation (Figure 5). AM colonisation was highest in the unamended control and FMS at 1.5%. It was lowest with CP and FYS at 1.5%. Increasing the rate of addition of FYS and CP decreased AM colonisation whereas in FMS, AM colonization was higher at 1.5% than at 0.5%.

AM colonisation decreased with increasing available P concentration (Figure 5). However, across all treatments, the relationship between available P and AM colonization was weak (r^2= 0.24) (Figure7). The correlation was much stronger when only the inorganic P treatments were considered (r^2=0.64) (Figure8) and it was weaker when only the residue treatments were included (r^2=0.22) (data not shown). Among residue treatments, low colonisation rates were found at high available P (for example; FYS added at 1.5%w/w) and also at low available P (CP residues added at 1.5% w/w). At ≤10 mg P kg^{-1}, AM colonisation varied between less than 2% with CP at 1.5% w/w and 17% with FYS at 1.5% w/w. AM colonisation was also negatively correlated with P uptake among all treatments (r^2= 0.37) (inorganic and residue additions). In inorganic P fertiliser treatments, AM development was negatively correlated with wheat shoot dry weight (r^2= 0.49), available P (r^2= 0.64), P uptake (r^2= 0.82) and shoot P concentration (r^2= 0.48) (data not shown). However, in residue treatments, AM colonisation was only negatively correlated with P uptake (r^2= 0.37).

Discussion

This study showed that addition of legume residues can increase available P and shoot growth, P uptake and AM colonisation in wheat, but it also highlighted substantial differences among residues and addition rates. Moreover, while the expected negative correlation between available P and AM colonisation was found in the treatments with inorganic P, this was not the case for the residue treatments, suggesting that residue addition affects AM colonisation by factors other than available P.

The application of FYS residue, which had the highest P concentration among the residues (Table 1), increased wheat growth and P uptake to levels similar to or even higher than those of high rates of inorganic P (Table 2). The high total P content of faba bean is in accordance with Nuruzzaman et al. (2005a) who reported that faba bean shoot

P content was two-fold higher than in field pea and white lupin (Nuruzzaman *et al.* 2005a).Therefore, FYS appears to be a good P source for plants. Additionally, N is added with the residues which may explain the higher shoot dry weight compared to high inorganic P addition rates. Similarly, higher wheat shoot dry weight was found after legumes than after barley, due to higher N content of legume residues remaining in the soil (Armstrong *et al.* 1997). However, addition of FYS decreased AM colonisation of wheat (Figure 5), probably due to the high available P concentrations (Figure 4). In addition to inhibition of AM colonisation through increasing available P concentrations in the soil, FYS may also has contributed to a decrease in AM colonisation by increasing wheat shoot P concentrations (Mosse *et al.* 1973). The P concentration in plant tissues has a more direct impact in suppressing AM colonisation than soil P availability (Grant *et al.* 2005; Mosse *et al.* 1973). Indeed, AM colonisation can be high in the presence of high P concentration in soil, when P concentration in root is low (Menge *et al.* 1978). Further, while this did not affect wheat growth in the present experiment, low colonisation could have negative effects on uptake of other poorly mobile nutrients such as Zn (Bolan 1991).

The other two residues, FMS and particularly CP decreased wheat growth compared to the unamended control, and at the higher rate CP residues also reduced wheat shoot P concentration (Table 2). The poor growth of wheat in soil with these two residues added can be explained by their low P concentration which resulted in available P concentrations that were similar to the unamended control (Figure 4). Thus, although 31.5 and 10.5 mg P kg^{-1} had been added with 1.5% FMS and 1.5% CP respectively, P availability remained low (Figure 4). This could suggest low residue decomposition rates. However, the increased shoot N concentration in these treatments indicates that the residues were decomposed. Most likely, P was immobilised in the microbial biomass during decomposition of the mature residues. The high C: P ratios,

210 for FMS and 617 for CP (Table 1), may result in P immobilisation since net immobilisation is likely at C:P ratios greater 100 (Cheshire and Chapman 1996). In FMS, low P availability was accompanied by high AM colonisation rates (Figures 4, 5). However, the high AM colonisation rates did not improve growth and P uptake of wheat in our short-term experiment (Table 2 and Figure 5). A poor correlation between AM colonisation and P uptake in young wheat was also found by (Li *et al.* 2005), however in that study, AM colonisation improved uptake of P and Zn in mature wheat as well as P uptake into the grain. Moreover, even without a significant effect on wheat growth, more than 50% of P uptake may be due to AM fungi (Li *et al.* 2006).

The high AM colonisation rates with CP at 0.5% (Figure 5) can be explained by the low P availability (Figure 4). However, at the higher addition rate, CP reduced AM colonisation despite low concentrations of available P. This suggests that higher addition rates of CP had a detrimental effect on AM colonisation. This may be due to phenolic compounds in residues that inhibit mycorrhiza development. It has been reported that AM colonisation is suppressed by the application of olive mill residues that contain phenolic compounds (Martin *et al.* 2002). A high concentration of phenolics in soil may have inhibitory effects, not only on AM development but also on plant growth (Fries *et al.* 1997), which would explain the poor growth of wheat with 1.5% CP.

The negative correlation between available P and AM colonisation in the inorganic P treatments as shown in Figure 8 is in agreement with other studies. For example, Baon *et. al* (1992) showed that AM colonisation of cereals decreased with P addition to the soil. As explained above, the negative effect of available P in soil on AM development could also be related to the increased shoot P concentrations (Grant *et al.* 2005; Mosse *et al.* 1973). However, there was no relationship between available P and AM colonisation in the residue treatments. Low colonisation rates were found at high

available P (FYS) and at low available P (CP1.5%). Therefore, the effect of residues on AM colonisation cannot be predicted by their effect on soil P availability.

Conclusions

Our results show that addition of residues with low C/P ratio, such as in green manuring, can be an alternative to inorganic P addition to improve wheat growth and P uptake. On the other hand, residues with low P concentration, for example mature residues remaining after harvest, had a negative effect on wheat growth and AM colonisation. However, these results are only for residues of two common legume species and in order to fully examine the potential of residues as alternatives to inorganic P fertilisers, more residues need to be tested. It should be noted that the present experiment was conducted over 6 weeks only. The longer term effect of the residues may change over time as they are decomposed and positive or negative effects found in the first 6 weeks may diminish. Therefore, longer term studies on the effects of residues on nutrient uptake and AM colonisation need to be conducted to evaluate the effects of these residues for the complete life cycle of the cereal crop.

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Figure 1. AM colonisation of wheat after 6 weeks growth in a loamy sand soil with different rates (0, 2.5, 5, 7.5, 10, 12.5, 15, 20, 25, 30, and 40 mg/kg) of added inorganic P (n=4). Available P was measured at the end of the experiment. Bars with the same letters are not significantly different at $P \leq 0.05$.

Figure 2. Relationship between available P and AM colonisation of 6 week old wheat.

Figure 3. Available P one week after addition of different types and amounts of legume residues (n=4). Bars with the same letters are not significantly different at $P \leq 0.05$.

Figure 4. Available P 6 weeks after addition of inorganic P at a rate of 0, 15, 40,or 80 mg kg^{-1}or legume residues [fababean young shoots (FYS), faba bean or chickpea mature shoots (FMS, CP)] at either 0.5% or 1.5% w/w (n=4). Bars with the same letters are not significantly different at $P \leq 0.05$.

Figure 5. AM colonisation (%) of wheat after 6 weeks of growth in a soil with different sources of P, added either as inorganic fertiliser (P15, P40, P80) or in legume residues (n=4). For abbreviations see Figure 4. Bars with the same letters are not significantly different at $P \leq 0.05$.

Figure 6. Phosphorus uptake by wheat shoots after 6 weeks of growth with different P sources (n=4). Bars with the same letters are not significantly different at $P \leq 0.05$.

Figure 7. Relationship between available P and AM colonisation in treatments with addition of inorganic P and residues.

Figure 8. Relationship between available P and AM colonisation of treatments with addition of inorganic P only

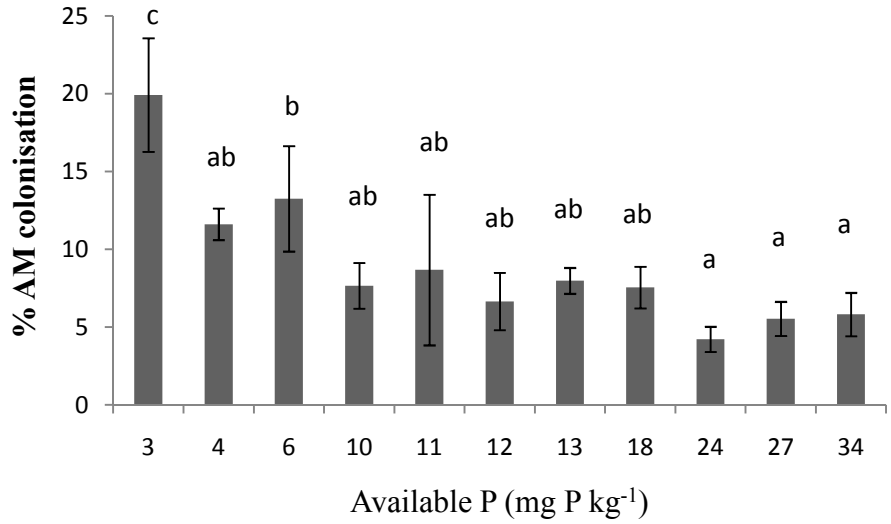


Figure 1

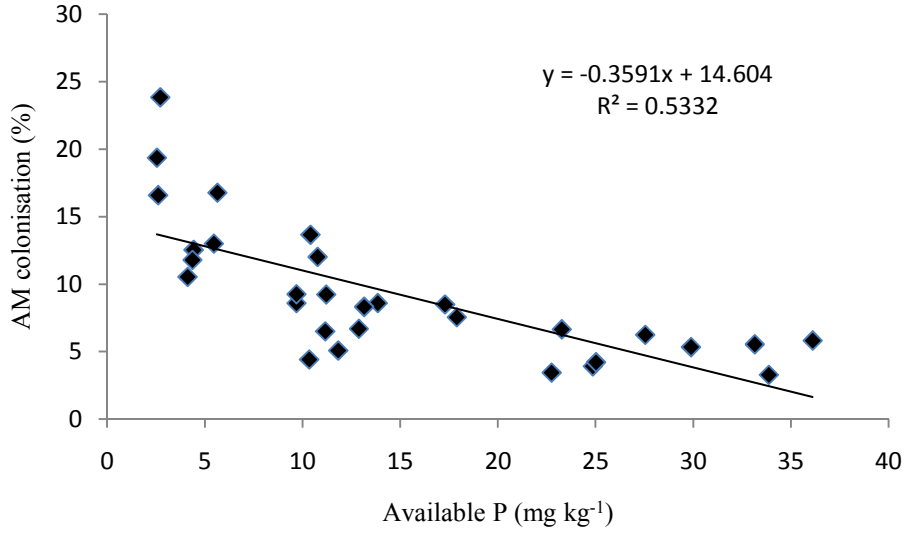
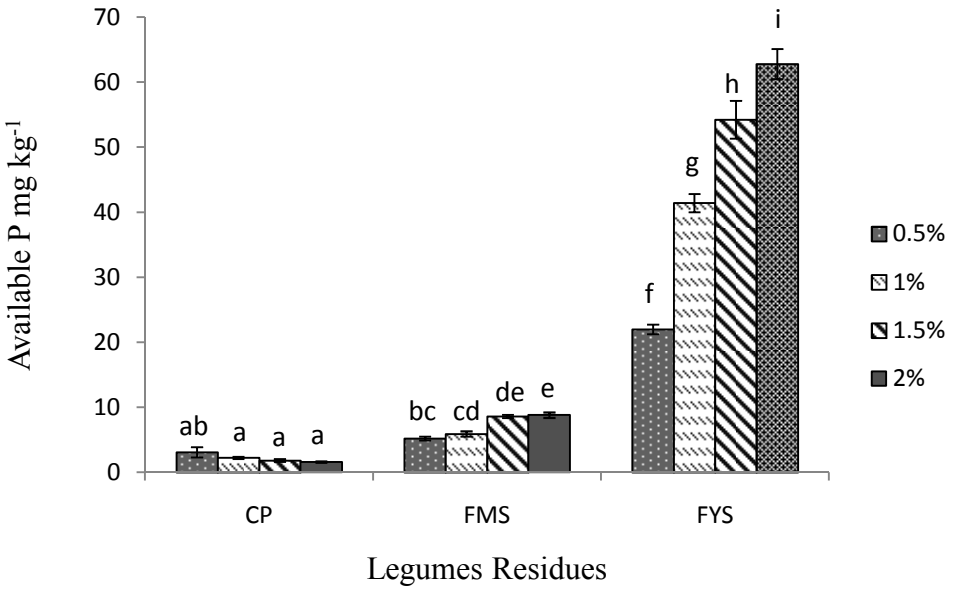


Figure 2



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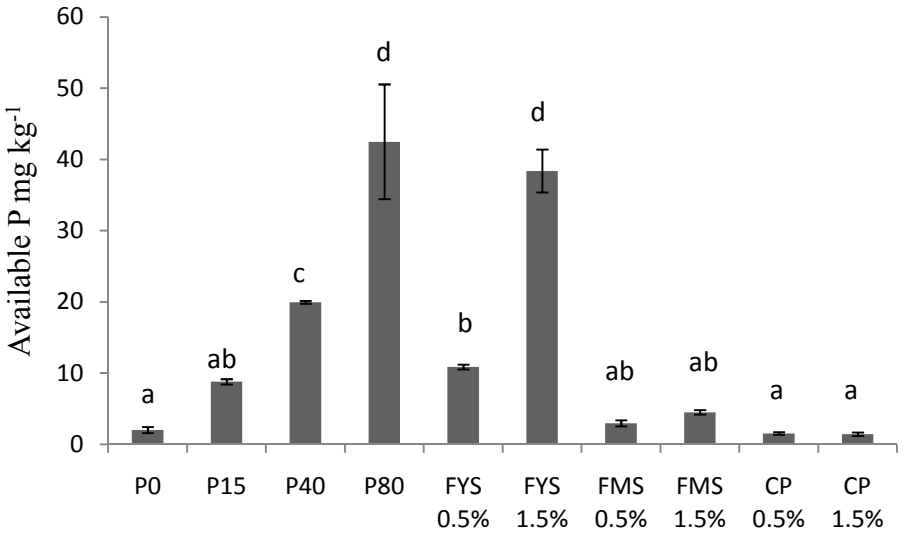

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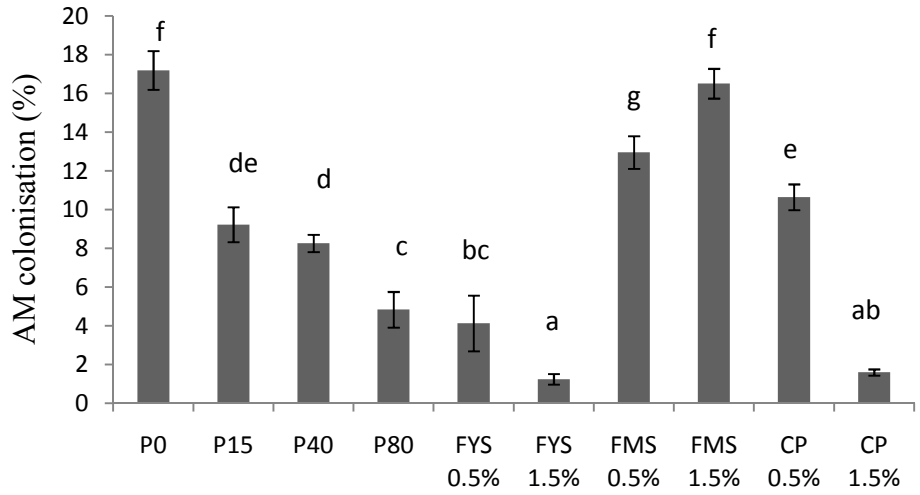
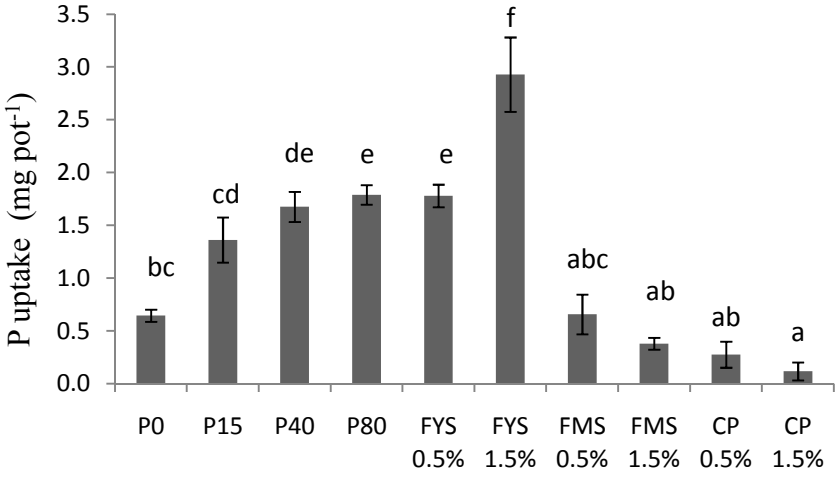


Figure 5



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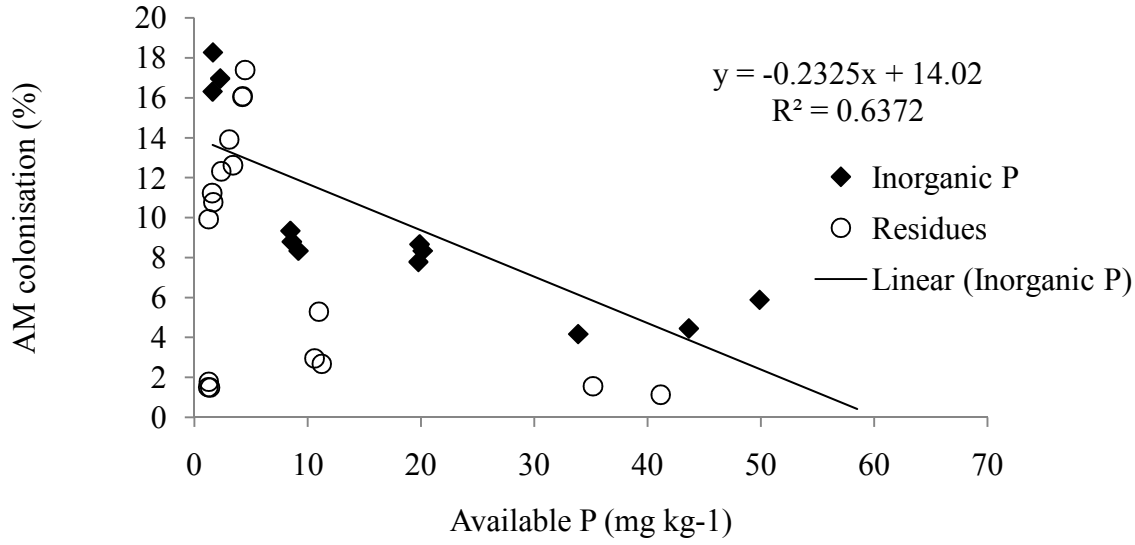

Figure 7

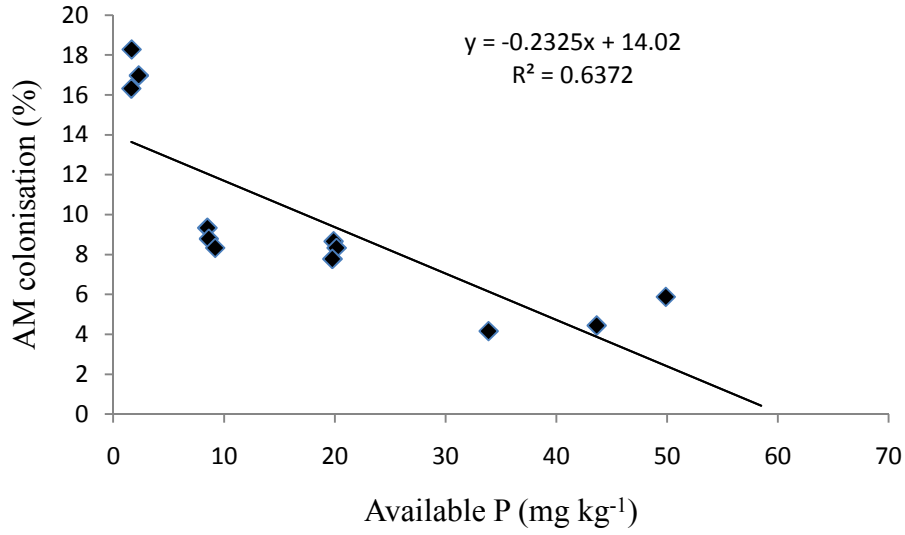

Figure 8

Table 1. Nutrient concentration in residues

Residues	**Total P g kg^{-1}**	**Total N g kg^{-1}**	**Total C g kg^{-1}**	**C/N**	**C/P**
Chick pea (CP)	0.67	10.6	414.9	39.1	617.7
Faba bean young shoots (FYS)	6.55	34.5	417.2	12.1	63.7
Faba Bean mature shoots (FMS)	2.09	23.8	439.8	18.5	210.5

Table 2. Wheat shoot and root dry weight and shoot P and N concentration after 6 weeks growing in loamy sand soil amended with inorganic P fertiliser (at 0, 15, 40 or 80 mg/kg) or legume residues (young faba bean shoots or mature faba bean or chickpea shoots) at 0.5 or 1.5 % w/w (n=4) Values followed by the same letters are not significantly different at P≤ 0.05.

Treatments	Rate mg kg^{-1} or % w/w	Shoot dry weight (g pot^{-1})		Root dry weight (g pot^{-1})	Shoot P concentration (mg g^{-1})	Shoot N concentration (mg g^{-1})	
Inorganic P	0	0.44	a	0.24abcd	1.8bc	11.902	abc
	15	0.57	f	0.31e	1.8bc	16.134	abc
	40	0.59	cd	0.32f	2.9cd	11.669	ab
	80	0.56	c	0.28bcd	3.1d	12.070	a
Residues	FYS 0.5%	0.68	de	0.58h	2.5bcd	17.656	abc
	FYS 1.5%	0.70	e	0.38g	4.1e	32.370	d
	FMS 0.5%	0.31	b	0.25abcd	2.4bcd	17.270	d
	FMS 1.5%	0.16	a	0.16abc	2.3bcd	33.496	d
	CP 0.5%	0.15	a	0.09a	1.8ab	22.737	c
	CP 1.5%	0.12	a	0.10ab	0.7a	21.156	c

CHAPTER 3

LEGUME PRE-CROP AND RESIDUE EFFECTS ON ARBUSCULAR MYCORRHIZAL COLONISATION AND GROWTH AND PHOSPHORUS UPTAKE BY THE FOLLOWING WHEAT

Legume pre-crop and residue effects on arbuscular mycorrhizal colonisation and growth and phosphorus uptake by the following wheat

Abstract

Planting legumes prior to wheat has been shown to have several positive effects on the following cereal even in absence of N limitation for the cereal. However, it is unclear if this effect is due to the pre-crop alone or whether the legume residues (roots or shoots) play an important role in this positive effect on the following cereal. To address this knowledge gap, a study was conducted with four pre-crops (chickpea, faba bean, white lupin and wheat) which were grown for 10 weeks in a soil with low P availability. The legumes were inoculated with the respective Rhizobium strains, while wheat was supplied with inorganic N. At harvest, roots and shoots were removed and the soil mixed; root and shoot residues were dried and cut into 2 cm long pieces. Before planting wheat as the following crop, four treatments were imposed: both roots and shoots of the pre-crop were removed completely; only roots (0.04 % w/w) were added back into the soil; only shoot residues (0.24% w/w) mixed with soil; and the mixture of shoot and root residues (0.24% shoots + 0.04 % w/w roots) was added. Soil available P, growth and P uptake and AM colonisation of wheat were measured after 6 weeks. Among the legume pre-crops, only white lupin increased the growth and P uptake, but reduced AM colonisation of the following wheat compared with wheat as a pre-crop. This growth increase was not due to residue amendment and can be explained by the increased P availability after white lupin as pre-crop. Chickpea as pre-crop improved the growth and P uptake of the following wheat only in combination with shoot residues or the mix of root and shoot residues, suggesting that the P released from the chickpea residues was taken up by the wheat. On the other hand, faba bean as pre-crop decreased the growth of the following wheat compared to wheat as pre-crop particularly with

addition of the residues. However, faba bean pre-crop increased AM colonisation of the following wheat.

Keywords: legumes, mycorrhiza, pre-crop, P uptake, wheat

Introduction

Provision of sufficient amounts of phosphorus (P) is an important factor in wheat production in Australia. Even though the amount of total P in soil may be high, the concentration of available P in is usually low because over 90% of soil P is in forms that are poorly available to plants (Holford 1997). Many plants have the ability to absorb or mobilize the unavailable or less available soil P pool (Valsami-Jones 2004), particularly legumes. For example, *Lupinus albus* forms cluster roots which is mobilise P in soil with a low P content by release of citrate and other carboxylates, phosphates and decreasing the pH (Shane and Lambers 2005) (Kamh *et al.* 1999). Pigeon pea (*Cajanus cajan* (L) Millps.) releases exudates, especially piscidic acid that release P that fixed with iron in soil (Ae *et al.* 1990). The P mobilised by legumes may remain available in the soil and could be used by the following cereal (Nuruzzaman *et al.* 2005). Due to the mobilisation of soil P, some legumes also have high shoot P concentrations (Nuruzzaman *et al.* 2005), therefore P released from decomposing legume residues may also play a role in the positive effect of legume pre-crops on the following cereal.

Plant P uptake can be increased by symbiosis with mycorrhiza which increase the soil volume from which P can be taken up to several centimetres outside the rhizosphere (George *et al.* 1995; Li *et al.* 1997). Arbuscular mycorrhiza (AM) colonisation has contributes to P uptake by wheat in soil with a low available P (Li *et al.* 2006). In a study in African soil, legume pre-crops increased AM colonisation of sorghum compared with cereal following cereal (Bagayoko *et al.* 2000). Moreover, it has been demonstrated that the addition of lupin residues to soil increased AM

colonisation compared to soil with no residue addition or wheat residue amendment (Borie *et al.* 2002).

Despite several reports of positive effects of legume pre-crops on growth and P uptake of the following cereal (Hafner *et al.* 1993; Kamh *et al.* 2002; Nuruzzaman *et al.* 2005), it remains unclear which role the legume residues have in this pre-crop effect. Therefore the aim of this study is to differentiate between the effect of the pre-crop only and that of pre-crop and their residues on P uptake and AM colonisation of wheat.

Materials and Methods

Soil

Loamy sand soil (0-20 cm) from Monarto (35°05′ S and 139°06′ E), South Australia was used. The soil was air dried, thoroughly mixed and sieved to 2 mm. The soil properties are 1.26 % organic matter, 229.6 mg kg^{-1} total P, 4.5 mg P kg^{-1} resin P, 31.2 mg N kg^{-1} nitrate-N, 82.6 % sand, 10% silt, 7.5% clay, 15% water holding capacity, 8.82 pH (1:5 soil: water). During the experiment, the soil moisture was maintained at 70% of water holding capacity by weighing the pots and adding RO water several times a week.

Experimental design

The experiment was conducted in a glasshouse with 2 kg pots for the pre-culture. Chickpea (*Cicer arietinum*), faba bean (*Vicia faba* L.) and white lupin (*Lupinus albus* L.) and wheat (*Triticum aestivum* L.) (Krichauff) were grown for 10 weeks. Unplanted pots as controls were prepared and received the same nutrient amendments as the planted pots. Germinated seeds were sown at 2 cm depth and plants were thinned after 2 weeks to 2 plants per pot. A nutrient solution (μM) with NH_4NO_3, 200; K_2SO_4, 100; $MgSO_4$, 54; $CuSO_4$, 0.02; $MnSO_4$, 0.24; H_3BO_3, 2.4; $NaMO_4$, 0.03; and $ZnSO_4$, 0.1 was added at 40 ml per pot every week to ensure that these nutrients did not limit plant

growth (Nuruzzaman *et al.* 2005). Legumes did not receive inorganic N, but were inoculated with the respective Rhizobium strain. There were 4 replicates per treatment.

After 10 weeks, roots were carefully washed out of the soil. After drying at 70°C for 48 hours, roots and shoots were cut to 1- 2 cm length before mixing into the soil. All residues of a given species were combined with wheat having the lowest amount. The amount of residues added was the same for all pre-crops. Four treatments were imposed: (1) pre-crop only: both roots and shoots of the pre-culture were removed completely; (2) pre-crop and root residues: shoots of each pre-crop were removed, while roots (0.04 % w/w) were mixed into the soil; (3) pre-crop and shoot residues: roots were removed and shoot (0.24% w/w) mixed with soil; (4) pre-crop and residues of roots and shoots: a mixture of shoot and root residues 0.24% shoots + 0.04 % w/w roots) was added. Control soils without pre-crop were mixed in the same manner. There was no inorganic fertiliser application in the main crop (wheat). Wheat (cv Krichauff) was planted immediately after mixing the residues into the soil. Wheat growth and P uptake and AM colonisation, were assessed 6 weeks after planting.

Arbuscular mycorrhiza assessment

At harvest, roots washed from the soil. Root subsamples (~0.2 g) were cut approximately in 1 cm before putting in the cassettes and submerging using into KOH 10% (for clearing at the room temperature for 2-3 days and then stained using the modification of Vierheilig *et al.*'s methods (1998). Stained roots were scored to assess AM colonisation using gridline intersection method (Brundrett *et al.* 1996; Giovannetti and Mosse 1980) under a dissecting microscope at 40× magnification.

Soil and plant analyses

Total P concentration of shoots and roots was assessed using phosphovanado-molybdate method by Hanson (1950) after digestion in nitric: perchloric acid solution (6:1). Total

N concentration was analysed by the Kjeldahl method (Bradstreet 1965) and colorimetrically measured at 650 nm wavelength (Bremner and Black 1965). Soil available P was extracted using the anion exchange membrane method (Kouno *et al.* 1995) and colorimetrically measured at 712nm wavelength (Murphy and Riley 1962).

Statistical analysis

The experiment was conducted in a Completely Randomised Design and data was analysed using Genstat 11th edition (Windows) for analysis of variance (ANOVA). Means were tested using Tukey's at 95% confidence intervals.

Results

Among the pre-crops, shoot dry weight of white lupin was the lowest and faba bean was the highest (Table 1) and root dry weight was lowest in wheat. Wheat shoots had the highest total P concentration and the highest available P in soil. However, wheat showed the lowest total N concentration, whereas white lupin had the highest shoot N concentration which was twice as high as that of wheat and chickpea had the highest root N concentration being approximately 3-fold higher than in wheat. The available P in soil immediately after pre-crops was highest in the control soil and lowest after faba bean. AM colonisation of the pre-crops was very low. Roots of faba bean and white lupin were not colonised and AM colonisation of wheat and chickpea was 8 and 7%, respectively.

Shoot and root dry weights of the following wheat were greatest in the previously unplanted control (Table 2). The pre-crops chickpea and white lupin resulted in higher shoot and root dry weight of the following wheat than the pre-crops wheat and faba bean. In the pre-crops wheat, faba bean, and white lupin, addition of root residues had

no effect on dry weight of the following wheat, whereas addition of either shoots or the mixture of shoots and roots decreased the dry weight of the following wheat with the strongest effect for faba bean, where shoot dry weight of the following wheat was decreased by 49% compared to the pre-crop alone. In contrast, addition of chickpea roots and the mixture of roots and shoots increased dry weight of the following wheat by 32% and 44%, respectively, compared to chickpea as pre-crop only.

Pre-crops generally decreased shoot N concentration of the following wheat compared to wheat grown in the previously unplanted soil (Table 2). Addition of wheat or faba bean residues had no effect on the N concentration of the following wheat, whereas addition of shoot or mixture of shoot and root residues of chickpea and white lupin increased N concentrations of the following wheat by 47% for chickpea shoot addition and 26% with shoots from white lupin compared to the pre-crop alone.

Wheat shoot P concentration in the pre-crop treatments was generally higher than in wheat grown in previously unplanted soil except for wheat as pre-crop alone or with root residue addition (Table 2). Compared with pre-crop alone, wheat shoot P concentration was increased more strongly by addition of shoot residues than by adding root residues. Chickpea shoot addition had the strongest effect, increasing wheat shoot P concentration by 47% compared to pre-crop alone. P uptake of wheat following chickpea and white lupin, was generally higher than in wheat following wheat and faba bean and wheat in the previously unplanted control, especially with addition of residues (Figure 6). Compared to the pre-crops alone, addition of shoot residue increased P uptake of the following wheat in the pre-crops wheat, white lupin and chickpea, with strongest effect in chickpea where wheat P uptake was increased by 50% compared to pre-crop only. On the other hand, addition of residues of faba bean decreased wheat P

uptake compared to the pre-crop alone, leading to the lowest P uptake among all treatments.

Available P decreased from week 1 to week 6 in all treatments (Figure 2). Compared to the unplanted control, available P after 1 week was lower in all pre-crop treatments except white lupin pre-crop alone or with root residues. In general, available P among the pre-crops was lower after faba bean and chickpea than after wheat or white lupin. Residue addition had no effect on available P in faba bean and chickpea whereas in wheat as a pre-crop, shoot residue addition alone or in the mix increased available P compared to the pre-crop alone. In white lupin, addition of the mix of shoots and roots decreased available P compared to the pre-crop alone. After 6 weeks compared to the previously unplanted control available P was generally after the pre-crops, particularly after faba bean and chickpea. The only exception was the wheat pre-crop where addition of shoot residue alone or in the mix increased available P compared to the previously unplanted control. Compared to the pre-crop alone, addition of residues had no effect on available P after 6 weeks in faba bean and white lupin whereas in wheat addition of shoot residues and in chickpea, addition of root, shoot residues or the mixture of shoot and root residues increased available P.

Compared to the previously unplanted control, pre-crops increased AM colonisation of the following wheat between 2 and 10-fold (Figure 3). Nevertheless, AM colonisation of the wheat was quite low, ranging between 2 and 16%. Among the pre-crops, AM colonisation of the following wheat was generally higher after wheat and faba bean than after chickpea and white lupin. Residue addition had no significant effect on AM colonisation.

Discussion

Shoot and root dry weight in all pre-crop treatments was lower than in the previously unplanted control (Table 1). This may be due to lower P availability following faba bean and chickpea, but P availability is not lower following wheat and white lupin (Figure 1b). Residue addition may cause a decrease in available P due to immobilisation in the microbial biomass (Mafongoya *et al.* 2000; Schomberg and Steiner 1999). Having a low P concentration lower than the critical limit at 0.25% in residues may lead to P net immobilisation (Nziguheba *et al.* 1998). However, all treatments in current experiment showed lower P concentrations than the critical level, except for wheat shoot residue addition (0.27%) and white lupin root residue amendment (0.28%). Moreover, the N concentration of the following wheat was lower after pre-crops (except for white lupin with shoots or the mixture of shoots and roots), suggesting that pre-crops decreased growth by limiting N availability. The pre-crops took up N and even in the legumes, some of this would have originated from the soil. Among the pre-crops, N concentration was lowest in white lupin (101 g N pot^{-1}) whereas N concentration in chickpea was 25% greater and that in faba bean twice as high than in white lupin. However, the low N uptake in the following wheat occurred even when the pre-crop residues were added to the soil. This suggests that N was not released during decomposition of the residues. In contrast, Nuruzzaman et al.(2005) found that in a loamy soil wheat growth was significantly higher in soils with previously planted legumes than in unplanted soils. The negative effect of the pre-crops on the following wheat suggests rotations where wheat follows legumes immediately may not improve the growth of wheat compared to wheat planted after a fallow period. It should be noted though, that nutrients stored in the legume residues will be eventually released and may therefore be an important nutrient source for the following crop, for example released P from decomposing legume residues that can be used by the following cereals (Horst *et*

al. 2001). It is possible that this would have also occurred in the present study if wheat had been grown for longer.

Among pre-crops without residue addition, white lupin increased growth of the following wheat compared to the other pre-crops. This positive effect is in agreement with Asseng et al. (1998) who reported high yields of wheat following lupins (narrow leaf lupin and white lupin) compared to after barley, chickpea and field pea. These authors stated that lupins contributed to the positive effect N_2 fixation from the soil. This effect is unlikely in the present study as white lupin did not significantly increase N concentrations of the following wheat compared to the other pre-crops. On the other hand, Nuruzzaman et al. (2005b) found that without addition of residues, faba bean pre-crop increased the growth of the following wheat to a greater extent than white lupin The poorer growth of wheat after wheat, faba bean and chickpea may be due to the low P availability after the first week of wheat growth (Figure 1a). Although shoot P concentrations of the following wheat were not lower, P uptake was lower following wheat and faba bean. On the other hand, the available P concentration after the first week of wheat growth and P uptake of the following wheat was greatest after white lupin suggesting that white lupin mobilised P that was then available to the following wheat. The lack of increase in available P after white lupin can be explained by the greater P uptake of the following wheat, i.e. any P mobilised was taken up by the wheat. White lupin roots release large amounts of carboxylates e.g. citrate from its cluster roots (Marschner *et al.* 1987), that increase available P (Gerke *et al.* 1994). (Dinkelaker *et al.* 1989; Keerthisinghe *et al.* 1998),

In general, addition of root residue had no effect on growth or P uptake of the following wheat, which is probably due to the very small amount of residue and nutrients added (0.11-1.10 g P kg^{-1} compared to 3.2-6.6 g P kg^{-1} added with shoot residues).

The effect of addition of shoot residue or the mix on the growth of the following wheat compared to the pre-crops alone differed between the pre-crops: no effect in white lupin, decreased growth after faba bean and wheat, increased shoot dry weight after chickpea.

Addition of shoots or the mixture of shoots and roots of white lupin did not affect P availability, P uptake or AM colonisation of the following wheat, but increased wheat shoot N concentration hence increased N uptake. Due to the short growth period of the wheat in the present experiment, this did not improve the growth of the following wheat. However, the greater N uptake could increase wheat growth in the later stages of development. This is in agreement with a study showed that wheat yield was increased by 131% following lupins compared to wheat following cereal. This higher yield in wheat following lupins was explained by greater N uptake (Asseng *et al.* 1998). Other studies also have demonstrated that legumes can increase N uptake of the following wheat (Armstrong *et al.* 1997; Evans *et al.* 1991).

Addition of shoot residue or the mix of the faba bean and wheat pre-crops decreased the growth of the following wheat compared to the pre-crops alone. Although up to 11.4 g P kg^{-1} was added with the residues, P availability was only increased by about 2 mg kg^{-1} and wheat P uptake was 3-6 mg P pot^{-1}. This suggests that over the 6 weeks following the incorporation of the residues, there was only a small net release of P from the residues Shoot P concentration in the following wheat was increased by residue addition, however this did not increase the growth of the wheat, suggesting that another nutrient was limiting the growth of the wheat after residue addition. This is unlikely to be N because residue addition did not affect shoot N concentrations. Residue addition also did not affect AM colonisation of the following wheat. Therefore, the poorer growth of wheat after addition of faba bean or wheat residues cannot be due to depression of AM colonisation.

Other studies have demonstrated that the addition of residues, particularly of wheat and sorghum has negative effects on early growth (Jessop and Stewart 1983) and yield of wheat (Sidhu and Beri 1989). The adverse effects could be due to immobilisation of nutrients by the microbial biomass after addition of residues with low N or P concentrations (Cheshire and Chapman 1996). Shoot P concentration was only 1.6 g kg^{-1} in faba bean and shoot N concentrations of the wheat pre-crop was lower than in the other pre-crops.

Only in chickpea, the addition of shoot or mixed residues improved growth and P uptake of the following wheat compared to the pre-crop alone. This cannot be explained by a greater amount of P added as it was similar as with that added with residues of faba bean or wheat. However, P availability after 6 weeks was increased by residue addition compared to the pre-crop alone and residue addition increased shoot P concentrations and P uptake in the following wheat. This suggests that P was mobilised either from the chickpea residues or from the soil during decomposition of chickpea residues. It has been reported that legume residues may mobilise soil P by carboxylates released during decomposition and that organic matter from residues may block P-fixing sites (Ayaga *et al.* 2006). This increased P availability after addition of chickpea residue occurred although the amount of P added was similar as with W and FB residues, indicating that chickpea residues may be more easily decomposable. The P concentration in chickpea residues were greater than in faba bean residues and the N concentration were greater than in wheat residues. Moreoverdecomposition rate is not only affected by nutrient concentration, but also by cellulose, polyphenol and lignin contents in residues (Baggie *et al.* 2005).

AM colonisation of the following wheat was relatively low and did not differ consistently among pre-crops. Faba bean pre-crop alone resulted in the highest AM colonisation which may be due to the low P availability after 6 weeks in this treatment.

However, P availability was also low in chickpea and this did not result in higher AM colonisation. Thus, there was no relationship between AM colonisation and available P concentration. Residue addition also had no consistent effect on AM colonisation which may be due to the lack of strong changes in P availability by residue addition. This result is in agreement with our previous study with legume residue applications where there was no correlation between available P and AM colonisation when P was added with residues whereas AM colonisation decreased with increasing available P when inorganic P was added (Hasbullah, unpublished). In that study, low colonisation rates occurred not only at low available P concentration with chickpea residue amendment, but also at high available P concentration with faba bean young shoot addition. In a recent study, that residue application reduced AM colonisation in cereals (Duan et al.(2010). Different residues may have different effects on AM colonisation, due to effects on biological and chemical properties of the soil (Borie *et al.* 2002; Duan *et al.* 2010).

Conclusion

Among the legume pre-crops, only white lupin increased the growth and P uptake of the following wheat compared to wheat as pre-crop. This growth increase occurred in presence or absence of residues, suggesting that P mobilised by white lupin during its growth is available to the following wheat and that this P mobilisation was more important than any P released during decomposition of the white lupin residues. The growth increase of wheat following white lupin did not seem to be related to AM colonisation since it was in generally lower than in the other treatments.

Chickpea increased the growth and P uptake of the following wheat only after addition of residues which indicates that P released from the residues and not that mobilised in the soil during chickpea growth is responsible for the growth improvement of the following wheat.

In contrast to previous studies, faba bean as pre-crop did not improve the growth of the following wheat, in presence of the residues growth of wheat was even decreased despite the addition of P and N with the residues at levels similar to those added with the other legume pre-crops. This suggests that incorporation of faba bean residues may have a negative effect on the following wheat.

It should be noted that in the present study, all residues were added at the same rate. In the field, the amount of residue added will depend on the growth of the pre-crop; in that case, the results may be different from those shown here. Moreover, our results may be specific to the soil used. Experiments in different soil types and under field conditions are required to ascertain the effect of legume pre-crops on the following cereal.

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Table 1. Pre-crop shoot and root dry weight, P concentration, N concentration after 10 weeks and P added with residues

Pre-cops	**Plant parts**	**Dry weight g/pot**	**P concentration g kg^{-1}**	**N concentration g kg^{-1}**	**P added g kg^{-1}**
Wheat	shoots	2.77	2.67	15.0	6.62
	roots	0.50	0.21	14.5	0.11
	Mixture (roots&shoots)				8.93
Faba bean	shoots	4.86	1.60	25.7	3.85
	roots	2.71	2.36	37.9	0.85
	mixture (roots&shoots)				10.44
White lupin	shoots	2.73	1.36	30.6	3.27
	roots	0.54	2.75	33.7	1.10
	mixture (roots&shoots)				11.66
Chickpea	shoots	3.35	2.41	24.6	5.29
	roots	1.05	1.46	40.5	0.58
	mixture (roots&shoots)				9.94

Table 2. Shoot and root dry weight, P and N concentration of shoots of following wheat (6 weeks old) after pre-crops and amendment with root residues shoot residues and mixture roots and shoots (n=4).

Pre-crop and residue treatments	Shoot dry weight (g)	Root dry weight (g)	N concentration (g kg^{-1})	P concentration (g kg^{-1})
Control	1.70	0.49	21.5	2.84
Wheat				
Precrop only	1.09	0.29	15.9	3.04
Root residues	1.11	0.33	16.3	2.86
Shoot residues	0.89	0.26	17.8	4.01
Mixture (roots&shoots)	0.86	0.25	17.2	4.48
Faba bean				
Precrop only	1.17	0.29	14.9	3.71
Root residues	1.22	0.32	14.3	3.71
Shoot residues	0.85	0.16	16.9	5.16
Mixture (roots&shoots)	0.60	0.11	16.0	4.93
White lupin				
Precrop only	1.44	0.37	12.6	3.58
Root residues	1.54	0.43	16.6	3.70
Shoot residues	1.19	0.25	21.2	4.50
Mixture (roots&shoots)	1.36	0.35	22.8	3.60
Chickpea				
Precrop only	1.14	0.34	14.0	3.61
Root residues	1.50	0.37	13.7	3.06
Shoot residues	1.32	0.33	19.6	5.29
Mixture (roots&shoots)	1.64	0.37	18.8	3.70

Figure 1. Wheat shoot P uptake (6 weeks old) after pre-crops and amendment with root residues shoot residues and mixture roots and shoots Line indicates values of the previously unplanted control (n=4)

Figure 2. Available P in soil one week (**a**) and 6 weeks (b) after addition of different types and amounts of legume residues and available P 6 weeks after pre-crops and amendment with root residues shoot residues and mixture roots and shoots Line indicates values of the previously unplanted control (n=4)

Figure 3. AM colonisation (%) of wheat (6 weeks old) after pre-crops and amendment with root residues shoot residues and mixture roots and shoots Line indicates values of the previously unplanted control (n=4).

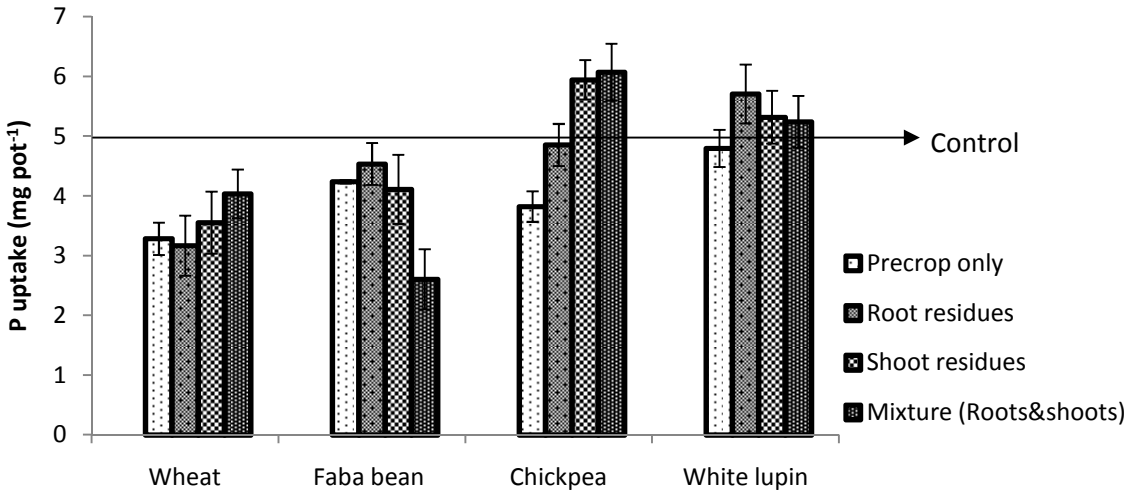
7
6
5
4
3
2
1
0
P uptake (mg pot-1)
Control
Precrop only
Root residues
Shoot residues
Mixture (Roots&shoots)
Wheat
Faba bean
Chickpea
White lupin

Figure 1

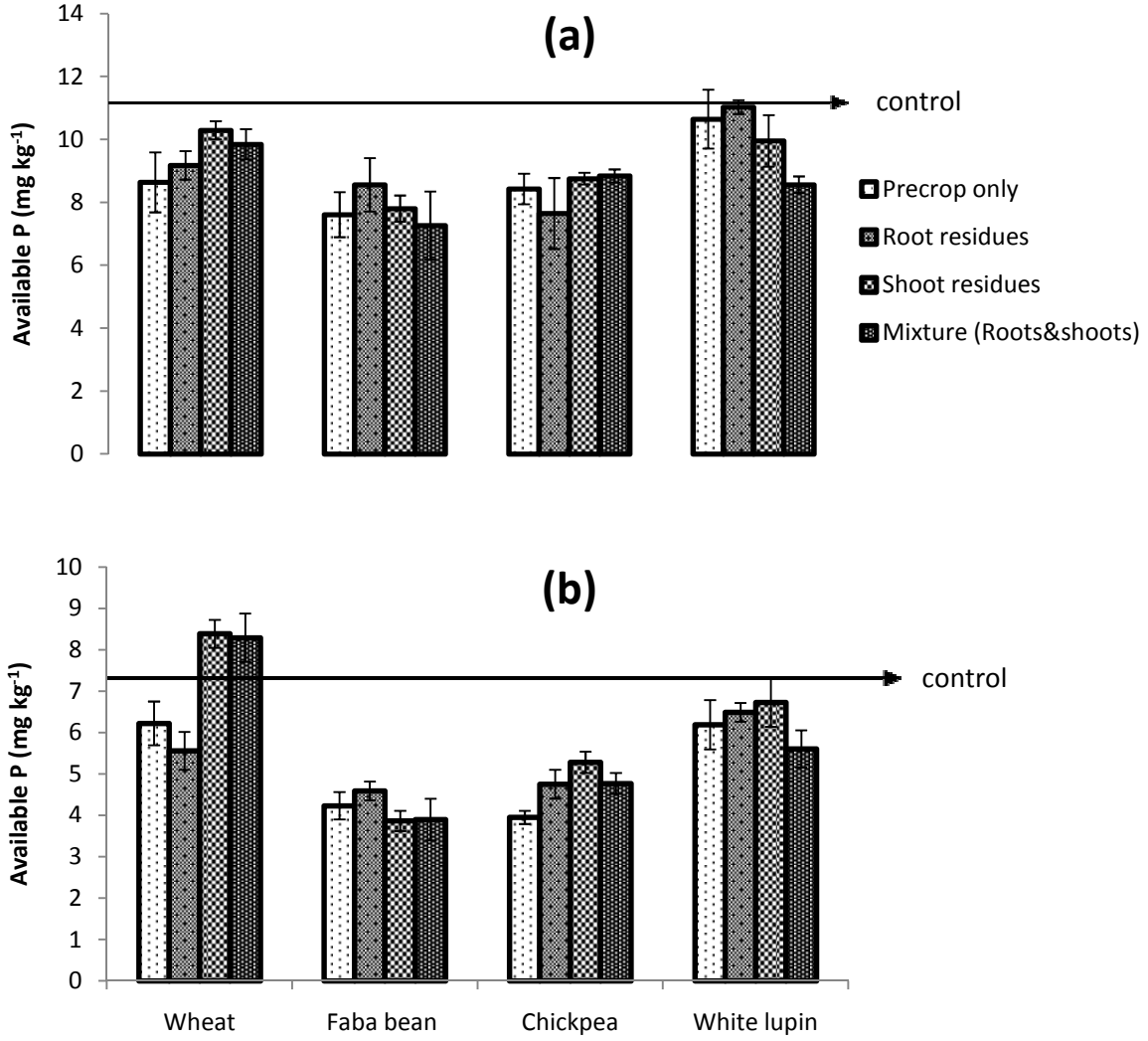
(a)
Available P (mg kg-1)
14
12
10
8
6
4
2
0
control
Precrop only
Root residues
Shoot residues
Mixture (Roots&shoots)
(b)
Available P (mg kg-1)
10
9
8
7
6
5
4
3
2
1
0
control
Wheat
Faba bean
Chickpea
White lupin

Figure 2

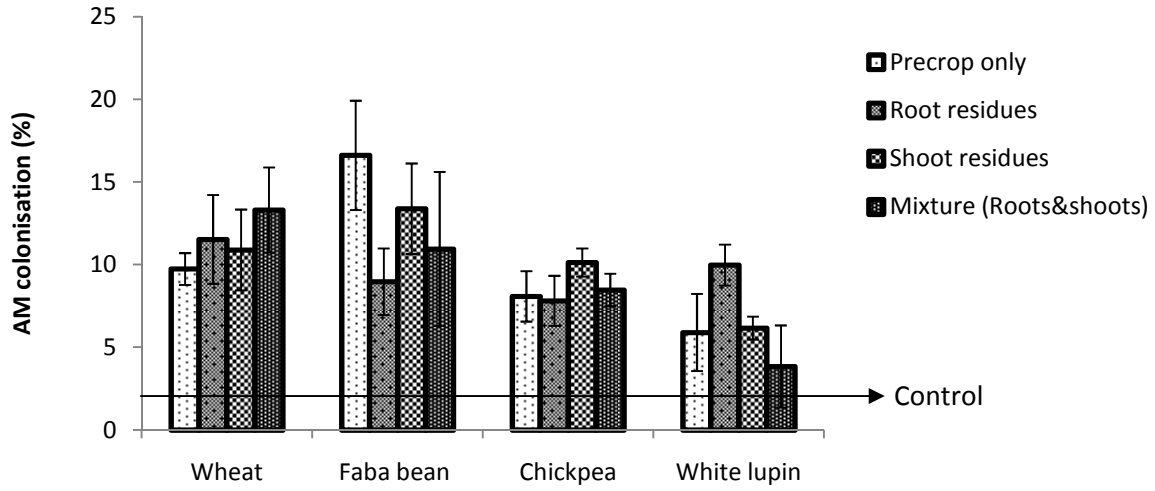
25
20
15
10
5
0
AM colonisation (%)
Precrop only
Root residues
Shoot residues
Mixture (Roots&shoots)
Control
Wheat
Faba bean
Chickpea
White lupin

Figure 3

CHAPTER 4

The effects of moisture regime between harvest of the precrop and planting of wheat on arbuscular mycorrhizal colonisation and phosphorus uptake by the following wheat

The effects of moisture regime between harvest of the precrop and planting of wheat on arbuscular mycorrhizal colonisation and phosphorus uptake by the following wheat

Introduction

Legumes are an important crops in Australian agricultural systems. Legume species have different abilities to use and mobilise P from the soil pools (Bagayoko *et al.* 2000). It has been demonstrated that Some have higher capacity for P uptake than cereals and to have access to P pools not available to wheat (Kamh *et al.* 2002; Nuruzzaman *et al.* 2005). However, there is scarce information about how soil moisture regime during fallow time affect P uptake and AM colonisation of the following wheat.

In the experiment described in chapter 3 and most pot experiments, the cereal was planted immediately after the pre-crop. However in the field, there is usually a fallow between the harvest of the legume and the following cereal, allowing pre-crop residue decomposition which may alter the effect of the pre-crop on growth and P uptake of the cereal.

The soil water content during this fallow time may affect the rate of decomposition as well as interactions of P with the soil. At sufficient water content, residue decomposition would be rapid, leading to mineralisation of P and N, however, the released P may also be sorbed to soil particles and become unavailable. At low water content, decomposition rates would be low, but the P released may remain available. A long dry period may also reduce the capacity of AM fungi to colonise the roots of the following wheat rapidly.

The aims of this experiment were to study the effect of the soil water content during harvest of the pre-crop and planting of wheat on arbuscular mycorrhizal colonisation and phosphorus uptake by the following wheat

Materials and Methods

Soil

A loamy sand (0-20 cm) from Monarto (35°05′ S and 139°06′ E), South Australia was used. The soil was air dried, thoroughly mixed and sieved to 2 mm before used. The soil properties are 229.6 mg/kg total P, 4.5 mg P/kg resin P, 31.2 mg N/kg nitrate-nitrogen, 82.6 % sand, 10% silt, 7.5% clay, 1.26 % organic carbon, 15% water holding capacity and 8.82 pH (25°C). During the growth of the pre-crops and the following wheat, soil moisture was maintained at 70% WHC by weighing the pots and adding RO water.

Experimental design

In the pre-crop phase,chickpea (*Cicer arietinum*), faba bean (*Vicia faba* L.) and white lupin (*Lupinus albus* L.) and wheat (*Triticum aestivum* L.) (Krichauff) were were grown in the soil in 2kg pots. Germinated seeds were sown at 2 cm depth and plants thinned after 10 days to 2 plants per pot and 4 replicates unplanted pots were the control pots.

After 10 weeks, roots were carefully washed out of the soil. After drying at 70^{o}C in 2 days, roots and shoots were cut to 1- 2 cm length before mixing into the soil. All residues of a given species were combined with wheat having the lowest amount. The amount of residues added was the same for all pre-crops. Four treatments were imposed: (1) pre-crop only: both roots and shoots of the pre-culture were removed completely; (2) pre-crop and root residues: shoots of each pre-crop were removed, while roots (0.04 % w/w) were mixed into the soil; (3) pre-crop and shoot residues: roots were removed and shoot (0.24% w/w) mixed with soil; (4) pre-crop and residues of

roots and shoots: a mixture of shoot and root residues 0.24% shoots + 0.04 % w/w roots) was added. Control soils without pre-crop were mixed in the same manner. There was no inorganic fertiliser application in the main crop (wheat).

Following the mixing with the residues, the soil was filled into 1 kg pots. Then the pots of each treatment were incubated for 30 days in the glasshouse at two different soil moisture regimes: (i) drying-rewetting (DR) in which the soil was allowed to dry and remained dry until day 31 when the following wheat was planted when the soil was rewet to 70% WHC and (ii) constant moisture (CM) in which the soil was maintained at 70% WHC. P availability, AM colonisation, growth and P uptake were assessed after 6 weeks of wheat growth. Shoot and root dry weight were determined after drying at 70°C for 48 hours.

Arbuscular mycorrhiza assessment

The roots were harvested and washed from the soil. Root subsamples (~0.2 g) were cut approximately in 1 cm before putting in the cassettes and submerging using into KOH 10 (2-3 days) for clearing at the room temperature then stained using the modification of Vierheilig *et al.*'s methods (1998). Roots were stained roots and scored to assess AM colonisation using gridline intersection method (Brundrett *et al.* 1996; Giovannetti and Mosse 1980) under a dissecting microscope at 40× magnification.

Soil and plant analyses

Total P concentration of plant shoots and roots was assessed using phosphovanado-molybdate method by Hanson (1950) and total N concentration was analysed by kjeldahl method (Bradstreet 1965) and colorimetrically measured at 650 nm wavelength (Bremner and Black 1965). Soil available P was extracted using anion exchange

membrane method (Kouno *et al.* 1995) and colorimetrically measured at 712nm wavelength (Murphy and Riley 1962).

Results

The dry weight of the pre-crops was greatest in faba bean and lowest in wheat (Table 1). Shoot P concentrations in the pre-crops ranged from 2.4 to 3.6 mg kg-1 and were higher in wheat and faba bean than in white lupin and chickpea. Shoot N concentrations ranged from 12.2 to 37.9 mg kg-1 and were lowest in wheat and highest in white lupin and faba bean.

Compared to the previously unplanted control, the wheat pre-crop reduced shoot and root dry weight (Table 1 and figure 1). Similarly, faba bean pre-crop reduced wheat shoot dry weight compared to the previously unplanted control except when root residues were added. Chickpea and white lupin pre-crops also reduced wheat shoot dry weight relative to the previously unplanted control in the treatment without residue addition and with root residues only. However, wheat shoot and root dry weight were similar or higher than in the previously unplanted control when shoot residues or the mixture of shoot and root residues were added.

Among all treatments, the application of faba bean shoot and the mixture (shoots and roots) resulted the lowest shoot dry weight of the following wheat, especially in drying-rewetting (DR) treatment (Figure 1b). Shoot dry weight of the following wheat was highest with after white lupin + root and shoot residue addition and in the control with DR (Figure.1d). The effect of the water content during in the time between pre-crop harvest and planting of the wheat on shoot dry weight of the following wheat varied with pre-crop treatment. In the previously unplanted control, wheat shoot dry weight was lower in the CM compared to the DR treatment (Figure 1c). In the pre-crop soils,

the water content on the shoot dry weight of the wheat had either no effect (chickpea, white lupin, wheat pre-crop only, or + root residues) or shoot dry weight of the following wheat was lower in DR than in CM (faba bean and wheat pre-crop + shoot residues or mixture.

Compared to the previously unplanted control, shoot P concentration was increased with faba bean as pre-crop with addition of shoot residues or the mixture and by wheat as pre-crop with addition of shoot residues in the DR treatment (Table 1). The other pre-crop treatments had either no effect (chickpea) or decreased wheat shoot P concentration (white lupin with residues). The soil water content during the time between legume harvest and planting of the following wheat had no effect on the P concentration in the shoots of the following wheat in the previously unplanted control, but in most pre-crop treatments, shoot P concentrations in the following wheat were lower in CM than CR except in white lupin where the reverse was true with addition of root residues or the mixture.

In all pre-crop treatments, the N concentrations in the following wheat were lower in CM than in DR with decreases up to 50% and more (Table 1). The P uptake of the following wheat showed a similar pattern among treatments as the shoot dry weight, with generally smaller differences between CM and DR than in shoot dry weight (Table 1)

At the time of harvest of the following wheat, the available P concentration was generally higher in the soil that had been left unplanted during the pre-crop phase than in the soil that had been planted with the pre-crops faba bean and chickpea with greater differences in DR than in CM (Table 1). In the previously unplanted control, available P was lower in CM than in CR and this was also the case in most white lupin treatments,

whereas the soil water content during the time between legume harvest and planting of the following wheat had no effect on available P in the other pre-crop treatments.

AM colonization of wheat was very low in the soil which was unplanted during the pre-crop phase. Among the pre-crops, it was generally highest in wheat after faba bean and lowest in wheat after white lupin. Residue addition increased AM colonisation of the following wheat compared to pre-crop only in wheat, whereas in faba bean AM colonisation of the following wheat was only increased with addition of root residues and in chickpea only with addition of shoot residues or the mixture. In wheat following white lupin, AM colonisation was decreased by the addition of residues. Compared to CM, AM colonisation of the following wheat was increased in DR in only some of the treatments: in the previously unplanted control, in pre-crop wheat with shoot residues, the faba bean pre-crop treatments (except faba bean + mixture), chickpea + shoot residues and in white lupin pre-crop only. In the other treatments DR decreased AM colonisation of the following wheat compared to CR (e.g. chickpea pre-crop only, white lupin + root residues) or had no effect.

Discussion

The results obtained in the present study are in general quite similar to those in the previous experiment where wheat was planted immediately after residue addition (Chapter 3). The dry weight of the pre-crops was similar while P and N concentrations of faba bean and white lupin were higher in this study compared to the previous experiment. Given that the same amount of residue was added in the two experiments, this would have resulted in a greater N and P addition with the residues of faba bean and white lupin. However, this did not lead to higher P availability at the harvest of the following wheat (figure 3b and 3d), greater shoot dry weight or P concentration of the following wheat, suggesting that the two experiments are comparable.

Shoot and root dry weights of the following wheat are in the same range in the two experiments as are the trends among pre-crop treatments (Table 1, Figure 1). Available P concentrations (Figure 3) and AM colonisation (Figure 2) are also in the same range in this and the previous study. However, there are also some differences. Faba bean as pre-crop resulted in the highest shoot P concentration in the following wheat in both experiments (Table 1), but whereas the shoot P concentration was higher with white lupin and chickpea as pre-crops compared to wheat as pre-crop in the previous experiment, there were no differences among these treatments in the present study. This indicates that a period of 30 days between residue addition and planting of wheat may reduce some differences in P release from residues compared to planting of wheat immediately after residue addition. This could be due to the fact that in the present study, there were 10 weeks between residue addition and harvest of the following wheat, whereas this period was only 6 weeks in the previous experiment. The longer period would allow more time for residue decomposition, but also sorption of P to soil particles. Available P concentrations were in the same range in both experiments, suggesting that wheat was able to take up some of the P released during decomposition of white lupin and chickpea residues when it was planted immediately after residue addition. On the other hand, when wheat was planted 4 weeks after residue addition, the P released from residues during this time and during wheat growth appeared to be less available to wheat.

The fact that AM colonisation was in a similar range and shows similar trends among pre-crop treatments in both experiments suggests that a 4 week period between the harvest of one host (pre-crop) and the planting of the next host (wheat) did not affect the AM colonisation potential of the soil. However, a longer period of fallow could have adverse effects on AM colonisation because AM propagule viability decreases over time (Thompson 1987). In cotton, AM colonisation decreased after a

long fallow time (McGee *et al.* 1997). Furthermore, disturbance during fallow such as drying-rewetting lowered AM colonisation (Pattinson and McGee 1997)

The most striking difference between the two experiments occurred with shoot N concentrations of the following wheat (Table 1). Whereas shoot N concentrations were similar between the previous experiment and the DR treatment in the present study, they were about 50% lower in the CM treatment. Thus, during the 4 weeks between harvest of the pre-crops and planting of the wheat, N became less available when the soil was moist. This may be due to N immobilisation in the microbial biomass, but also gaseous N loss via denitrification. Although the soil was maintained at 70% WHC and the soil appeared to be well-aerated at this water content, anaerobic conditions and thus denitrification may have occurred immediately after watering or in microsites. Denitrification rather than immobilisation seems to be the most likely reason, since the decrease in shoot N concentrations with CM also occurred in the soils without added residues where immobilisation was likely to be low due to lack of easily available C. The lower shoot N concentration in the CM treatment cannot be explained by a dilution effect since wheat shoot dry weight was not consistently higher in CM than in DR. In the DR treatment on the other hand, any N released from the decomposing residues and immobilised in the microbial biomass during the period when the soils dried, would have remained immobilised during the dry period. Rewetting would have released a proportion of microbial biomass N and resulted in higher microbial activity (Birch 1958, Xiang et al. 2008) and enhanced decomposition of residues during the growth of the following wheat. However, the lower shoot N concentrations did not seem to a negative effect on wheat growth because shoot and root dry were not consistently lower in CM than in DR.

From the comparison of this and the previous experiment it can be concluded that wheat growth and P uptake and AM colonisation are generally little affected by

delaying the planting of the wheat four weeks after pre-crop harvest compared to immediate planting. Moreover, this delay did not seem to have a strong impact on the effect of the various pre-crop treatments.

Except for the shoot N concentration in the following wheat, the soil water content during the time between pre-crop harvest and planting of the following wheat had no consistent effect on the measured parameters, but some differences could be detected. Compared to the soils with pre-crops, wheat shoot dry weight was substantially higher in the previously unplanted soil in DR whereas there were smaller or no differences in CM. This is also the case for available P. Moreover, wheat shoot dry weight in DR was similar as in the previous experiment where wheat was planted immediately after mixing, but lower in CM. This suggests that in the previously unplanted soil constant moisture during the time after mixing the soil and planting of the wheat has resulted in a decrease in P availability and thus growth of the following wheat. This effect was compounded by the lower N availability. In the previously unplanted soil, mixing may result in increased microbial activity and thus mineralisation of native soil organic matter and possibly also exposure of previously occluded P. This released P would have been available to the wheat planted immediately after mixing (Chapter 3) and, if the soil was dry after mixing, remained available or become available again after rewetting in the DR treatment in the present study. In the CM treatment on the other hand, the released P could have become fixed or immobilised due to the presence of water in the time after mixing and the planting of wheat. Similarly, Bramley *et al.* (1992) found that moist soil during incubation resulted in lower P availability compared to dry soil during incubation or planting of clover immediately after addition of P fertiliser.

On the other hand, constant moisture increased the growth of wheat with faba bean as pre-crop compared to DR (Figure 1). This was not related to increased P

availability (Figure 3) or higher shoot N concentration (Table 1). In the DR treatment and in the previous experiment, faba bean pre-crop, particularly with addition of shoot residues, strongly decreased the growth of the following wheat compared to the previously unplanted soil, suggesting that compounds toxic to wheat may be released during faba bean growth and during decomposition of faba bean residues. The low soil moisture in the DR treatment would have inhibited decomposition of the residues or compounds released from faba bean roots during the pre-crop phase in the period between faba bean harvest and planting of wheat. But upon rewetting, the compounds released from the faba bean roots would be dissolved and the decomposition of the residues resumed. In the CM treatment on the other hand, decomposition of toxic root exudates and faba bean residues would have proceeded during the period until wheat planting. This finding suggests that although faba bean as pre-crop may inhibit the growth of the following wheat, this effect can be minimised if wheat is not planted immediately after faba bean harvest and if the soil is maintained moist during this period.

In some pre-crop treatments, AM colonisation was higher in DR compared to CM (figure 2). This is in agreement with Braunberger et al. (1996), who showed drying can increase the ability of several types of AM fungi to infect and colonise roots. Such a direct effect the soil moisture between pre-crop harvest and planting of the wheat on infectivity of AM is more likely the reason for the higher AM colonisation in DR since P availability (another factor which often affects AM colonisation) was generally little affected by soil moisture. This is supported by the lack of correlation between P availability and AM colonisation.

Conclusions

In general, a four week period between pre-crop harvest and planting of the following wheat had little effect on the measured parameters compared to planting wheat immediately after the pre-crop. The trends between the pre-crop treatments were quite similar except for a smaller difference between the previously unplanted soil and the pre-cropped soil in terms of wheat growth. This suggests that to take advantage of the relatively higher nutrient availability that accumulated in the unplanted soil, the soil should not be left unplanted for long periods of time (in our studies more than 10 weeks). The results further suggest that toxicity to the following crop that may arise from a pre-crop can be reduced if the soil is maintained moist between the harvest of the pre-crop and the following crop.

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Table 1.Root dry weight, P and N concentration of shoots of the 6 week-old wheat after pre-crops with and without addition of residues and with different soil moisture regime during the 30 day period between pre-crop harvest and planting of wheat: dry and rewet (DR) and constant moist (CM), (n=4).

Pre-crop and residue treatments	Root dw (g)		P utake (mg pot^{-1})		P concentration (g kg^{-1})		N concentration (g kg^{-1})	
	DR	CM	DR	CM	DR	CM	DR	CM
Control	0.76	0.53	5.04	3.76	2.94	2.86	14.6	6.65
Wheat			2.19	2.44				
Precrop only	0.45	0.43	2.17	2.22	2.74	2.78	11.7	6.30
Root residues	0.37	0.40	1.88	2.51	2.75	2.95	11.9	6.86
Shoot residues	0.25	0.37	2.20	2.33	3.44	3.10	13.3	10.7
Mixture (roots&shoots)	0.33	0.34	5.04	3.76	3.52	2.98	16.5	8.7
Faba bean								
Precrop only	0.50	0.43	2.42	2.83	3.16	2.97	12.4	7.06
Root residues	0.30	0.49	2.73	3.04	3.36	2.83	12.2	8.84
Shoot residues	0.11	0.23	1.85	3.40	5.44	3.66	34.9	16.4
Mixture (roots&shoots)	0.09	0.14	1.44	2.55	5.72	4.10	23.3	22.5
Chickpea								
Precrop only	0.44	0.49	2.51	2.54	2.98	2.77	11.1	9.5
Root residues	0.49	0.55	3.48	3.71	3.06	2.83	11.9	9.4
Shoot residues	0.67	0.54	4.53	4.15	3.10	2.75	14.6	13.0
Mixture (roots&shoots)	0.69	0.62	4.10	4.11	2.69	2.61	26.0	13.9
White lupin								
Precrop only	0.52	0.57	3.41	3.40	2.84	2.92	18.8	10.1
Root residues	0.61	0.49	2.74	3.41	2.19	2.57	20.3	10.7
Shoot residues	0.60	0.50	3.85	3.68	2.57	2.49	26.5	12.9
Mixture (roots&shoots)	0.59	0.46	4.02	3.89	2.22	2.44	25.2	9.3

Figure 1. Shoot dry weight of 6 week-old wheat after pre-crops with and without addition of residues and with different soil moisture regime during the 30 day period between pre-crop harvest and planting of wheat: dry and rewet (DR) and constant moist (CM), (n=4).

Figure 2. AM colonisation of wheat (6 weeks) after pre-crops with and without addition of residues and with different soil moisture regime during the 30 day period between pre-crop harvest and planting of wheat: dry and rewet (DR) and constant moist (CM), (n=4).

Figure 3. Available P of 6 week-old wheat after pre-crops with and without addition of residues and with different soil moisture regime during the 30 day period between pre-crop harvest and planting of wheat: dry and rewet (DR) and constant moist (CM), (n=4).

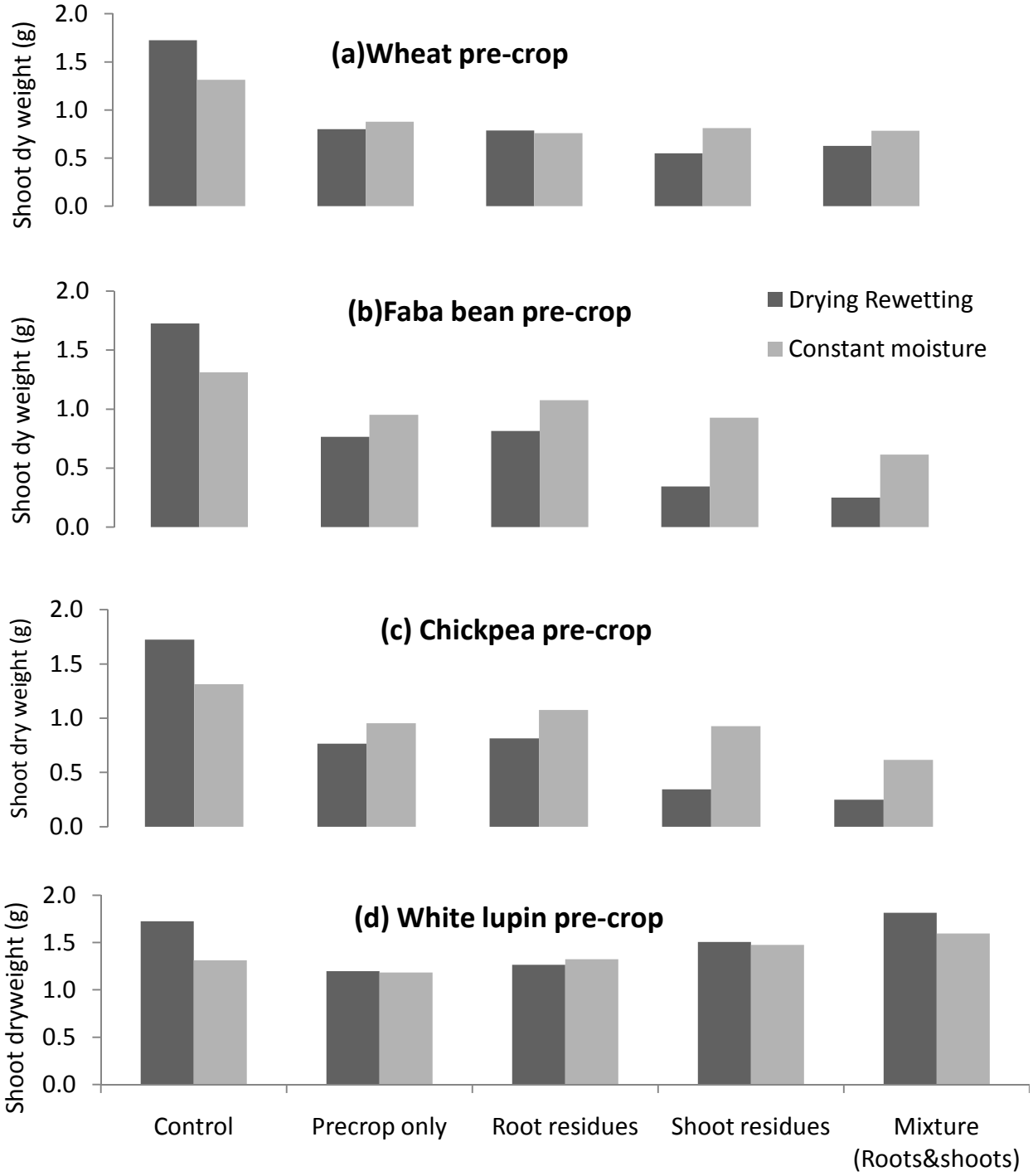
(a)Wheat pre-crop
(b)Faba bean pre-crop
(c) Chickpea pre-crop
(d) White lupin pre-crop
Shoot dy weight (g)
Shoot dry weight (g)
Shoot dryweight (g)
2.0
1.5
1.0
0.5
0.0
Drying Rewetting
Constant moisture
Control
Precrop only
Root residues
Shoot residues
Mixture (Roots&shoots)

Figure 1.

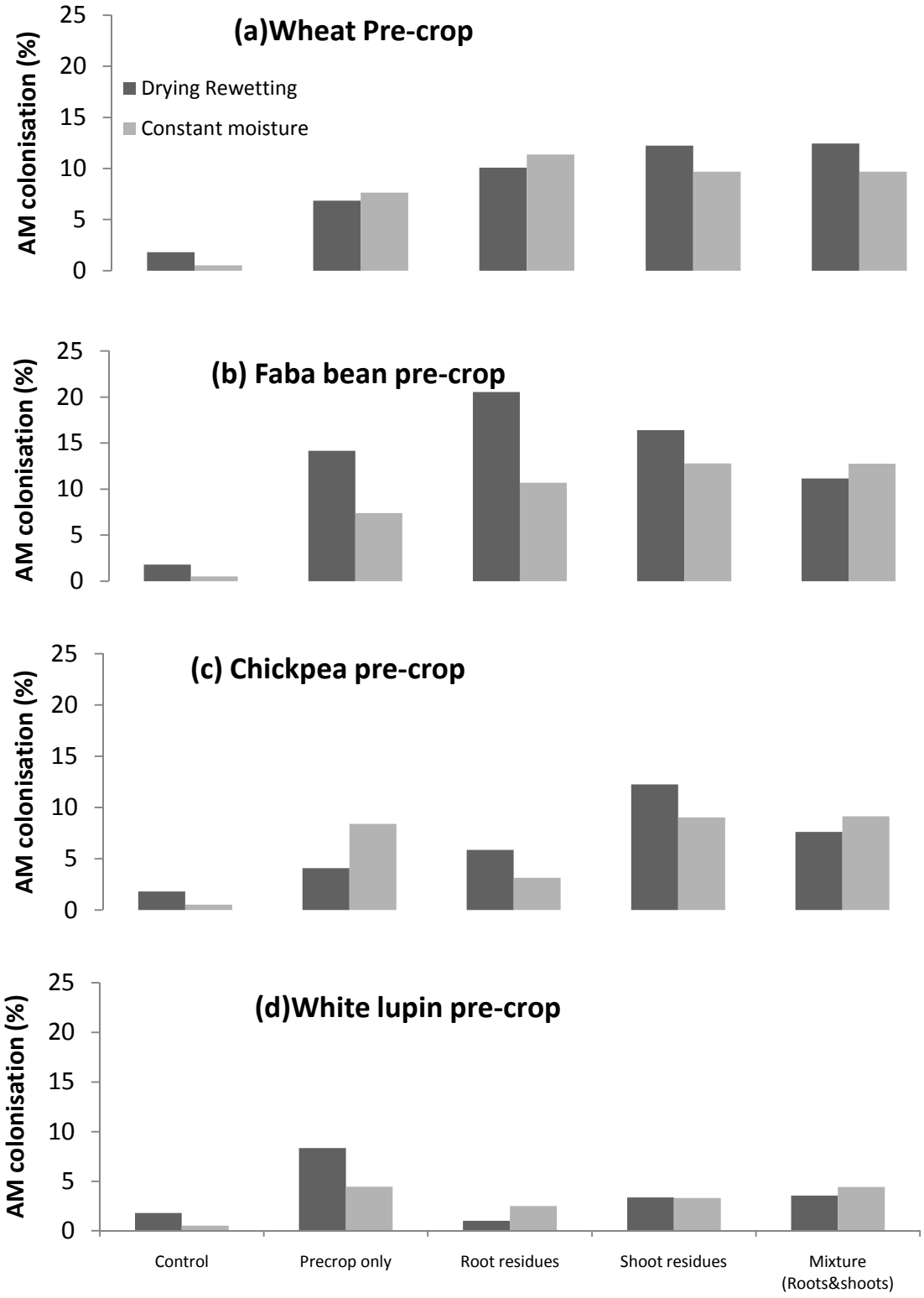
(a)Wheat Pre-crop
Drying Rewetting
Constant moisture
AM colonisation (%)
25
20
15
10
5
0
(b) Faba bean pre-crop
(c) Chickpea pre-crop
(d)White lupin pre-crop
Control
Precrop only
Root residues
Shoot residues
Mixture (Roots&shoots)

Figure 2

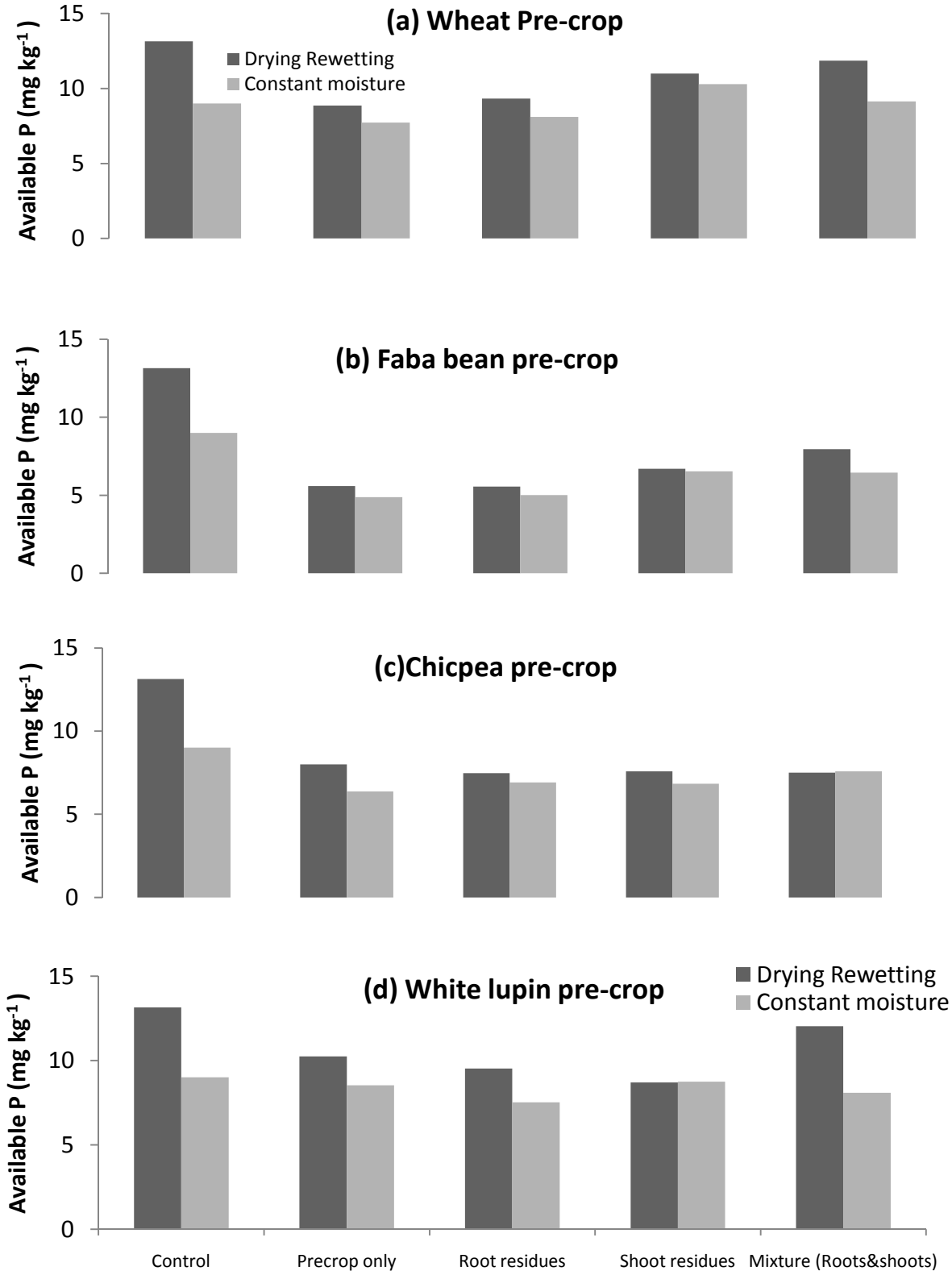
(a) Wheat Pre-crop
Drying Rewetting
Constant moisture
Available P (mg kg-1)
15
10
5
0
(b) Faba bean pre-crop
(c)Chicpea pre-crop
(d) White lupin pre-crop
Control
Precrop only
Root residues
Shoot residues
Mixture (Roots&shoots)

Figure 3

Chapter 5 SUMMARY AND CONCLUSIONS

1. Summary and implications

It is well-known that legumes are important components of sustainable crop rotations, increasing the growth of the following cereal compared to cereal after cereal (Bagayoko *et al.* 2000; Marschner *et al.* 2004). The growth improvement and yields benefits from legumes to cereals are thought to be mainly due to increased N availability through N fixation by the legumes (Armstrong *et al.* 1997; McEwen *et al.* 1990; Mubiru and Coyne 2009). However, other studies suggested that legumes may also increase the growth of the following cereal in absence of N limitation for the wheat and that this effect was, in part, due to improved P uptake by the cereal. The improved P availability was explained by P mobilisation by the legume root exudates which could either affect soil P availability even during the growth of the following wheat, or would lead to high P uptake by the legumes and that this P would become available during decomposition of the legume residues (Braum and Helmke 1995; Kamh *et al.* 2002; Nuruzzaman *et al.* 2005). However, it is not clear which of these two mechanisms, pre-crop effect alone or decomposing residues, is more important or if there relative importance depends on the legume species.

Other positive effects of legumes were demonstrated in African soils where legume pre-crops resulted in earlier colonisation of AM fungi and reduced soil pathogens in cereals (Bagayoko *et al.* 2000; Marschner *et al.* 2004). Moreover, Borie et al. (2002) suggested that the application of legume residues may lead to higher AM colonisation. Since AM fungi may increase plant P uptake, earlier and higher AM colonisation in the cereal could explain the positive effect of legume pre-crops and their residues on the following

cereal. However, there is scarce information about how legumes and their residues affect AM colonisation of the following wheat in Australian soil. The aim of the studies described in this thesis were to determine the effects of legumes as pre-crop alone or with residues on growth, P uptake and AM colonisation of the following wheat.

The first study (Chapter 2) showed that although there was the expected negative correlation between available P and AM colonisation of wheat when inorganic P was added (Jasper *et al.* 1979; Nagahashi *et al.* 1996)., AM colonisation is not related to available P when residues were added. This suggests that factors other than P availability influence AM colonisation during residue decomposition. Residues from mature faba bean and chickpea, which had a low P concentration, not only inhibited wheat growth and P uptake but also AM colonisation, although P availability was low. This negative effect of these residues on wheat growth and AM colonisation suggests that toxic compounds are released during faba bean and chickpea residue decomposition.

In the second and third study, legumes or wheat were grown as pre-crops, their residues removed or added back into the soil and then wheat was planted either immediately after residue addition (Chapter 3) or 4 weeks later (Chapter 4). The results of the two experiments were quite similar, suggesting that, compared to immediate planting, a 4 week period between pre-crop harvest and planting of wheat has little effect on the impact of the pre-crops on the following wheat. All pre-crops reduced the growth of the following wheat compared to the previously unplanted control, which is probably due to nutrient uptake by the pre-crops. This effect was observed not only when all residues of the pre-crops were removed, but also when they were added to the soil. Hence, the nutrient taken up by the pre-crops were apparently not completely released from the

decomposing residues during the 6-10 weeks following the incorporation of the residues.

In both studies, mature faba bean residues negatively affected wheat growth compared to wheat as a pre-crop, confirming the hypothesis from Chapter 2 that compounds toxic to the following wheat are released by decomposing faba bean residues, but additionally this suggests that such compounds are also produced during the growth of the faba bean. The soil moisture content during the 4 weeks between pre-crop harvest and planting of the following wheat had generally little impact on the effect of the pre-crops, exceptions being a strongly reduced shoot N concentration and improved growth after faba bean in the following wheat when the soil was maintained moist compared to dry soil during this period. In the field, fallow periods between pre-crops and the following crops vary with climatic conditions. In Australia, this fallow period may last several months due to insufficient soil moisture after pre-crop harvest (during summer), but in temperate climates, the fallow period can be very short.

Regarding the relative importance of the pre-crop only and their residues, the studies showed that this depends on the pre-crop species. White lupin and chickpea increased the growth and P uptake of the following wheat particularly when the residues were returned to the soil, suggesting that nutrients released by the decomposing residues were an important nutrient source for the following wheat. In the first rotation experiment (Chapter 3), white lupin increased the growth of the following wheat also in absence of residues, suggesting that P mobilised during the growth of white lupin may still be available to the following wheat if it is planted immediately after harvest of the white lupin. However, if wheat was planted 4 weeks later (Chapter 4), wheat growth after whie lupin was improved only if the residues were incorporated. This suggests that the effect of the white lupin exudates on P availability for the following wheat may be short-lived.

In faba bean and wheat as pre-crops, addition of residues had little effect on the growth of the following wheat. The pre-crop wheat resulted in poorer growth in the following wheat compared to white lupin and chickpea which occurred although AM colonisation and P availability did not differ between these pre-crops. The poorer growth of wheat following wheat may be due to presence of pathogens. As mentioned above, faba bean as pre-crop consistently reduced growth of the following wheat which may, in part be due to the lower P availability but also presence of inhibiting compounds in the soil after faba bean.

The effect of the pre-crops on AM colonisation of the following wheat was quite small except for a stimulation of AM colonisation by all pre-crops compared to the previously unplanted control. This can be explained by the fact that AM propagules may die during prolonged absence of host plants (Thompson 1987). Regarding the pre-crops, there was no consistent relationship between AM colonisation and the growth of the following wheat. Faba bean pre-crop resulted in high AM colonisation but decreased wheat growth, whereas white lupin pre-crop lead to low AM colonisation and increased wheat growth. These inconsistent results suggest, that in the soil used here, AM colonisation is not important for the effect of pre-crops on the following wheat and that wheat growth is not related to AM colonisation. This could be due to the fact that AM colonisation of wheat in this soil was relatively low (maximal about 20%). The effect of the pre-crops on AM colonisation of the following wheat and the impact of AM colonisation on wheat growth may be different in a soil with a greater AM infection potential.

2. Suggestions for further studies

Future studies, that could address some of the questions arising from this work include:

a. In this study, the following wheat was grown for only for 6 weeks. The effect of the pre-crops and particularly their residues may be different if wheat is grown for a longer period of time, e.g. to maturity. The longer growth period may allow more nutrients to be released from the residues and may also result in higher AM colonisation. The pre-crops and their residue may also improve micronutrient availability. Hence shoots of the following wheat should also be analysed for micronutrients. Due to the limited time of the study, the fallow time was only 4 weeks. Experiments with longer period between harvesting of the pre-crop and planting of the following wheat should be conducted to allow enough time for residue decomposition which may alter their effect on the following wheat.

b. In the two rotation experiments, the same amount of residues was used for all pre-crops to allow comparison between the pre-crops. However, in the field, the amount of residues added to the soil would depend on the growth of the pre-crop. Thus, our results and other studies suggest, that because of the greater biomass, more residues would be added after faba bean than after wheat. This may alter the effect of the residues on the following wheat. To mimic the field situation, all residues produced by a given pre-crop could be added back to the soil. However, this would make the comparison between the pre-crops more difficult as very different amounts of nutrients would be added. Future studies should also investigate the effect of the pre-crops on soil P pools by sequential fractionation after pre-crop growth and after the harvest of the following wheat to assess if the pre-crops are able to mobilise certain P pools and increase their availability to the following wheat.

c. Only one soil type was used in the present studies. Pre-crop decomposition rate and thus nutrient release, sorption processes as well as root growth of the wheat may differ with soil type. Therefore, research with different soil types and under field conditions are needed to ascertain the influence of pre-crops to the following wheat.

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