Phosphorus acquisition characteristics of cotton (*Gossypium hirsutum* L.) compared with other crop species

Submitted by

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List of publications

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List of abbreviations

Al	Aluminium
Al-P	$AlPO_4$
AM	Arbuscular mycorrhizae
Ca	Calcium
Ca10-P	Hydroxyapatite
Ca-P	$Ca(H_2PO_4)_2$
Day 30	Plant harvested 30 days after sowing
Day 45	Plant harvested 45 days after sowing
Fe	Iron
Fe-P	$FePO_4$
HCl-P	1 M HCl-extractable phosphorus
K	Potassium
K-P	KH_2PO_4
N	Nitrogen
$NaHCO_3$-P_i	0.5 M $NaHCO_3$-extractable inorganic phosphorus
$NaHCO_3$-P_o	0.5 M $NaHCO_3$-extractable organic phosphorus
NaOH-P_i	0.1 M NaOH-extractable inorganic phosphorus
NaOH-P_o	0.1 M NaOH-extractable organic phosphorus
Ndff %	The proportion of nitrogen in the plant derived from nitrogen fertilizer
Ndff mg	Total amount of nitrogen in the plant derived from nitrogen fertilizer
NH_4-N	Nitrogen source as NH_4^+
NO_3-N	Nitrogen source as NO_3^-
OC	Organic carbon
P	Phosphorus
P0	Zero phosphorus
P20	Phosphorus applied at the rate of 20 mg P kg^{-1} soil
PBC	Phosphorus buffering capacity
Pdff %	The proportion of phosphorus in the plant derived from phosphorus fertilizer
Pdff mg	The amount of phosphorus in the plant derived from phosphorus fertilizer
PdfS %	The percentage of phosphorus in the soil fraction derived from phosphorus source
PrecF %	The recovery of phosphorus sources into each soil phosphorus fractions
P_i	Inorganic phosphorus
P_o	Organic phosphorus
SA	Specific activity
$\sum P_f$	Sum of all phosphorus fractions

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Summary

The low responsiveness of cotton (*Gossypium hirsutum* L.) to phosphorus (P) fertilizers in the field where soils have low soil test values for P, suggests that cotton is capable of utilizing soil P pools that are less labile or are below the depth of P fertilizer placement. The objective of this thesis is to understand how the cotton plant acquires P from less labile and subsoil P pools.

The first experiment was conducted using rhizoboxes to examine whether cotton is physiologically efficient at acquiring less labile P pools, through comparison with wheat (*Triticum aestivum* L.) and white lupin (*Lupinus albus* L.). Under P-deficient conditions, cotton released negligible carboxylates into the rhizosphere. The P depletion zone of cotton from rhizosphere soil was less than 2 mm, and predominantly from the relatively labile $NaHCO_3$-P_i and NaOH-P_o pools. In contrast wheat and white lupin markedly depleted the $NaHCO_3$-P_i and less labile HCl-P pools, and the depletion zone extended to 3 mm. The depletion of NaOH-P_o pools by cotton was associated with increased activity of phosphatases in the rhizosphere soil.

A second sand culture experiment investigated the ability of cotton to access P from the sparingly soluble P sources of $AlPO_4$, $FePO_4$ and hydroxyapatite. The experiment revealed that cotton was inefficient in accessing P from all these sparingly soluble P sources. In contrast, wheat showed an outstanding ability to use $AlPO_4$. The ^{32}P reverse dilution technique was then used to determine the variation between these species in accessing sparingly soluble $AlPO_4$ and hydroxyapatite in a Vertosol when N was supplied as different forms. The inefficiency of cotton to access P from these sources was again documented. The percentage of hydroxyapatite recovered by the cotton plant was 8 and 10 times lower than that recovered by wheat and white lupin respectively, while the availability of $AlPO_4$ to cotton was 10 times lower than wheat but 3 times higher than white lupin. Addition of N as NH_4-N elevated total plant P uptake from all P sources except in the case of wheat fed with Al-P. The fate of these P sources in the soil was examined in a separate incubation experiment. Sparingly soluble Al-P was more 'labile' in Vertosols, in comparison to $FePO_4$ and hydroxyapatite which showed little change in availability with time.

Hydraulic lift can be a desirable characteristic for P acquisition by crops growing in areas that experience frequent drying of the topsoil. A glasshouse experiment using specialized soil columns tested whether cotton could hydraulically lift water and whether this hydraulically-lifted water facilitates P acquisition from dry topsoil. Hydraulic lift was detected in a Vertosol for 7 days after water was withheld from the topsoil. However, the hydraulic lift detected for the cotton did not aid P acquisition from the dry topsoil. Therefore, it appears that if the topsoil dries out, P uptake by the cotton plant would rely mainly on P acquisition from P sources in the subsoil.

The contribution of subsoil P sources to plant uptake was investigated in two field surveys. Soil samples at various soil depths were collected from continuously-cultivated cereal and cotton paddocks, and corresponding virgin sites, in north-western NSW. Regardless of P fertilizer history, organic and residual P pools at depths of 0 to 45 cm had been invariably depleted following long-term continuous cotton and cereal cropping. With a negative P balance, the HCl-P pool below 10 cm also declined significantly. Thus, routine soil P tests, using alkaline bicarbonate extraction solutions with soil samples collected from the topsoil layers (above 10 cm), were less accurate in estimating P responsiveness of cotton to P fertilizers applied on Vertosols. The effect of cropping system, expected from species variation in root morphological and physiological characteristics, on the soil P accumulation and depletion profile was undetectable.

The results suggest that the low responsiveness of cotton to P fertilizers can be attributed to a number of causes. These include the utilization of organic P pools, subsoil P exploration or possibly mycorrhizal symbioses, rather than any efficient P acquisition from sparingly soluble inorganic P pools in the topsoil. Further research should focus on accurately estimating the contribution of organic P and subsoil P pools to P uptake by cotton plants.

Statement of authorship

Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis or any other degree or diploma.

No other person's work has been used without due acknowledgment in the main text of the thesis.

This thesis has not been submitted for the award of any degree of diploma in any other tertiary institution.

Melbourne, November 2009
Author

Xiaojuan Wang

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Chapter 1: General introduction

Cotton (*Gossypium hirsutum* L.) is a major crop species in the semiarid cropping areas of eastern Australia where Vertosols (Isbell 1996) are a major soil type. Traditionally, cotton production on Vertosols required no phosphorus (P) fertilization because P was rarely limiting in these soils (Trudgian *et al.* 1977). Over the past 25 years, lint yields of irrigated cotton have increased steadily to more than 2000 kg ha^{-1}, which is 2.6 times the world average (Rochester 2007). These high yields of cotton impose a high demand for nutrients like P, and thus P fertilizer had been increasingly applied in order to maintain both crop production and soil P fertility (Dorahy *et al.* 2004). Nevertheless, the response of cotton to applied P is unpredictable and frequently low. Low responsiveness of cotton to P fertilizers had been detected on soils with low soil test P values (Bronson *et al.* 2001; Funderburg *et al.* 1996). Dorahy *et al*. (2004) reported that only 3 out of 17 cotton field sites in Australia showed increases in lint yield from P fertilizer application. This occurred when Colwell-P concentrations fall below 6 mg kg^{-1} in the surface 30 cm soil (Dorahy *et al*. 2004).

Factors responsible for the low responsiveness of cotton to P fertilizer have been investigated by Dorahy *et al*. (2007; 2008). These authors found that P fertilizer applied to the Vertosols remains available for at least two months after application (Dorahy *et al*. 2007). Thus, reduced P availability from soil P fixation is not likely to be a contributor to the low responsiveness of cotton to P fertilizer. By using ^{32}P and ^{33}P radioisotopes, Dorahy *et al*. (2008) also revealed that more than 95% of P in the cotton originated from soil beyond the P fertilizer bands. However, little is known about where and how these soil P reserves are being utilized by the cotton. The reported critical concentrations of Colwell P for cotton in Vertosols are from 6 to 12 mg kg^{-1} (Dorahy *et al.* 2004; Hibberd *et al.* 1990), which are much lower than those for wheat (21 mg kg^{-1}) and barley (18 mg kg^{-1}) on similar soils with low P sorption capacity (Reuter *et al.* 1995). This suggests that cotton may be able to access P from stable soil P pools. Thus there is a need for further research to identify the P acquisition characteristics of cotton from soil P pools of varying availability.

As a tropical species, cotton is normally cultivated in warm to hot climates. Rapid drying of furrow-irrigated soils from evaporation is quite common with cotton production (Muchow and Keating 1998; Singh *et al.* 2006). Decreased soil moisture could lead to a poor response by cotton to shallow P placement (Singh *et al.* 2005). Piper and de Vries (1964) and Jarvis and Bolland (1991) also reported that the restricted uptake of surface-applied P, due to surface dryness, occurred in wheat and lupins respectively. Where P in the topsoil contributes less to crop demand, the importance of subsoil P nutrition may increase. The ability of cotton roots to absorb both water and nutrients present in lower soil layers was found to be critical for better cotton growth in a semi-arid environment (Georgen 1986). Understanding the contribution of subsoil P pools to the P nutrition of cotton would also provide an insight into the responsiveness of cotton in soils with varying P status and cropping history. In the cotton farming systems in Australian, the cotton crop is normally rotated with wheat. Cotton has a tap root system in contrast to the fibrous root system of wheat, which also raises the issue of P removal from different soil depths by cotton and wheat crops.

This thesis aims to investigate whether cotton is physiologically efficient at acquiring P from sparingly soluble P sources, such as $AlPO_4$ and hydroxyapatite. In these studies, cotton will be compared with wheat and white lupins which differ in both root morphological and physiological properties. The availability of sparingly soluble P sources to these species will then be quantitatively determined by using the ^{32}P reverse dilution technique. A further experiment will examine whether cotton is able to redistribute soil water from deeper layers to the drier surface layer, and whether this strategy, known as hydraulic lift, can assist P uptake by cotton from the dry surface soil. Finally, the impact of long-term cropping on P fractions in soil layers in Vertosols, will be investigated by collecting soil samples from continuously cultivated cotton and wheat sites, and comparing them with corresponding virgin sites. All these experiments will test the hypothesis that the low responsiveness of cotton to surface-applied P fertilizer in soils testing low in P, is due to (i) P uptake from stable P pools in the surface soil not measured by traditional soil tests (e.g. Colwell P); (ii) or P uptake from subsoil layers (below 10 cm).

Chapter 2: Literature review

2.1. Introduction

Phosphorus (P) is the second most limiting macronutrient for plant growth in many soils (Schachtman *et al.* 1998). The continuing depletion of global P reserves has prompted concern regarding continued availability of soil P for plant uptake. Due to its chemically reactive nature, P in the soil mainly exists as mineral P, specifically adsorbed P to the surfaces of soil particles or as precipitated P. In addition, organic P constitutes 20 to 80% of total P content in the surface soil (Jungk *et al.* 1993; Richardson 1994). Only a marginal proportion of soil P (0.1~10 µM) is present as phosphate ions in the soil solution, and is therefore in the form available for direct plant uptake (Frossard *et al.* 2000; Mengel and Kirkby 1987). Nevertheless, some species are well documented for their capacity to use different soil P pools due to either proton release or organic anion exudation (Hinsinger 2001; Richardson *et al.* 2009).

This review will provide an understanding of the chemistry and availability of P from soil P pools. Focus will then be given to the root morphological and physiological adaptations of plant for overcoming P-deficient conditions. A review of long-term cropping effects on P dynamics of Vertosols, the main soil type studied in this thesis, will also be presented. A brief review will then be provided on P acquisition efficiency of cotton species from field applied P fertilizers.

2.2. Phosphorus in the soil

The total concentration of P in soil usually ranges from 200 to 5000 mg P kg^{-1} with an average of 600 mg P kg^{-1}, depending on the nature of parent material, degree of weathering and rate of P fertilization (Lindsay 1979). Considering that the majority of this P is held on the soil solid phase, replenishment of soil solution P (i.e. P immediately available for plant uptake) depends on the dissolution or desorption of inorganic P or the mineralization of organic P.

2.2.1. Mineral P

2.2.1.1. Formation

The ultimate source of soil P is the primary mineral apatite [$Ca_{10}(PO_4)_6F_2$ or $Ca_{10}(PO_4)_6(OH)_2$]. During soil development, apatite P is weathered and transformed to secondary P minerals such as Al and Fe phosphates in acidic soils and Ca phosphates in neutral to alkaline soils (Lindsay *et al.* 1962; Sanyal and De Datta 1991). Mineral P also accumulates following reaction of fertilizer P sources with soil, and may constitute a significant proportion of soil P in fertilized soils. Lindsay *et al.* (1989) found that the type of mineral P formed will depend on soil pH, and contents of Al, Fe and Ca in the soil. In acid soils where trivalent Fe and Al occur in high concentrations in the soil solution, P ions will precipitate as Fe and Al phosphates, e.g. strengite ($FePO_4.2H_2O$), variscite ($AlPO_4.2H_2O$) and various minerals of the plumbogummite group. In contrast, in neutral to alkaline soils, P ions precipitate as Ca phosphates since Ca is the dominant cation in the soil solution. The formation of various Ca phosphates occur in the following sequence: soluble monocalcium phosphate [$Ca(H_2PO_4)_2$], the less soluble dicalcium phosphate dihydrate ($CaHPO_4.2H_2O$); octocalcium phosphate [$Ca_8H_2(PO_4)_6.5H_2O$], and finally the least soluble, hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] or fluorapatite [$Ca_{10}(PO_4)_6F_2$] (Lindsay *et al.* 1989; Syers and Curtin 1989). Evidence for the formation of these diverse Ca phosphates had been provided by electron microscopy observations (Freeman and Rowell 1981; Wang and Tzou 1995).

2.2.1.2. Dissolution

The dissolution of P minerals is directly governed by the precipitation-dissolution equilibrium. Increasing pH increases the solubility of Fe and Al phosphates while decreasing the solubility of Ca phosphates. As a result, P availability is normally greatest around pH 6.0 to 7.0. Furthermore, the removal of soluble products by plant uptake or by complexation with organic ligands, from the zone of dissolution, is able to shift the chemical equilibrium towards mineral P dissolution (Hinsinger 2001). The effect of plant root-induced chemical changes on the solubility of P minerals will be addressed in detail in Section 2.4.2.

2.2.2. Adsorbed P

2.2.2.1. Adsorption

In most soils, the major processes that control the soil solution P concentration are adsorption and desorption. Ligand exchange reactions act as the principal mechanism by which P is adsorbed to variable-charge surfaces of Al, Fe-oxides or clay minerals (Hingston *et al.* 1967). Initially, P is bound to the surface of adsorbents by ligand exhchange (Figure 2.1) and remains relatively labile and available to plant through desorption processes (Rajan and Fox 1972). Then, P becomes less available due to the molecular rearrangement of adsorbed P (Figure 2.1) (Munns and Fox 1976). With time, P adsorbed initially on the surfaces of Al and Fe oxides and hydroxides can migrate into the molecular structure of the oxides and become physically encapsulated or occluded (Barrow 1983; Bolland *et al.* 2003; Smeck 1985), or partially precipitate as amorphous and crystalline phosphates (van Riemsdijk and Lyklema 1980). In calcareous soils, P adsorbed on the surface $CaCO_3$ commonly can precipitate into different Ca phosphate minerals. One can therefore conclude that the availability of soluble P fertilizers, such as superphosphates and diammonium phosphate, decreases as the time of contact between soil components and applied P increases.

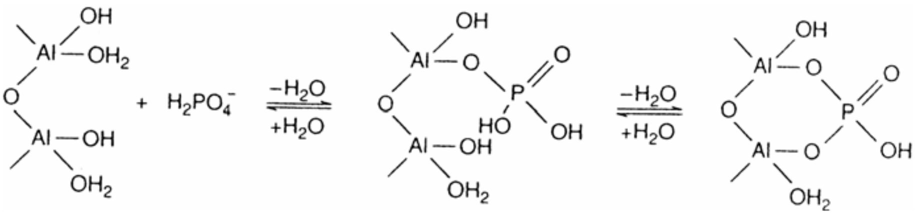


Figure 2. 1. Mechanism of P adsorption on Al oxide surface

Aluminium and Fe oxides are particularly active in the adsorption process due to their net positive charge in most soils and high specific surface areas (Norrish and Rosser 1983; Schwertmann and Taylor 1989). Even in neutral-alkaline soils, Al- and Fe-oxides contribute to P adsorption, and Al-P and Fe-P are amongst the most important P forms in these soils (Beauchemin and Simard 1999; Bertrand *et al.* 2003; Samadi and Gilkes 1998). On the other hand, the negatively charged $H_2PO_4^-$ and HPO_4^{2-} anions can also be simply adsorbed through electrostatic attraction to the positive surface of Al and Fe oxides and $CaCO_3$ (i.e. when soil pH is below the point of zero charge (ZPCs)). Retention of the P anion by Al and Fe-oxides will increase with decreasing soil pH due to increased positive charges at low pH (Barrow 1984; Quang *et al.* 1996; Strauss *et al.* 1997).

2.2.2.2. Desorption

Similar to adsorption, desorption of P from soil surfaces occurs mostly through ligand exchange reactions. However, desorption of P is much slower than adsorption, and some of the P that is originally sorbed can be prevented from desorbing, e.g. P occluded in Fe and Al oxides or hydroxide structures. However, given enough time, all adsorbed P is potentially desorbable (Lookman *et al.* 1995). Several studies have revealed that P adsorbed at low activation energies is desorbed quite rapidly while P held at higher activation energies takes longer to be desorbed (Barrow and Shaw 1975; Sharpley *et al.* 1981).

Inorganic and organic ligands, such as sulphate, bicarbonate and organic anions can compete with P for similar adsorption sites on surfaces of soil particles (Hue 1991; Ohno and Crannell 1996). In this respect, the role of organic amendments in reducing P sorption has been normally attributed to the competition of organic acids produced during mineralization for the same sites as P adsorption (Lessa and Anderson 1996; Lyamuremye *et al.* 1996; Ohno and Crannell 1996). However, due to the stronger affinity of metal oxide surfaces and clay minerals for P ions (Nagarajah *et al.* 1968), large concentrations of organic ligands (1-10 mM) are generally required for P ions to be desorbed to any significant extent (Staunton and Leprince 1996; Ström *et al.* 2002). Considering that the concentration of organic anions encountered in the bulk soil solution rarely exceed 10 μM, competitive inhibition of P sorption by organic acid is only likely to be significant in the rhizosphere (Guppy *et al.* 2005a; Hinsinger 2001). In addition, recent studies provide evidence contradicting the long held assumption that decomposing organic matter residues reduce P sorption by soil (Guppy *et al.* 2005b; Gutiérrez Boem *et al.* 2008). Improved P phytoavailability following organic matter addition can be simply attributed to the P released from the organic matter itself (Guppy *et al.* 2005b).

2.2.3. Phosphorus buffering capacity (PBC)

The soil property controlling the relationship between the soil solid phase P and solution P is defined as P buffering capacity (PBC). It reflects the capacity of soil to buffer against an increase in P concentration after fertilization or against a decrease in concentration during plant uptake. In the context of the reactivity of P fertilizer with soil, buffering capacity is synonymous with P sorptivity. The higher the P buffering capacity, the less effective the P fertilizer will be. The ability of the soil to replenish P during plant uptake depends on the quantity of the labile P existing in the solid phase, and ease with which P is released to soil solution. The PBC in this case is inversely related to the ease of P desorption and P diffusion through the soil.

Normally it is hard to differentiate between weakly-sorbed P and sparingly soluble mineral P as replenishment sources. Nevertheless, Holford (1983) suggests that it is the sparingly soluble mineral P that acts as the main buffering source in acid soils while adsorbed P is more important in replenishing the soil solution P in neutral and alkaline soils. In addition, adsorption appears to regulate dissolved phosphate at low P concentrations, whereas phosphate mineral precipitation controls P solubility at high P concentrations (Lindsay *et al.* 1989).

The PBC is usually measured from adsorption isotherms. These isotherms describe the relationship between P adsorbed to the soil surface and solution P concentration (Holford 1997; Webber and Mattingly 1970). By fitting a suitable equation, such as the Langmuir, Freundlich, multi-surface Langmuir and Tempkin equations, PBC can be determined. Reviews and comparison of various equations can be found in Barrow (Barrow 1978; 1999). In addition to these multi-point sorption isotherms, several single-point indices of PBC had been investigated by Barrow (2000). For a routine laboratory analysis, the P sorption indices (PBI) recently evaluated by Burkitt *et al*. (2002) is considered to be a simpler and more accurate single-point index for estimating PBC in a wide range of soil types. As demonstrated by many studies, PBC decreases as fertilizer P is continually applied (Barrow *et al.* 1998; Bolland and Allen 2003). The two possible reasons are: (i) P sorption sites on soil surfaces become saturated, or (ii) sorption of negatively charged phosphate ions increases the negative surface charge making it more difficult to sorb subsequently applied phosphate ions.

2.2.4. Organic phosphorus

2.2.4.1. Forms

Organic P forms (P_o) identified in soils are mainly monoesters such as inositol P, and diesters such as nucleic acids and phospholipids. Inositol P (phytates) represents 50 to 60% of the total P_o while nucleic acids and phospholipids constitute only about 5% of the total P_o (McLaren and Cameron 1996). The remaining proportion of P_o (30-40%) in the soil still remains unidentified (Magid *et al.* 1996; Turner *et al.* 2003b). The magnitude of the concentration of different P_o forms in soil reflects the relative stability of this form in the soil, against microbial degradation. The dominance of the monoester, mainly as inositol P, in the soil is attributed to the bonding of -OH groups with the inorganic colloids protecting P groups from microbial attack (Celi *et al.* 1999; Turner *et al.* 2002). In contrast, the -OH groups of diester P are linked to two carbon chains, and are weakly adsorbed by soil colloids, resulting in easier decomposition by microorganisms (Guggenberger *et al.* 1996; Miltner *et al.* 1988). Although phytates are still considered the most recalcitrant and abundant P_o compounds in soils, an overestimation of this P_o form possibly occur in many studies due to inappropriate assignment of certain NMR resonance peaks to phytate (Smernik and Dougherty 2007). On the other hand, microbial biomass itself constitutes from 3 to 20% of soil P_o (Brookes *et al.* 1984). Smernik and Dougherty (2007) suggest that, in some pasture soils, microbial-P rather than phytate-P acts as the dominant P_o. Microbial growth can be stimulated by the addition of microbial substrates such as crop residue, and release of microbial P occurs as a result of cell lysis or predation (Bünemann *et al.* 2004; McLaughlin and Alston 1986; Oberson and Joner 2005).

2.2.4.2. Mineralization

Mineralization of organic P in soils includes biological and biochemical processes which are mainly mediated by microbial activity and exocellular enzymes, respectively (Frossard *et al.* 2000; McGill and Cole 1981). Biological mineralization is closely linked to carbon mineralization and is driven by the need of microorganisms for energy. In contrast, the biochemical mineralization is driven by the need of microorganisms for P (McGill and Cole 1981). The rate of mineralization is generally related to factors that affect microbial activity. These factors include moisture, temperature and utilizable substrate (Chen *et al.* 2003; Soon and Arshad 2002; Stewart and Tiessen 1987). Noticeably, the total P and C:N ratio of the substrate have a great effect on the net P mineralization rate (Kwabiah *et al.* 2003). For example, no net P mineralization occurs if the decomposing organic material has low P and a high C:N ratio (Bünemann *et al.* 2004; Friesen *et al.* 1997). In contrast, addition of organic matter sources with high total P and low C:N ratios, such as green manures and animal manures, result in an increased P content in soil labile P pools (Iyamuremye *et al.* 1996; Nziguheba *et al.* 1998). Guppy and McLaughlin (2009) further suggest that upon addition of organic matter, recalcitrant organic P fractions may transfer to labile P pools.

Mineralization of P also depends largely on the activity of soil phosphatase and phytase enzymes, which in turn can be controlled by the solution P concentration (McGill and Cole 1981). The synthesis and excretion of phosphatase by microorganisms declines as the P concentration in the soil increases (Wanner 1996; Wykoff *et al.* 1999). The presence of plant roots also contributes to a significant increase in phosphatase activity in soil, notably in the rhizosphere (George *et al.* 2008; Hayes *et al.* 1999; Richardson *et al.* 2000). The role of plant-released phosphatase in increasing plant-available P will be addressed further in Section 2.4.2.3.

2.3. Estimation of plant available P in the soil

Estimation of plant available P in the soil involves identifying and measuring the quantity of P in the soil solid phase which buffers the solution concentration. This reservoir comprises variable and unknown proportions of the adsorbed and sparingly soluble mineral P. Methods adopted for the determination of these labile P pools include the use of a single chemical extraction, anion exchange resins, ^{32}P isotopic exchange method and sequential P fractionations.

2.3.1. Chemical extraction methods

The principle of using extraction methods is to immerse soil in moderately acidic or alkaline solutions which extract P associated with the mineral phase without significantly solubilizing minerals. Alternately, combined with the pH change, specific anions are introduced to bring P into the solution by competing with, and desorbing P ions from adsorption sites, or by chelating cations that bind P in the soil. Worldwide, the most commonly used methods are the alkaline bicarbonate extract (pH 8.5) of Olsen *et a*l. (1954), the acid ammonium fluoride extract (pH 3.0) of Bray I (Bray and Kurtz 1945) and the more acidic (pH 1.25) Mehlich I method (Nelson *et al.* 1953). Other common tests include Colwell, Bray II, Mehlich II and III methods, which are modified versions of the original Olsen, Bray I and Mehlich I, respectively (Colwell 1963; Fixen and Grove 1990).

The effectiveness of extractants in extracting available P in soil depends on their chemical nature and soil properties. Extractants containing an anionic extractant (e.g the Bray test) are more effective than those containing only acid (Mehlich) (Holford 1997). The very acidic test (Bray II) would be superior to the moderately acidic test (Bray I) for available P extraction on slightly calcareous soils. In addition, the more effective soil tests are those which remove a proportion of labile P that is weakly correlated with exchangeable P but strongly and inversely related to PBC (Holford 1997). In this case, the Bray test had been shown to be the most effective in extracting soil available P, followed by the Olsen and Colwell tests, and least with the Mehlich test (Holford 1997). Nevertheless, the Bray methods which extract Al phosphate as well as adsorbed P are restricted to tropical acid soils, as they are considered unreliable on alkaline soils (Holford and

Crocker 1988). In contrast, the Olsen method has been used successfully on a wide range of neutral to alkaline soils (Watanabe and Olsen 1965). The quest for a universal soil P test procedure is unsuccessful to date and will no doubt continue for a long time.

It had been recommended by White and Beckett (1964) that bioavailable P in soil should be characterized using three factors. These are an intensity factor (the P concentration in the soil solution), a quantity factor (the amount of P in the solid phase that equilibrates with the solution P during the life of the plant), and a capacity factor (which indicates the ease of P release from the soil solid phase). When using the single extraction method, various P sources are likely to be extracted, and available P is described only by a quantity factor (Conyers and Moody 2009). More accurate prediction of available P requires measurement of PBC. This is especially true in the case of the Colwell test, which extracts significant amounts of soil P due to its wider soil solution ratio and longer extracting time than the Olsen test, and subsequently its extraction values are less correlated to soil buffering capacity (Holford 1980; Holford and Mattingly 1979). Usually, the critical value of the Colwell test, used for predicting yield response, elevates as the soil P sorptivity increases (Burkitt *et al.* 2002; Moody 2007; Reuter *et al.* 1995).

2.3.2. Ion-sink method

Conventional extraction methods modify the soil during the extraction procedure and thus might not closely relate to plant P uptake. In order to more accurately characterize the available P, ion-sink methods, such as FeO-coated papers or strips (Chardon *et al.* 1996; Menon *et al.* 1996), and anion-exchange membrane and resin bags (Fernandes and Warren 1995; Lajtha 1988) have been developed as alternatives. The main advantage of the ion-sink extraction method is its ability to extract P from various soils in the same manner as plant roots do (Sharpley *et al.* 1994). The ion-sink does not react with soil and only adsorbs P entering the soil solution from the exchangeable P pools, as well as from some of the more soluble precipitated P forms (Raven and Hossner 1993). Among various ion-sinks, the anion-exchange membrane is widely used and is generally considered the simplest method for evaluating available P in soils (Saggar *et al.* 1990; Schoenau and Huang 1991). Nevertheless, the newly developed FeO-impregnated paper test is considered to be more suitable for the routine laboratory use due to its lower costs (Chardon *et al.* 1996). Recently, the method of DGT (diffusive gradients in thin-films) which measures the diffusive supply of P under infinite-sink conditions (Fe-oxide containing gel) has been promoted as an effective tool to predict P bioavailability (Degryse *et al.* 2009; Mason *et al.* 2005; Menzies *et al.* 2005).

2.3.3. ^{32}P isotopic exchange method

Isotopic exchange methods allow the measurement of the P fraction that can exchange with phosphate anions in the soil solution over a given time (Fardeau and Guiraud 1972; Frossard *et al.* 1994). The shorter the period allowed for exchange, the more labile is the quantity of P measured. There is no definite limit to the amount exchanged, and given sufficient time most of the soil P would be exchanged (Nye and Tinker 1977). This method, unlike extraction methods, does not modify the equilibrium among various soil P pools, and determines available P without its direct extraction from the soil.

Three experimental procedures, the so-called E value (Wiklander 1950), the A value (Fried and Dean 1952) and the L value (Larsen 1952) can be normally used for the determination of the size of the isotopically exchangeable soil P pool. The E value procedure is based on the measurement of the isotopic composition of phosphate ions in the soil solution (the ratio of ^{32}P or ^{33}P to ^{31}P) after the addition ^{32}P or ^{33}P to a soil suspension (Wiklander 1950). The L value has been used to measure the isotopic composition (specific activity) of a plant grown in a soil labeled with ^{32}P or ^{33}P (Larsen 1952). Basically, the plant will not discriminate between isotopic forms of P when taking up P from the soil, and the isotopic composition in the plant will be identical to that of the P present in the soil solution when soil P supply is adequate (Bühler *et al.* 2003; Frossard *et al.* 1994; Morel and Plenchette 1994). This means that the L value equals the E value unless rhizosphere processes enable the plant to draw P from non-labile P sources. The A-value determination involves the measurement of P taken up from two sources of P, which are the available soil P pool and ^{32}P or ^{33}P-labeled P fertilizer. The P taken up is proportional to the respective amounts of P available in these two pools. The A value always varies with the type of fertilizer used as standards, the rate, the method of fertilizer application, and the original soil P fertility. It only serves as an index of the availability of soil P (Fardeau and Guiraud 1972).

Application of E and L values to soils with high P-fixing capacity or containing very little P has not been successful (Salcedo *et al.* 1991; Wolf *et al.* 1986). The possible reasons include (i) the specific adsorption of ^{32}P or ^{33}P ions on soil surfaces, (ii) the failure for the isotopic equilibrium to be reached between the ^{32}P or ^{33}P ions and soil P adsorption sites after a given time, (iii) the P concentrations in the soil solution are near the detection limits of standard colorimetric methods (for E value determination), and (iv) the large interference from P derived from seed reserves which is sometimes comparable to total P uptake by plants (for L value determination). Bühler *et al.* (2003) reported that, in order to obtain a reliable L value, the total P taken up by the plant shoot needs to be at least one order of magnitude greater than the P originating from the seed. Nevertheless, methods have been developed to measure the seed P contribution and low P concentrations in the soil solution (Ohno and Crannell 1996; Pypers *et al.* 2006). In addition, a period for P equilibration prior to sowing is recommended to establish the isotopic equilibrium (Probert 1972).

2.3.4. Phosphorus fractionation

The time frame over which the soil P supply is measured is important. In this respect, ion-sink and isotopic exchange methods are considered to provide an evaluation of the ability of the soil solid phase to replenish P in soil solution over a relatively short term. In contrast, soil P availability over the longer term is normally assessed by different sequential P fractionation schemes, as summarized recently by Pierzynski *et al.* (2005). Such schemes are based on the selective extraction of operationally defined P fractions by using single extractants in a sequential manner.

The most widely used sequential extraction method was developed by Chang and Jackson (1957) and Hedley *et al.* (1982a), in addition to a specially designed scheme for calcareous soils (Jiang and Gu 1989) (Table 2.1). These have subsequently been modified (Sharpley *et al.* 1987; Tiessen *et al.* 1983; Williams *et al.* 1967). A recent modification of Hedley's method (Guppy *et al.* 2000) increases the ease of use of this sequential scheme due to a simplified procedure. Trasar-Cepeda *et al.* (1987) concluded that the extractants adopted by Hedley's scheme were less able to extract the more strongly retained phosphates than the procedure described by Chang and Jackson (1957). Nevertheless, Hedley's scheme allows P to be removed with a series of successively stronger reagents, giving separate soil P fractions that vary in their availability to plants. Thus, this method had been more frequently used in the examination of P dynamics involved with long-term cropping practices (Cross and Schlesinger 1995).

Most studies seem to agree that the resin-P and the $NaHCO_3$-extractable P ($NaHCO_3$-P), which are removed first with Hedley's scheme, are plant available (Agbenin and Goladi 1998; Blake *et al.* 2003; Sharpley *et al.* 1987). However, the availability of other fractions is less certain. In slightly weathered soils, all P pools could act as P sources under intensive cropping with minimal P inputs (Guo and Yost 1998; Vu *et al.* 2008). The main pools buffering available P in highly weathered soils are extractable by NaOH (NaOH-P) (Beck and Sanchez 1994; Guo and Yost 1998; Tiessen *et al.* 1992). Noticeably, the residual pool separated by the same sequential method declined in slightly weathered soils (Agbenin and Goladi 1998; Hedley *et al.* 1982a; McKenzie *et al.* 1992a; McKenzie *et al.* 1992b), but increased in highly weathered soils following long-term cropping (Guo *et al.* 2000). Obviously, P pools separated by the same extractants are not of equal availability to plants in different soils. The nature of the availability of the residual pool in the slightly weathered soil is open to various interpretations. For example, Zhang and Mackenzie (1997a; 1997b) and Tiessen *et al.* (1992) attribute the contribution of the residual P fraction to plant uptake to its organic portion, whereas Guo *et al.* (2000) postulated that it was the P mobilized from P bonded with Ca.

Table 2. 1. The P fractionation schemes

Sequential extraction method developed by Hedley *et al.* (1982c)

P fraction	Chemical nature of soil P
Resin-P_i	Soluble and ion-exchangeable forms
0.5 M $NaHCO_3$ for 16 h	Labile P_i and P_o
$CHCl_3$-released 0.5 M $NaHCO_3$-P	Microbial P
0.1 M NaOH for 16 h	P_i and P_o associated with Al and Fe
Sonication and extraction with 0.1 M NaOH for 16 h	Occluded P_i and P_o
1 M HCl for 16 h	Ca-P
Digested with concentrated H_2SO_4 and H_2O_2	Residual P

Sequential extraction method developed by Chang and Jackson (1957)

Extraction steps	Chemical nature of soil P
0.5 M NH_4F for 1 h (soil was washed with 1 M NH_4Cl before extraction)	Al-P
0.1M NaOH for 17 h (soil from previous extraction was washed with1 M NH_4Cl)	Fe-P
0.5 M H_2SO_4 for 1 h (soil from previous extraction was washed with1 M NH_4Cl	Ca-P
0.3 M Na citrate + 1 g $Na_2S_2O_4$ heated at 85 °C	Reductant soluble P

Sequential extraction method developed by Jiang and Gu (1989)

Extraction steps	Chemical nature of soil P
0.25 M $NaHCO_3$ for 1 h	Dicalcium phosphate
0.5 M NH_4Ac, left unshaken for 4 h and then shaken for 1 h (soil from previous extraction was washed with 95% alcohol	Octacalcium phosphate
0.5 M NH_4F for 1 h (soil from previous extraction was washed twice with saturated NaCl	Al-P
0.1 M NaOH-0.1 M $NaCO_3$ for 2 h, left unshaken for 16 h, and then shaken again for 2 h	Fe-P
0.3 M sodium citrate solution + 1 g sodium dithionite, heated at 80 °C for 15 min and shaken continuously. After centrifuging, the supernatant was digested with 3-acid solution (H_2SO_4:$HClO_4$:HNO_3 = 1:2:7)	Occluded phosphate
0.5 M H_2SO_4 for 1 h	Apatite type

2.4. Phosphorus acquisition strategies by plants

There is overwhelming evidence suggesting that plants use various strategies to gain access to previously unavailable soil P reserves (Hinsinger 2001; Lynch 2005; Richardson *et al.* 2009). This includes altered root architecture, dry matter partitioning that favors growth of roots, promotion of root hair formation, establishment of the mycorrhizal symbiosis, and the ability of roots to modify the chemistry of rhizosphere soil by exuding P-mobilizing compounds such as protons, organic acids and phosphotase enzymes. Plant species that can grow well in soils with low P status possess at least one of these strategies.

2.4.1. Morphological strategies

2.4.1.1. Root architecture

The relative immobility of P in the soil means that root morphological traits are particularly important for P acquisition from the soil (Hodge 2004; Lynch 2005; Raghothama 1999). Root architecture is principally controlled by the root gravitropism which determines how much of the root systems remain in the topsoil. Since the labile P concentration is normally higher in the topsoil than in the subsoil, species or genotypes with relatively shallower root systems for topsoil foraging, should be superior for P uptake from the topsoil (Ge *et al.* 2000; Liao *et al.* 2001; Lynch and Brown 2001). Under low P conditions, roots of some genotypes of common bean (*Phaseolus vulgaris*) have less sensitivity to gravitropism, resulting in a shallower root system than other genotypes (Bonser *et al.* 1996). This topsoil foraging attribute has been found to be strongly related to the adaptation of the common bean genotype for acquiring P from low-P soils (Bonser *et al.* 1996; Liao *et al.* 2001; Lynch and Beebe 1995). High root length density of wheat in the upper soil layers is also considered to be an important trait associated with improved P uptake from the soil (Manske *et al.* 2000).

However, species or genotypes that possess shallow roots might be at a disadvantage in acquiring resources located deeper in the soil profile, such as soil water (Sponchiado *et al.* 1989). It is likely that species or genotypic variation in root architecture is a tradeoff between the acquisition of two primary resources with very different spatial and temporal distributions (Ho *et al.* 2004; Ho *et al.* 2005). Increased topsoil foraging is adapted for P acquisition, while deep rooting is an adaptation when water becomes more limiting than P availability. Deep rooting of annual crop species such as soybean, maize and wheat, for overcoming drought stress has been extensively studied (Proffitt *et al.* 1985; Reicosky *et al.* 1972; Sharp and Davies 1985).

Alternatively, roots in dry topsoil are able to maintain appreciable water and P uptake if water can be lifted from deeper wetter soil through the roots to drier top soil layers. This phenomenon, termed hydraulic lift, serves as another drought-tolerance mechanism (Wan *et al.* 2000; Yoder and

Nowak 1999). Evidence in the literature suggests that although varying in magnitude and timing, many plant species are able to hydraulically lift water (Valizadeh *et al.* 2003; Wan *et al.* 2000; Yoder and Nowak 1999). Drying of topsoil restricts the uptake of surface-applied P due to reduced P diffusion rates and root uptake capacity (Jarvis and Bolland 1991; Nye and Tinker 1977; Schnek and Barber 1979; Simpson and Pinkerton 1989). The occurrence of hydraulic lift prevents a net loss of fine roots, and maintains relatively higher soil moisture for diffusion of P toward the root (Huang 1999; Valizadeh *et al.* 2003). Many studies have reported that a significant uptake of immobile nutrients such as P from dry soil layers can occur as long as other parts of the root system have access to the wet deeper soil layers (Matzner and Richards 1996; Rose *et al.* 2008; Valizadeh *et al.* 2003). Thus, root plasticity is advantageous in enabling plants to respond to different environmental stresses. In natural environments, when several resources become limiting for plants at the same time, a well-adapted plant will possess an ability to optimize the use of these resources.

2.4.1.2. Root to shoot ratio

Allocation of more carbon to roots is a typical response of plants to P deficiency. This can result in increased root growth, enhanced lateral root formation, and increased root hair length and numbers (Liao *et al.* 2001; Lynch and Brown 2001). Under low-P conditions, long and dense lateral branching had been observed in bean (Lynch 1995; Lynch and Beebe 1995). This subsequently leads to an enhanced total root surface area available for soil exploration and P acquisition. In addition, P-efficient genotypes exposed to P deficiency stress generally retain more P in their roots than shoots, and subsequently develop efficient root systems for nutrient uptake (Adu-Gyamfi *et al.* 1990; Akhtar *et al.* 2008; Snapp and Lynch 1996).

2.4.1.3. Root hairs

Root hairs can confer a competitive advantage to plants in low-P conditions. They intercept P diffusing towards the roots and thus expand the P depletion zone around the roots. In many cases, root hairs can contribute up to 70% of the total surface area of roots, although the density and length of root hairs vary greatly within and among species (Hofer 1996; Jungk 2001). The significance of root hairs in P acquisition is well documented (Gahoonia *et al.* 1997; Gahoonia and Nielsen 1998). Root hair formation and growth are extremely plastic in response to soil P availability. Foehse and Jungk (1983) demonstrated that root hair lengths of rape, spinach and tomato were inversely related to the solution P concentration. Similar effects have been reported for legumes and *Arabidopsis* (Bates and Lynch 1996; Jungk *et al.* 1990).

2.4.1.4. Mycorrhizae

When root hairs are short there may be more mycorrhizal infection (Baon *et al.* 1994). The major type of mycorrihize in agriculture crops is arbuscular mycorrhizae (AM) fungi (Smith and Read 2008). The external hyphae of AM fungi, which are 100 times finer than wheat roots and 10 times finer than root hairs, contribute to a more thorough exploration of the soil volume by accessing pores normally not penetrable by roots or root hairs (Haymann and Mosse 1972). The increased P uptake by AM hyphae is normally attributed to an increased surface area for absorption and decreased distance for P diffusion (Bolan 1991; Schnepf *et al.* 2008). Moreover, AM hyphae have a higher affinity for P ions and are able to absorb P at lower solution concentrations than plant roots (Bolan 1991; Cavagnaro *et al.* 2005). Earlier work is equivocal as to whether AM hyphae are able to solubilise soil P that is unavailable to the plant, although the majority of the evidence suggests they are not (Joner and Johansen 2000; Li *et al.* 1991; Tinker and Nye 2000). Under conditions of P deficiency, the compatibility of plants to AM fungi is enhanced and acts as another P starvation-induced P acquisition strategy (McArthur and Knowles 1993; Schwab *et al.* 1991).

Nevertheless, the benefits of AM associations come at a considerable carbon cost to the plant. In common bean, root carbon costs of a mycorrhizal plant could reach 40% of the daily carbon budgets under P stress (Nielsen *et al.* 1998). It has been suggested that the increased carbon cost in mycorrhizal roots is mainly due to increased maintenance and growth of the fungal tissue (Nielsen *et al.* 1998). Thus, it is only worthwhile to have AM infection, which consumes a significant portion of photosynthetic assimilates, when P is the primary limiting factor for growth. The net effect of AM association depends on whether the benefits of increased P acquisition outweigh the respiratory costs of AM infection.

2.4.1.5. Cluster roots (proteoid roots)

In addition to mycorrhizal infection, cluster roots can be regarded as one of the major adaptations in some plants for enhancing P acquisition from P impoverished soils (Pate and Watt 2001; Skene 2000). Cluster roots are formed in most members of the Proteaceae and other plant families adapted to habitats of extremely low soil fertility (Watt and Evans 1999). The roots are bottlebrush-like structures consisting of densely packed rootlets arising from secondary roots. This unique root structure enables these species to use P efficiently by increasing root surface area, and by mobilizing P through concentrated root exudates in localized patches (Gardner *et al.* 1983). Detailed information on the structure and function of cluster roots had been reviewed by Lamont (2003) and Shane and Lambers (2005).

2.4.2. Physiological strategies

Alternatively, in response to P deficiency, plants exhibit a range of physiological adaptations to 'chemically mine' the soil P pools through the exudation of protons, organic anions, phosphatase enzymes and other compounds.

2.4.2.1. Proton release

The main reason for root proton release is to maintain electroneutrality inside the plant, under conditions of excess cations intake over anions intake (Hinsinger *et al.* 2003). In this case, the forms of N affect the root cation-anion balance, and thus rhizosphere pH change. For example, NH_4-N assimilation is associated with rhizosphere acidification, in contrast to the alkalization effect from NO_3-N uptake (Kirk *et al.* 1999; Tang *et al.* 2004; Tang and Rengel 2003; Zoysa *et al.* 1998). Legumes normally absorb more cations than anions through N_2 fixation and thus acidify rhizosphere soil (Raven *et al.* 1990; Sas *et al.* 2002b). In addition, soil acidification occurs as a response to P deficiency due to a decrease in NO_3-N uptake (Bertrand *et al.* 1999; Neumann and Römheld 1999; Tang *et al.* 2001b). Many plant species, such as rape, tomato, chickpea and white lupin, have the capacity to acidify their rhizosphere under P-deficient conditions (Neumann and Römheld 1999; Sas *et al.* 2001a).

Rhizosphere acidification does not always result in an enhanced P availability across all soil types. Its effectiveness depends on the presence of acid-soluble P forms such as calcium phosphate. Many reports have shown that plant rhizosphere acidification enhances the effectiveness of phosphate rock utilization (Hinsinger and Gilkes 1996; Sas *et al.* 2002b; Zoysa *et al.* 1997), and the depletion of acid-extractable P (HCl-P) in the soil (Gahoonia *et al.* 1992). Nevertheless, its effect in mobilizing P from highly weathered soils containing minimal amounts of acid soluble P is questionable. Hedley *et al.* (1994) found that the amount of P uptake was less correlated to rhizosphere acidification in a strongly weathered Ultisol. In fact, the availability of P in Al and Fe phosphate-dominated acid soils is expected to increase with an elevated rhizosphere pH, as demonstrated by many studies (Gahoonia *et al.* 1992; Gahoonia and Nielsen 1992). For soybean and *Phaseolus vulgaris*, P deficiency decreased proton release of N_2-fixing plants and increased hydroxyl release of NO_3-N fed plants (Tang *et al.* 2004; Tang *et al.* 2009; Tang *et al.* 2001a). Further studies are needed to determine if decreased proton and increased hydroxyl ion release act as a strategy for these species to mobilize P from acid soils.

2.4.2.2. Organic anions release

Evidence of organic anion release in response to P deficiency has been increasingly reported for many species (Hoffland 1992; Imas *et al.* 1997; Pearse *et al.* 2006a; Zhang *et al.* 1997). This is especially the case for the model species, white lupin (Dinkelaker *et al.* 1989; Johnson *et al.* 1994). While citrate is the main carboxylate exuded by species such as white lupin and alfalfa (Johnson *et al.* 1994; Lipton *et al.* 1987; Watt and Evans 1999), malate was shown to be the dominant carboxylate in the root exudates of plants such as maize, wheat, oilseed rape or tomato (Hoffland 1992; Jones 1998). Root exudates of oxalate and piscidate have been demonstrated to play a significant role in the acquisition of inorganic soil P by sugar beet and pigeon pea, respectively (Ae *et al.* 1990a; Gerke *et al.* 2000b). The important role of organic anions exuded from plant roots in promoting P mobilization, results from the complexation of metal cations bound with P, or the displacement of P from the soil matrix by ligand exchange (Gardner *et al.* 1982; Gerke *et al.* 2000a; 2000b; Jones 1998). In general, organic compounds containing tri-carboxylates, such as citrate, are more effective at releasing inorganic P than mono and di-carboxylates, due to their higher affinity for trivalent and divalent metals and soil absorption sites (Dinkelaker *et al.* 1989; Jones 1998; Jones *et al.* 2003).

Relatively high concentrations of organic anions (>10 μmol per g soil) are generally required for significant P mobilization (Gerke *et al.* 2000a; 2000b). Except in some rather extreme cases, such as those reported for proteoid roots of white lupin, organic anions exuded by plant roots represent a rather small flux (Jones 1998; Jones and Brassington 1998; Jones and Darrah 1994b). Furthermore, they can be rapidly metabolized by rhizosphere microflora, and have a limited diffusion distance due to their adsorption onto soil constituents such as Al and Fe oxides (Jones 1998; Jones and Brassington 1998; Staunton and Leprince 1996). Thus, the efficiency of organic anions in improving P acquisition is still a question of debate (Jones 1998**).** In some soils, no P appears to be mobilized upon the addition of organic acids (Jones and Darrah 1994b; Lan *et al.* 1995). Using sand culture, Tang *et al.* (2007) concluded that the release of organic acids did not correlate with P uptake by soybean from different sparingly soluble P compounds such as $AlPO_4$, $FePO_4$ and hydroxyapatite. Pearse *et a*l (2007) also showed that root exudates do not relate consistently to the ability of a crop species to take up P precipitated with Al, Fe or Ca. It is likely that a number of factors, such as proton and organic acid release, contribute to the P mobilization from rhizosphere soil.

Strong complexes between organic acids and Al and Fe compounds can only be formed at an acid pH range of 4 to 5 (Hue *et al.* 1986; Kerven *et al.* 1991; Wang *et al.* 2001). Organic anions such as citrate, malate and oxalate have little or no complexation ability with Fe above pH 6.8 (Jones *et al.* 1996; Parker *et al.* 1995). Also, the efficient desorption of P only occurs at the pH that corresponds roughly to the second pK value of the organic acids (pH 4-6 for many organic acids) (Nagarajah *et al.* 1970). Thus, efficient P mobilization by carboxylates should only be expected if the soil pH encountered in the experiment is within the efficient pH range for the chelation of cations.

2.4.2.3. Phosphatase release

For organic P sources to be used by plants, they must first be hydrolysed by phosphatases. Phosphatases are adaptive enzymes, and plant roots in general secrete acid phosphatases under P starvation (Chen *et al.* 2002; George *et al.* 2002b; Gilbert *et al.* 1999; Richardson *et al.* 2005). However, the ability of plants to secrete acid phosphatases differs greatly among species. Tadano *et al*. (1993) found that the amount of acid phosphatase released by white lupin was about 14, 15 and 4 times higher than wheat, rice and soybean respectively. There was no evidence for an increased activity of alkaline phosphatases associated with P deficiency (Tadano and Sakai 1991).

Evidence for the direct contribution of phosphatases to organic P utilization is provided by a number of studies (George *et al.* 2006; Li *et al.* 1997; Tarafdar and Jungk 1987). Using a rhizosphere slicing method, Tarafdar and Jungk (1987) found a significant correlation between increased phosphatase activity and depletion of organic P. Similarly, Li *et al*. (1997) demonstrated that elevated acid phosphatase activity in the rhizosphere of P-deficient white lupin caused an appreciable depletion of organic P in the soil. Nevertheless, a high phosphatase activity alone does not confer great mineralization of organic P if the enzyme substrate is limited or not available (George *et al.* 2002b; Hedley *et al.* 1994; Hedley *et al.* 1982b).

2.4.3. Species variation in utilizing sparingly soluble P sources

Phosphorus precipitated as insoluble P minerals has been identified as the main reaction product in freshly fertilized soil (Lindsay *et al.* 1962; Sanyal and De Datta 1991). Recent evidence suggests that sparingly soluble P sources such as Ca phosphates, Al and Fe phosphates are not equally available to plants, and plant species differ in their ability to utilize these different P sources (Gahoonia and Nielsen 1992; Hocking *et al.* 1997; Pearse *et al.* 2007). Usually, synthetic P compounds have been used as representatives of different mineral P compounds with similar solubility in soil. Most studies examining the availability of various sparingly soluble P sources to plants have utilized biological assays or isotopic (^{32}P and ^{33}P) techniques.

2.4.3.1. Biological assay

For biological evaluation, varying quantities of the P sources are added to soil and their effects on plant growth and P uptake are compared to that resulting from a soluble P source, such as superphosphate (Bolan *et al.* 1987; Pearse *et al.* 2007; Taylor *et al.* 1960). However, this approach is unable to differentiate between P derived from the soil, and that from P sources, and does not allow an accurate assessment of contribution of P sources to plant uptake when soil P concentrations are high. In order to overcome this problem, coarse river sand, although quite different from soil, has been adopted as an alternative plant growth medium (Pearse *et al.* 2007;

Pearse *et al.* 2006b; Perez Corona *et al.* 1996; Tang *et al.* 2007). Species variation in P acquisition from the sparingly soluble P sources is expressed in relation to its response to soluble P sources, or a control without P addition.

2.4.3.2. Isotopic (^{32}P or ^{33}P) technique

The principal advantage of isotopic ^{32}P or ^{33}P techniques is that the availability of sparingly soluble P sources can be accurately assessed at relatively high background soil P levels. In addition, while total dry matter yield and P uptake may be significantly affected by environmental factors, isotopic parameters, such as specific activity (^{32}P/^{33}P ratio), are less sensitive to variation in environmental conditions. By labeling the P sources, the proportion of P in the plant derived from P sources (Pdff %), is determined directly by using equation (2.1) (Armstrong *et al.* 1993; Fried 1954; Kucey and Bole 1984). Specific activity (S.A.) in the plant or fertilizer is determined as radioactivity (^{32}P) per amount of P (^{31}P) (Becquerel µg^{-1} P).

$$\text{Pdff \%} = \frac{S.A.\ plant\ material}{S.A\ labelled\ \ fertilizer} \times 100 \qquad \text{(Equation 2.1)}$$

However, labeling of sparingly soluble P sources always involves using expensive neutron irradiation, and some sparingly soluble P sources, such as rock phosphates, cannot be labeled without drastically changing their chemical and physical characteristics (Armstrong *et al.* 1993). Thus, an indirect method, reverse dilution, which labels the soil P rather than the P sources, is often used to evaluate the availability of sparingly soluble P sources such as rock phosphate (Di *et al.* 1994; 1995; Fardeau *et al.* 1996; Kato *et al.* 1995; Zapata and Axmann 1995). This approach consists of the determination of the uptake of P from a ^{32}P or ^{33}P labeled soil in the presence and absence of the unlabelled P source. It requires the addition of unlabelled P sources at a high rate, in order to provide a significant dilution of the radioisotope content in the plant. The lower the S.A. of the species fed with P sources, when compared with control without adding P sources, the higher the availability of these P sources to the plant (Di *et al.* 1995; Kato *et al.* 1995).

In order to determine Pdff % by using ^{32}P or ^{33}P reverse dilution technique, a pair of treatments is often required. In the case of treatment 1 (standards), in absence of an unlabelled P sources, the S.A. of plant material is a measure of the S.A. of the labeled source or the S.A. of the bioavailable P, and

$$\text{S.A. plant in absence of P sources} = \text{S.A. labeled soil} \qquad \text{(Equation 2.2)}$$

In the treatment 2, in the presence of an unlabelled P sources, Pdff % is determined by:

$$\text{Pdff \%} = [1\text{-}(\frac{S.A.\ plant\ in\ presence\ of\ \ P\ sources}{S.A.\ labelled\ soil})] \times 100 \quad \text{(Equation 2.3)}$$

Substituting Equation 2.2 into 2.3:

$$\text{Pdff \%} = [1\text{-}(\frac{S.A.\ plant\ in\ presence\ of\ P\ sources}{S.A.\ plant\ in\ absence\ of\ P\ sources})] \times 100 \qquad \text{(Equation 2.4)}$$

2.4.3.3. Species variation

Species variation in accessing P from sparingly soluble P sources is mostly attributed to physiological or morphological traits, or sometimes cation and anion uptake patterns (Ae *et al.* 1990a; Bekele *et al.* 1983; Zhang *et al.* 1997). Species with increased proton excretion activity would enable an increased use of P bonded with Ca. For example, buckwheat (*Pagopyrum esculentum*) and dicots such as legumes and oilseed rape (*Brassica napus*) were reported to be efficient in utilizing rock phosphate due to their ability to excrete protons (Aguilar and van Diest 1981; Bekele *et al.* 1983; van Ray and van Diest 1979). Particularly, white lupin (*Lupinus albus*) and narrow-leaf lupin (*Lupinus angustifolius*) are known to excrete large amounts of protons in their rhizosphere and therefore are possibly the most efficient species in utilizing Ca phosphates (Dinkelaker *et al.* 1989; Hinsinger and Gilkes 1995). The availability of rock phosphate for buckwheat and rape can also be explained by the high rate of Ca uptake by these species, which then shifts the equilibrium towards rock phosphate dissolution (Bekele *et al.* 1983; Khasawneh and Doll 1978).

In contrast, release of carboxylates is responsible for the efficient utilization of less soluble P sources by other species. Pigeonpea *(Cajanus cajan* L.), a legume cultivated in semi-arid regions, was found to be more efficient in utilizing Fe-P than several cereals and other leguminous crops due to the secretion of piscidic acids from its roots (Ae *et al.* 1990a). Zhang *et al.* (1997) also found that P-starved radish utilized P from Al phosphate efficiently when tartaric acid was detected as the major form of organic acid being exuded. By using the ^{32}P isotopic technique, Braum and Helmke (1995) and Hocking *et al.* (1997) showed that white lupin can access up to six times more P from stable P pools when compared to a range of crop species, due to its ability to develop proteoid roots and secrete carboxylates such as citrate. Recently, Pearse *et al.* (2007; 2006b) found that wheat (*Triticum aestivum* L.) was significantly better than white lupin at accessing P from Al phosphate, possibly due to the release of malate in the presence of high level of Al in the rhizosphere. It appears that for efficient P uptake from sparingly soluble P sources, plants need to perceive a change in both P availability and P form, and respond by secreting different carboxylates.

Knowledge on species variation in taking up P from different P sources might help understanding the role of intercropping or rotation in improving soil P sustainability. For example, intercropping of organic-anion secreting crops such as white lupin and pigeon pea with non-secreting species such as canola, soybean and wheat would optimize use of soil P sources since these species access P from different soil P pools (Braum and Helmke 1995; Hocking *et al.* 1997; Li *et al.* 2003). Similarly, grain legume crops, such as white lupin and chickpea, which are capable of mobilizing P through a variety of root traits, could increase the use of residual soil P by cereal corps in the rotation (Nuruzzaman *et al.* 2005a; 2005b). Further research is required to test the feasibility of these intercropping or rotation practices under field conditions.

2.5. Phosphorus acquisition of cotton

Cotton (*Gossypium hirsutum* L.) production is one of the most important agricultural industries in Australia. Lint yields from flood-irrigated cotton crops have increased steadily over the past 25 years and impose a high demand for nutrients (Rochester 2007). Although cotton lint is composed of primarily cellulose, considerable amount of nutrients can be removed with cotton seed (Dorahy *et al.* 2004; Rochester and Peoples 1998). In Australia, cotton production requires regular P fertilizer input (~ 20 kg P ha^{-1}) in order to maintain soil P fertility and high lint yield, especially in the last 25 years (Dorahy *et al.* 2004). Sufficient P nutrition can promote early boll development, hasten maturity and maintain high lint yields and lint quality. In the literature, knowledge on the P acquisition by cotton plants has invariably focused on root morphological traits. Root physiological strategies of cotton are poorly understood. The effectiveness of different P placement methods on cotton growth is reviewed next in order to reveal where in the soil profile efficient P uptake occurs.

2.5.1. P acquisition strategies

Like many species, P stress causes a preferential distribution of dry matter and P content to the roots of cotton (Gill *et al.* 2005). The P-deficient cotton can retain 35% of total P in its roots, compared with only 14% in P-sufficient cotton (Ahmad *et al.* 2001). Ahmad *et al.* (2001) also found that the tolerance of cotton genotypes to P stress was due to their efficiency in absorption of soluble P and P utilization for biomass synthesis. Cotton crops grown in Australian are known to be well infected with AM fungi (Rich and Bird 1974), and poor P uptake of cotton on virgin soil in tropical Australia had been related to the lack of an association with AM (Duggan *et al.* 2008). Plant species like cotton with less branched and coarse root systems could be highly dependent on mycorrhizal association for P acquisition compared to species with finely branched roots (Graham and Syvertson 1985). In addition, the peak uptake of P by the cotton plant occurs later in the growing season (first peak bloom) when the root system is fully developed (Schwab *et al.* 2000), which possibly indicates that P acquisition of cotton mainly depends on root exploration.

2.5.2. Response to surface-applied P

Surface P banding beneath or beside the seed is usually considered the most effective way of applying P fertilizer in the Australian cotton industry. However, low responsiveness of cotton to surface applied P fertilizers has been frequently detected on soils with low soil test P values (Bronson *et al.* 2001; Dorahy *et al.* 2004; Funderburg *et al.* 1996). Dorahy *et al.* (2008) demonstrated that the cotton seedling derived most of its P from the fertilizer band. Nevertheless, at a later growth stage (36 days after sowing), soil P pools beyond the fertilizer band showed a significant contribution (more than 90%) to the total P uptake by cotton (Dorahy *et al.* 2008). When P was applied in a pot at a fixed amount, Mullins (1993) found that P uptake by the cotton plant reached a maximum when 25% to 50% of the soil volume was fertilized with P, compared to that when less than 25%, or more than 50% of the soil volume, received fertilizer P. It is possible that efficient P application requires a compromise between maintaining high P availability and high root-P contact during the entire growth period.

On the other hand, even with irrigation, transient or ongoing drying of topsoil is quite common during cotton production as a consequence of either high evaporation rates or long intervals between irrigations (Carmi *et al.* 1993; Muchow and Keating 1998; Singh *et al.* 2006). Phosphorus fertilizer becomes less effective if the surface soil is subjected to rapid soil drying. Poor responses of cotton to shallow P placement have been observed under frequent surface drought (Hibberd *et al.* 1990; Singh *et al.* 2005). Decreased availability of P in a rapidly drying soil was attributed to reduced P diffusion and poor root P uptake capacity (Nye and Tinker 1977; Schnek and Barber 1979).

2.5.3. Response to deeply applied P

Since topsoil is prone to drying, deep banding of P fertilizer has been advocated in recent years. The benefits of deep P application in increasing P availability and crop production had been reported by a numbers of studies (Sander and Eghball 1999; Teutsch *et al.* 2000). Under frequent drought conditions, seed cotton yield in northern Australia showed a significant increase (17%-67%) to the P applied in subsurface (10-15 and 25-30 cm deep) than that applied at shallow depth (7-10 cm) (Singh *et al.* 2005). Root length density of cotton in a vermiculitic soil was greatest in the 10-20 cm soil layer but least in the surface 0-10 cm (Brouder and Cassman 1990). Schwab *et al.* (2000) found that approximately half of the cotton root system was below the surface 15 cm layer throughout the growing season. In addition, the temporary drying of the topsoil promoted more root growth in the deeper soil layers (Klepper *et al.* 1973; Taylor and Klepper 1974). Thus, it is likely that tap-rooted crops such as cotton with a relatively higher proportion of roots in the subsoil were superior in accessing immobile nutrients like P from the deeper soil layers, compared with shallow-rooted species. Nevertheless, whether P fertilizer should placed deep or shallow, will depend to a large extent on the soil's wetting and drying patterns during the growing season.

2.6. Phosphorus in Vertosol: sinks and sources

The specific soil type used most extensively in the cotton industry is the heavy clay soil in the order Vertosol (Isbell 1996). Vertosols are clay soils (>35% clay) with the ability to shrink and swell, and form deep cracks when dry (Isbell 1996). The total P contents depend on the type of parent material. Probert *et al.* (1987) reported that Vertosols formed on some basalts have higher P contents than on other basalts, or many sedimentary rocks, alluvial sandstones and siltstones. For Vertosols with neutral or alkaline pH, inorganic P occurs mainly as Ca phosphate due to the relatively high pH and the presence of dominant cations such as Ca (López-Piñeiro and Garcia-Navarro 2001; Singh *et al.* 2003). Thus, acid-extractable P in a sequential fractionation scheme that extracts mainly Ca phosphates, was found to be the most abundant fraction in these Vertosols (Pundarikakshudu 1989; Reddy *et al.* 1999; Soils and Torrent 1989; Vu *et al.* 2008).

2.6.1. P sinks

Amorphous Al and Fe-oxides contribute to the apparent P sorption capacity in Vertosol soils (Beauchemin and Simard 1999; Bertrand *et al.* 2003; Tran and Giroux 1987). Assimakopoulos *et al.* (1998) observed that when P fertilizer was applied to a Vertosol, about 50% of P applied was adsorbed onto the surface of amorphous Al and Fe-oxides. Consistently, sequential extraction techniques reveals that excess fertilizer P in the Vertosols is recovered mostly in the labile $NaHCO_3$-extractable inorganic P ($NaHCO_3$-P_i) and moderately labile NaOH-extractable inorganic P (NaOH-P_i) in the short term (Dorahy *et al.* 2007; Singh *et al.* 2001). $NaHCO_3$- and NaOH-extractable organic P pools ($NaHCO_3$-P_o and NaOH-P_o) are also increased due to microbial immobilization of the applied inorganic P (Boschetti *et al.* 2009; Perrott *et al.* 1992; Reddy *et al.* 1999).

Although the acid-extractable P fraction (HCl-P or H_2SO_4-P) remained constant in some Vertosols following P fertilizer application (Boschetti *et al.* 2009; Dorahy *et al.* 2007; Zhang *et al.* 2004), it increases significantly in soils with high Ca content (Guo *et al.* 2000; Reddy *et al.* 1996; Samadi and Gilkes 1998). This has been further verified by a positive correlation between acid-extractable P and the content of active $CaCO_3$ present in some Vertosols (López-Piñeiro and Garcia-Navarro 1997; 2001). In addition to a significant initial retention by Fe and Al oxides, slower precipitation of P with Ca possibly acts as an additional P sorption mechanism in alkaline and Ca-dominated Vertosols over the longer term. The predominance of the acid-extractable P fraction in Vertosols might further suggest that the applied P was eventually transformed to this fraction rather than to the NaOH-Pi pool.

2.6.2. P sources

It is well documented that the P extracted into the NaOH fraction in Vertosol soils is weakly adsorbed on Al and Fe oxide and is relatively plant-available (Dorahy *et al.* 2004; Holford and Mattingly 1975; Soils and Torrent 1989). This has been confirmed by the study of Reddy *et al.* (1999) who demonstrated that the $NaHCO_3$-P_i and NaOH-P_i fractions acted as the main P sources for crops in a soybean-wheat rotation. In contrast, HCl-P was identified as the major contributor to the labile P pool for a cotton crop in a Vertisol (Pundarikakshudu 1989). Guo *et al.* (2000) demonstrated that under intensive plant growth, both HCl-P and residual P acted as the main buffering pool for the plant available P in slightly weathered soils, such as Vertosols. Indeed, evidence has accumulated to support the contention that the residual P fraction in slightly weathered soils is partly plant-available (Guo and Yost 1998; Hedley *et al.* 1982a; McKenzie *et al.* 1992b; Vu *et al.* 2008). Thus, it appears that all inorganic P pools in the Vertosol soil are potentially plant available, although the dominant sources depend on the original distribution of soil P fractions, or the plant species involved.

The contribution of soil organic P to plant available P in Vertosols is controversial in the literature. Studies from Pinto (1974) and Samadi and Gilkes (1998) observed that organic P was more soluble in alkaline and neutral soils than in acid soils and is thus likely to be more available to plants in the former soils. This is further supported by studies from Dalal (1997) and López-Piñeiro and Garcia Navarro (2001) who found that organic P was an important fraction for supplying available P in the Vertosol. However, no relationship was derived between organic P and plant P uptake in studies by Boschetti *et al.* (2009) and Dorahy *et al.* (2007), despite the fact that organic P comprised a significant proportion of the total P in these soils. Factors such as the availability of inorganic P, the level of phosphatase activity and the plant species etc, are likely to account for differences in the contribution of organic P to plant uptake (George *et al.* 2006; Guo *et al.* 2000; Tadano *et al.* 1993).

2.7. Conclusion

It is clear that the availability of soil P is not only soil- and time-specific, but also crop-specific. Adequate predictions of bioavailable P in soils require knowledge of both the chemical nature of soil P and the P acquisition characteristics of the plant species. Future recommendations for fertilizer P applications should be based on fertilizing the soil and the crop. While the ability of plant species to utilize less labile P sources has been extensively studied for many crops, such information is lacking for cotton. The issue is whether the cotton plant displays specific mechanisms for accessing different P forms, like other species. Such information is necessary for developing a sustainable P fertility management plan for long-term cotton production. On the other hand, little attention had been given to the subsoil P pool in the Vertosol soil, where cotton is normally grown, even though the P dynamics in the surface layer of Vertosol has been extensively studied. This thesis aims to identify the P acquisition characteristic of cotton from various P pools and soil depths, and compare them to the P acquisition strategies of other species.

Chapter 3: Phosphorus acquisition characteristics of cotton (*Gossypium hirsutum* L.), wheat (*Triticum aestivum* L.) and white lupin (*Lupinus albus* L.) under P deficient conditions

3.1. Introduction

Phosphorus (P) deficiency occurs in soils low in native P or with high P-fixation capacities**.** A well-adapted plant species develops various strategies to enhance the acquisition of P from soils. In contrast, growth of a poorly-adapted species will generally rely on P fertilizer input. Efforts to identify P-efficient plant species and their adaptive strategies will enhance plant productivity on low-P soils with less P fertilizer input.

Strategies that lead to enhanced uptake or acquisition of P vary with plant species. Some species develop more extensive root systems and root hairs allowing the plants to explore a larger volume of soil (Gahoonia and Nielsen 1998; Lynch and Brown 2001) while others alter the chemistry or biochemistry of the rhizosphere allowing the uptake of P from insoluble P forms (Braum and Helmke 1995; Gilbert *et al.* 1999; Watt and Evans 2003). Bhadoria *et al.* (2001) reported that the P efficiency in wheat (*Triticum aestivum* L.) was predominantly related to its higher root to shoot ratios. The members of the family Proteaceae are well-documented for their efficiency in utilizing P from stable pools by excreting large amounts of carboxylates (Lambers *et al.* 2006). Among other species, rape (*Brassica napus* L.) accesses P from calcium phosphate pools through rhizosphere acidification under P-deficient conditions (Bertrand *et al.* 1999; Moorby *et al.* 1985; Moorby *et al.* 1988). The secretion of carbohydrates and amino acids by maize (*Zea mays* L.) roots under P-stress increases its P influx (Jones and Darrah 1994a).

Little is known, however, about the P acquisition capacity of cotton (*Gossypium hirsutum* L.). Some authors have reported low responsiveness of cotton to P fertilizers on alkaline soils with low soil test P values (Bronson *et al.* 2001; Dorahy *et al.* 2004; Funderburg *et al.* 1996). Phosphorus uptake of cotton was strongly correlated with the Al- and Fe-P fractions at early flowering under field conditions (Dorahy *et al.* 2004). This indicates that cotton has the potential to utilize P from relatively stable pools. If different crop species possess different root strategies for acquiring P from soil, they may differ in their ability to access certain unavailable P pools. For example, proton release will facilitate P acquisition from acid-extractable pools, whereas the effect of carboxylates is associated with P mobilization from Al and Fe oxides. Understanding the P acquisition strategies of different crop species may provide insight into responsiveness in soils with varying P status and cropping history.

This chapter investigated whether cotton is physiologically efficient at acquiring P, and in particular whether cotton acquires P by releasing protons, phosphatases enzymes or carboxylates. In eastern Australian farming systems, wheat is regularly used in rotation with cotton. Unlike tap-rooted cotton, with a less-branched and coarser root system, wheat has a fibrous root system which allows greater exploration of P in soil. Therefore, it is likely these two species would differ in their P acquisition strategies. White lupin (*Lupinus albus* L.), a model plant known as a P-efficient species, has been included as a reference. By using rhizoboxes, it is possible to detect the changes in P pools within a relatively short period and with distance from the root surface. Determining the pools of P in the rhizosphere soil which are utilized by different crop species should help explain which chemical or biochemical strategies in the rhizosphere are used by the three species.

3.2. Materials and methods

3.2.1. Soils

A soil was collected at a depth of 10-30 cm (to ensure P deficiency) along the fence line of a long-term experiment site at Horsham, Victoria, Australia (36º 42' 36" S, 142º 11' 23" E). The soil is classified as Grey Vertosol (Isbell 2002) or Vertisol (USDA) and had the following properties: organic carbon 1.48%, pH 7.5 (0.01 M $CaCl_2$), NO_3-N 20 mg kg^{-1}, NH_4-N 3 mg kg^{-1}, Colwell P 13 mg kg^{-1}.

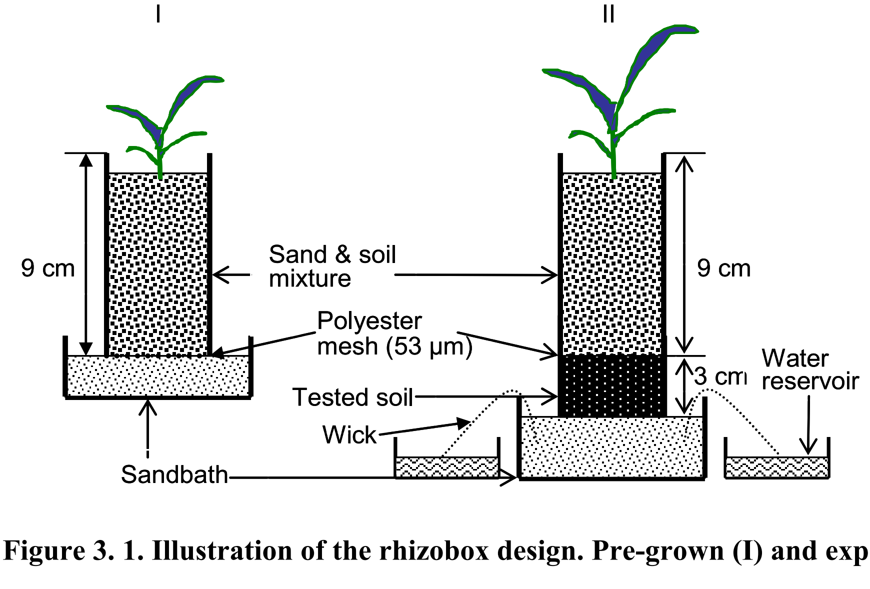


Figure 3. 1. Illustration of the rhizobox design. Pre-grown (I) and experimental set-up (II)

3.2.2. Plant growth

A glasshouse experiment consisting of three plant species, two levels of P supply (0 and 20 mg P kg^{-1} soil) and four replicates was established. The rhizobox used to create rhizosphere soil was modified from the design of Nuruzzaman *et al.* (2006). Each rhizobox consisted of two compartments (PVC tube) which were separated by a polyester mesh (53 µm) impervious to roots (Figure 3.1). Cotton (*Gossypium hirsutum* L. cv. Sicala 60 BR), wheat (*Triticum aestivum* L. cv. Yitpi) and white lupin (*Lupinus albus* L. cv. Kiev) were pre-grown in the upper compartment (length 9 cm, diameter 10 cm) filled with a mixture of siliceous sand (Colwell P = 2.6 mg kg^{-1}) and 18% soil described above. All basic nutrients had been mixed thoroughly with the soil and sand mixture in the following composition (mg kg^{-1}): K_2SO_4, 140; $CaCl_2.2H_2O$, 150; $MgSO_4.7H_2O$, 20; $MnSO_4.H_2O$, 15; $ZnSO_4.7H_2O$, 9; $CuSO_4.5H_2O$, 2; H_3BO_3, 0.7; $Na_2MoO_4.2H_2O$, 0.2; FeEDTA, 5.5. Nitrogen was applied weekly as Ca $(NO_3)_2$ at the rate of 32 mg N kg^{-1} after sowing. Two P treatments were applied as KH_2PO_4 into the upper compartment in order to create P-deficient and sufficient plants before the upper compartment was transferred to the lower soil compartment (length 3 cm, diameter 10 cm).

Wheat and white lupin were planted in October in a glasshouse with a diurnal temperature range of 15-30 ºC. Cotton was planted in December in the same glasshouse (18-36 ºC) in order to meet its optimal light and temperature requirement. One week after sowing, cotton was thinned to 5 plants per pot, wheat 6 plants per pot and white lupin 3 plants per pot to achieve similar root biomass and root mat coverage. Cotyledons of white lupin were removed two weeks after sowing to minimize the effect of P supply from the seeds. After two weeks, only two true leaves were present on cotton seedlings, thus cotyledons were not removed.

Prior to transfer to the lower soil compartment of the rhizobox, the moisture of the upper compartment was maintained at 80% field capacity by weighing and placing them on separate small sand baths which were watered daily. A preliminary experiment showed that watering from the bottom was essential to both produce good root mat coverage and avoid adverse leaching effects. When the plants receiving no P began to show P deficiency, namely visible reduction in shoot growth by comparison with the plants supplied with 20 mg P kg^{-1} (18 d after germination for wheat, 25 d for cotton and white lupin), the upper compartment was transplanted onto the lower compartment filled with 100% Vertosol subsoil collected from the fence line. The lower compartment was placed on a small sand bath fitted with a wick dipping into a reservoir of distilled water to maintain relatively constant moisture in the soil. The whole system was weighed daily and amount of water added to the upper compartment was calculated as the difference between the total water loss and water loss from the sand bath. The upper compartment was watered twice daily to 80% of field capacity by gentle spraying on the soil surface. Rhizobox devices were randomly arranged in the glasshouse. Four rhizoboxes without plants were also included as the control.

3.2.3. Sampling of the rhizosphere soil

The plants were harvested 10 d after transfer to the lower compartment. Shoots were severed at the soil surface and the root mats were photocopied in order to estimate the root coverage. After taking off the polyester mesh, the root mat was carefully cut off, washed and weighed. All washed roots (including root mat) and plant tissues were dried at 70 °C for 48 h and weighed. The soil column of the lower compartment was then sliced into thin layers (1 mm thick per layer) parallel to the rhizoplane by using a hand-made slicing device to obtain rhizosphere soil samples at distances of 1-10 mm from the mesh surface. The accuracy of the slicing technique was monitored by recording the weight of each slice. Finally, a bulk soil sample was taken by slicing a further 13 mm from the soil face. A fresh subsample was taken to assay phosphatase activity, while the remaining soil was air-dried, ground to pass a 0.5 mm sieve and used for pH measurement and P fractionation. The pH was measured using a Thermo Orion 720 pH meter after extraction in 0.01 *M* $CaCl_2$ solution (1:5 w/v soil: solution ratio) by shaking for 17 h on an end-over-end shaker.

3.2.4. Collection of root exudates

Details on collecting root exudates were described by Shen *et al.* (2003). Briefly, at the time of harvest, the upper compartment was separated from the lower compartment. The upper compartments with intact plants were leached six times with 600 mL of deionised water to wash out previous rhizosphere products. Then, root exudates were collected through polyester mesh by continuously percolating the soil with 80 mL of deionised water for 2 h under sunlight during 9:30-11:30 am. The volume of eluted solution was recorded. Two drops of concentrated acetic acid were added to the collected root exudates to inhibit the activity of microorganisms. A sub-sample of 10 mL from the collected solution was kept at -20 °C for subsequent analysis of carboxylates using LC-ESI-MS (with detection limits 20-30 μg L^{-1}) (Chen *et al.* 2007). Working standards of tartaric, formic, malic, malonic, lactic, maleic, citric, succinic, fumaric, pyruvic and t-aconitic acids were used.

3.2.5. Analysis of phosphatase activity

The activity of phosphatase was determined by measuring the release of para-nitrophenol from para-nitrophenyl phosphate following exposure to soil in a modified universal buffer (MUB) at pH 6.5 for the assay of acid phosphatise activity, and pH 11 for the assay of alkaline phosphatase activity, as described by Tabatabai and Bremner (1969). The reaction mixture contained 1 g soil to which 1 mL para-nitrophenyl phosphate (0.025 M) and 0.2 mL of toluene were added. After incubation at 37 °C for 90 min, the mixture was centrifuged at 1800 g for 5 min. The p-nitrophenol formed was determined by spectrophotometer at 400 nm.

3.2.6. Sequential P fractionation

A modified version of the Hedley's P fractionation scheme was used to sequentially fraction soil P (Guppy *et al.* 2000). Aliquots of the bicarbonate and hydroxide extracts were digested in an autoclave at 103 kPa, 120º C for 1 h using acid potassium persulfate (Rowland and Haygarth 1997) and analyzed for total P colorimetrically. Organic P of these two fractions was calculated as the difference between total and inorganic P. Inorganic phosphate in the extracts was determined colorimetrically using malachite green (Motomizu *et al.* 1980). No significant differences were observed in pH, phosphatase or P concentration between 4 and 10 mm from the root mat; hence data were presented only from 1 to 4 mm.

3.2.7. Calculation and Statistical analysis

Root mat coverage was estimated by scanning the root mat surface in black and white and calculating % white space (roots) in the digitized image using a 1 x 1 mm grid with Adobe Photoshop 6.0. Growth and P uptake data, and changes in phosphatase and P fractions with distance from the root mat were subjected to a two-way ANOVA and multiple comparisons with the least significant difference by using software R 2.3.0 (R Development Core Team 2006 R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

3.3. Results

3.3.1. Plant growth and P uptake

Biomass and P uptake was greatest for cotton (35 d) and lowest for wheat (28 d) (Table 3.1). Wheat root growth was vigorous, and had a significantly higher root-to-shoot ratio (1.4) compared with both cotton (0.4) and white lupin (0.3) (Table 3.1). From visual observations, wheat appeared to have longer and denser root hairs than the other species. In response to P application, shoot dry weight increased by 38% in cotton, 90% in wheat and 14% in white lupin (Table 3.1). Root dry weight increased by only 18% in cotton, 90% in wheat and 10% in white lupin (Table 3.1). Phosphorus addition significantly increased P concentration in shoot and roots of all three species although the P concentrations in plants supplied with P were marginal to adequate levels (Reuter and Robinson 1997). Such an increase was less marked for white lupin than for the other species (Table 3.1). Total P uptake where P was not added was 45%, 30% and 65% of that achieved where P was applied for cotton, wheat and white lupin, respectively. The root-to-shoot ratio remained relatively unchanged for wheat and white lupin under P stress. However, P-deficient cotton tends to have 15% higher root-to-shoot ratio when compared with the P-fed cotton.

Table 3. 1. Shoot and root growth characteristics for wheat, cotton and white lupin grown for 18, 25 and 25 days in the soil and sand mix supplied without and with 20 mg P kg^{-1}, and then grown on the soil compartment for 10 days.

Crop Species	P supply (mg kg^{-1})	Shoot dry weight (g)	Root dry weight (g)	Shoot P concentration (%)	Root P concentration (%)	Total P uptake (mg pot^{-1})	Root/shoot ratio	Root mat weight/ Total root weight (%)	Root mat coverage (%)
Cotton	0	3.04	1.35	0.09	0.10	4.26	0.44	11	55
	20	4.21	1.60	0.17	0.17	9.79	0.38	13	65
Wheat	0	0.60	0.83	0.12	0.13	1.87	1.38	26	60
	20	1.14	1.58	0.29	0.19	6.03	1.39	42	90
White Lupin	0	2.81	0.87	0.09	0.12	3.56	0.31	14	50
	20	3.21	0.96	0.12	0.15	5.49	0.30	14	50
LSD(P=0.05)		0.21	0.16	0.01	0.03	0.81	0.10	6	8
P levels		***	***	***	***	***	n.s.	***	***
Crop species		***	***	***	n.s.	***	***	***	***
P × Species		***	***	***	n.s.	***	n.s.	***	***

n.s., $P > 0.05$; ***, $P \leq 0.001$

Wheat plants formed a dense root mat, and root coverage on the polyester mesh reached 90% and 60% with and without P application, respectively (Table 3.1). The formation of the wheat root mat comprised more than 25% of the total root dry weight. In contrast, less than 15% of total root weight contributed to the formation of root mat for cotton and white lupin, and the root coverage on the mesh was approximate 60% for cotton and 50% for white lupin. At harvest, 1-2 root clusters were observed on the root mat of both P-deficient and P-fed white lupin.

3.3.2. Rhizosphere pH and root exudates

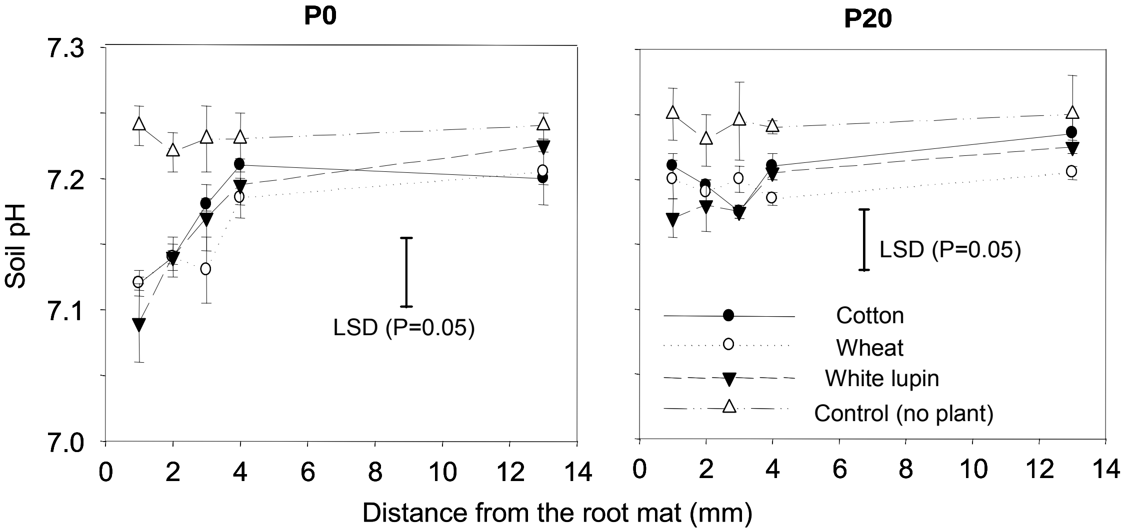


Figure 3. 2. Changes in soil pH with distance from the root mat of cotton, wheat and white lupin with 0 and 20 mg kg^{-1} P application. Error bars represent ± standard error of means of 4 replicates. For each panel, the vertical bar indicates the LSD (*P*=0.05) for the treatment ×distance interaction.

In response to P stress, rhizosphere pH under the root mat of all species decreased by about 0.1 units when compared with the soil distant from the root mat (13 mm) and the control pot without plants (Figure 3.2). The acidification extended to approximately 3 mm for all species. With adequate P supply, rhizosphere pH in all planted treatments remained relatively unchanged away from the root mat. However, the average pH in soil layers immediately below the mesh surface of the control pot was generally higher than in the equivalent layers for the pots with plants (Figure 3.2).

Carboxylates were detected only in the root exudates of white lupin. Malate was identified but was present only at very low concentration: 0.5 µmol h^{-1} pot^{-1}. The amount of citric released by P-deficient white lupin was 25.8 ± 1.2 µmol h^{-1} pot^{-1} (29.7 µmol h^{-1} g^{-1}dry roots), which was almost double that of P-sufficient plants (16.4 ± 1.5 µmol h^{-1} pot^{-1} or 17.1 µmol h^{-1} g^{-1}dry roots). There were no other carboxylates detected in the root exudate samples.

3.3.3. Phosphatase activity and depletion of organic P (P_o) in the rhizosphere

When no P was applied, the activity of acid phosphatase in the first 1-mm soil layer away from the root mat of cotton and wheat was 20% higher than that in the bulk soil which was measured in soil that was 13 mm from the root mat and the control pot. It then decreased gradually away from the root mat, with the decline being less marked for the wheat plant (Figure 3.3). Wheat was the only species which induced an increased activity of acid phosphatase near its root mat when P was applied. Regardless of their relatively lower root coverage, the P-starved cotton and wheat (P0) elevated acid phosphatase activity by 20% and 5% as compared with that of the P-fed cotton and wheat (P20) respectively, at the first mm away from the root mat. For white lupin, the activity of acid phosphatase remained unchanged and was not affected by distance from the root mat or by P treatment (Figure 3.3). The activity of acid phosphatase in the control pot did not change away from the root mat.

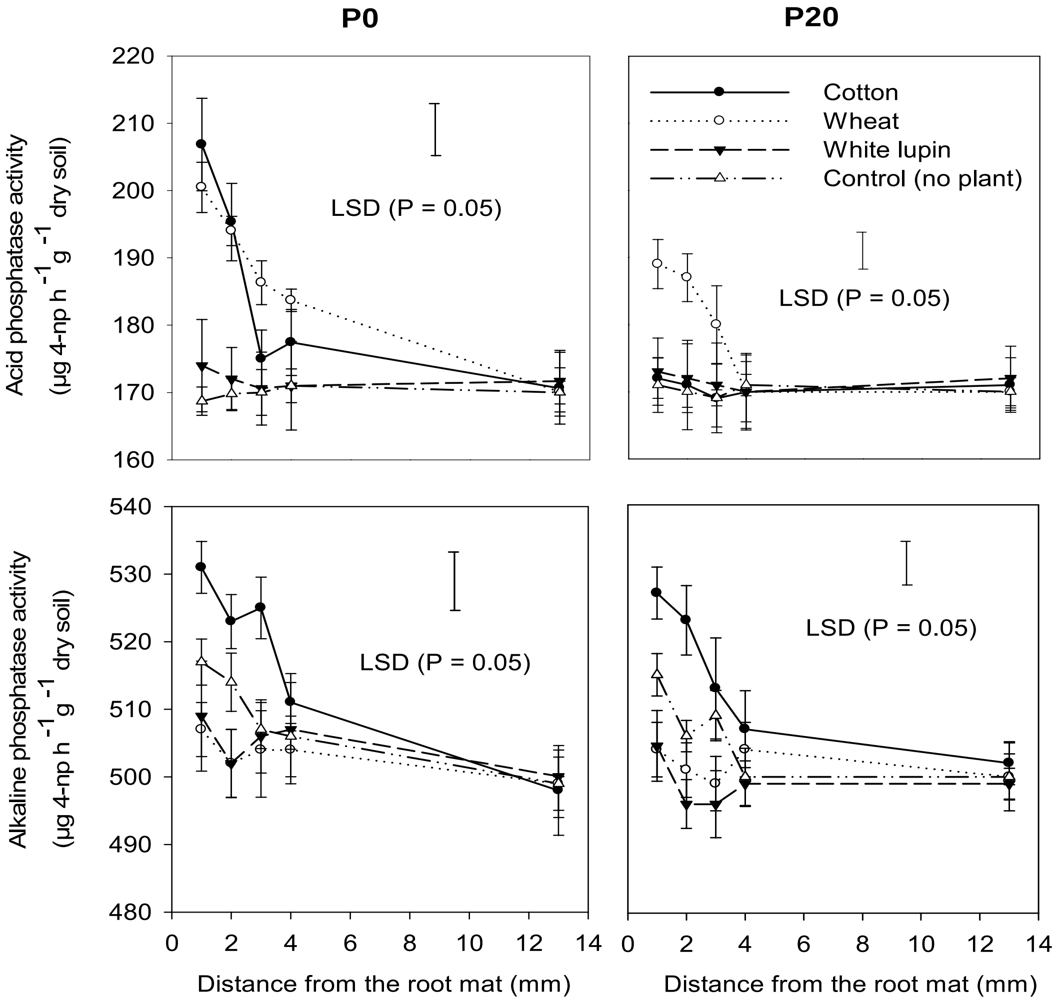


Figure 3. 3. Changes in acid and alkaline phosphatase activity with distance from the root mat of cotton, wheat and white lupin with 0 and 20 mg kg^{-1} P application. Error bars represent ± standard error of means of 4 replicates. For each pane, the vertical bar indicated the LSD (*P*=0.05) for the treatment × distance interaction.

The level of alkaline phosphatase activity in the rhizosphere was more than twice that of acid phosphatase for all species (Figure 3.3). Enhanced activity of alkaline phosphatase was only detected near the rhizoplane of the cotton, compared with the bulk soil and the control pot. Similar to acid phosphatase, the amount of alkaline phosphatase was inversely related to the distance from the root mat. However, there was no effect of P treatment on the activity of alkaline phosphatase near the root mat of cotton.

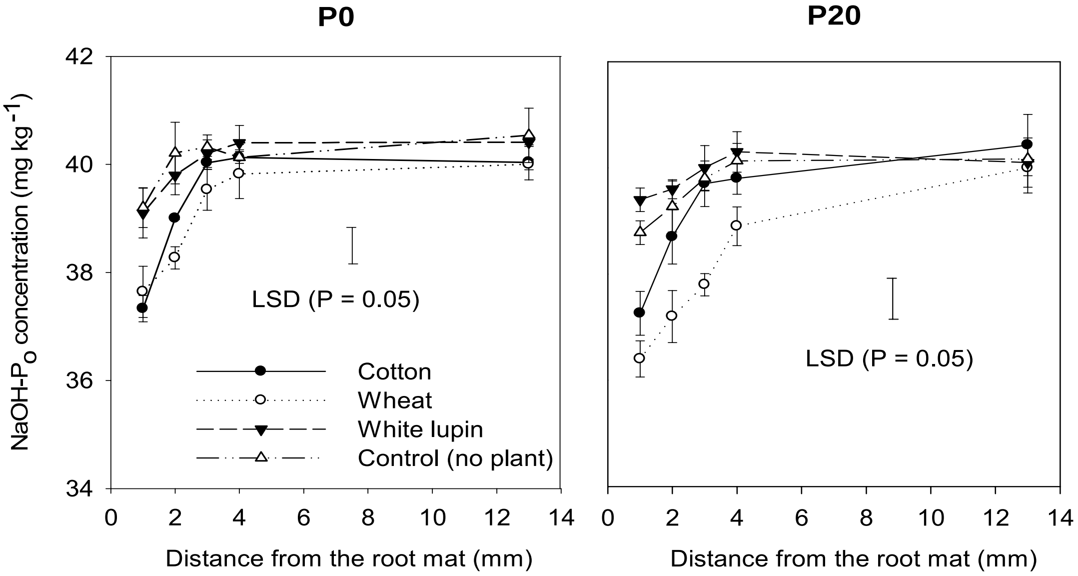


Figure 3. 4. Changes in concentration of NaOH-P_o with distance from the root mat of white lupin, wheat and cotton with 0 and 20 mg kg^{-1} P application. Error bars represent ± standard error of means of 4 replicates. For each panel, the vertical bar indicates the LSD (*P*=0.05) for the treatment × distance interaction.

3.3.4. Depletion of inorganic P (Pi) fractions in the rhizosphere

The concentration of $NaHCO_3$-extractable organic P ($NaHCO_3$-P_o) was less than 4% (about 10 mg kg^{-1}) of that for total P and showed no change with the distance to the root mat for all species (data now shown). On the other hand, the concentration of NaOH-extractable organic P (NaOH-P_o) decreased by 6% in the rhizosphere soil of P-deficient wheat and cotton relative to the bulk soil (Figure 3.4). Where P was supplied, the depletion of NaOH-P_o by wheat reached nearly 10%, followed by cotton with 8%, compared to the bulk soil. Minimal depletion of NaOH-P_o pool was demonstrated by white lupin.

There was a significant reduction of bicarbonate-extractable inorganic P ($NaHCO_3$-P_i) near the root mat (1 mm) of all P-stressed crop species compared to the bulk soil (13 mm) (Figure 3.5). The maximum depletion to this pool was achieved by white lupin, followed by wheat and cotton. The zone of $NaHCO_3$-P_i depletion (relative to the no-plant control) for the cotton root did not extend more than 2 mm from the rhizoplane. However, decreases in $NaHCO_3$-P_i concentration near the root mat of wheat and white lupin extended to about 3 mm. The NaOH-extractable inorganic P (NaOH-P_i) pool, theoretically, less available, decreased by about 15% of that in bulk soil, in the first 1-mm layer, for all species under P-stress (Figure 3.5). Beyond the first mm, this pool was not depleted by wheat and cotton roots. In contrast, the decline in NaOH-P_i was still detectable up to 4 mm for white lupin. The HCl-extractable P (HCl-P) pool at 1 mm was depleted

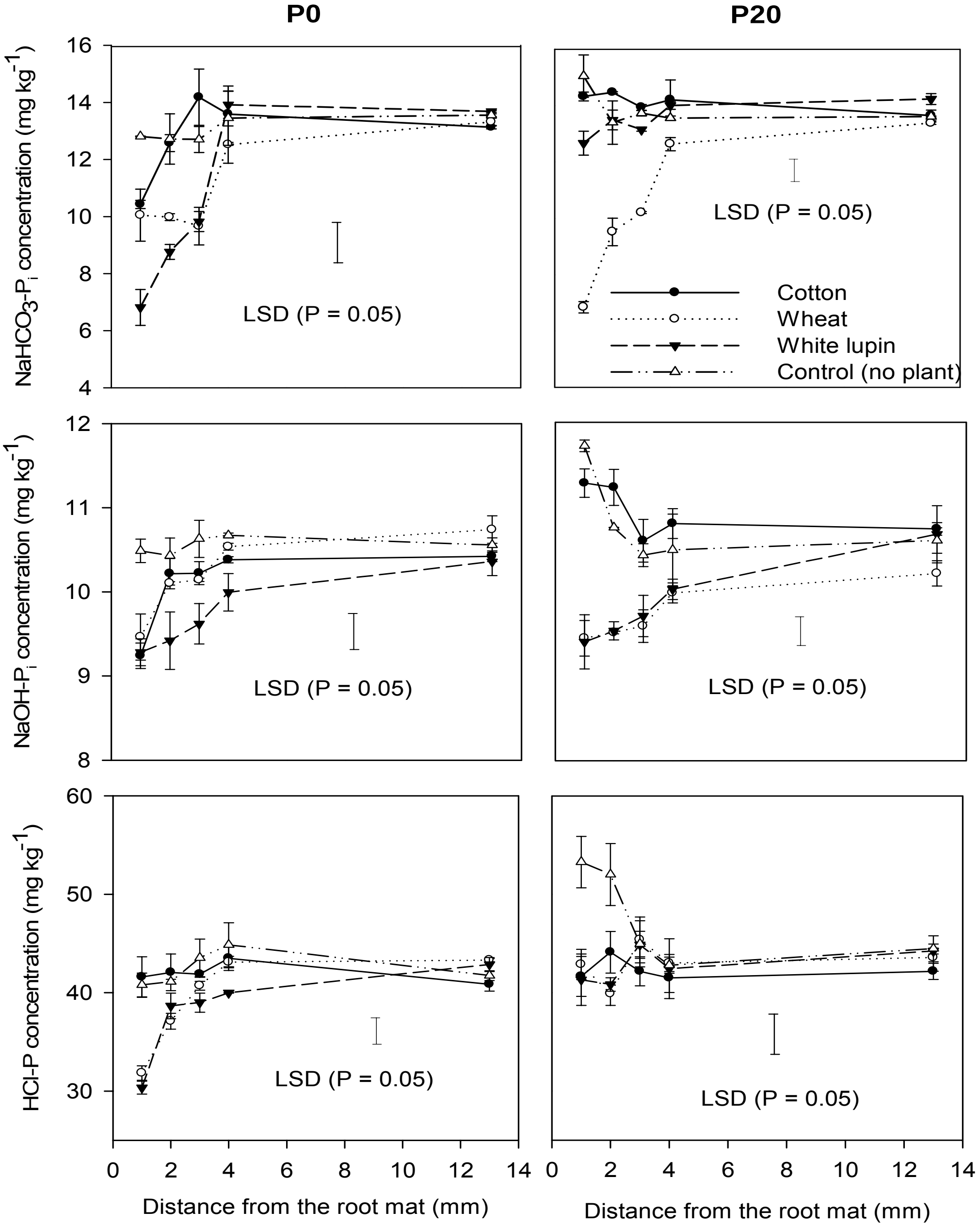


Figure 3. 5. Changes in concentration of $NaHCO_3$-P, NaOH-P_i and HCl-P with distance from the root mat of white lupin, wheat and cotton with 0 and 20 mg kg^{-1} P application. Error bars represent ± standard error of means of 4 replicates. For each panel, the vertical bar indicates the LSD (*P*=0.05) for the treatment × distance interaction.

significantly (25% of that in bulk soil) near the root mat of wheat and white lupin, but not for cotton. The depletion width of this pool was relatively narrower than other pools (Figure 3.5). None of the three crop species depleted the residual P fraction within 10 days (data not shown). Supply of P to the upper compartment resulted in the accumulation of NaOH-P_i and HCl-P pools in the lower soil compartment of the control (Figure 3.5). With plants, P accumulation into the NaOH-P_i pools was observed for cotton only. Unlike cotton and white lupin, wheat depleted significant amounts of $NaHCO_3$-P_i even with P supplied.

3.4. Discussion

To my knowledge, this is one of the few studies that attempt to determine the P acquisition mechanisms for cotton plants. By comparing cotton with wheat and white lupin differing in root morphology and root exudation (Nuruzzaman *et al.* 2006), this study did not detect carboxylates in root exudates of cotton plants irrespective of P supply. White lupin released considerable amounts of carboxylates under P deficiency with the values falling within the range reported previously for this species (Neumann and Römheld 1999; Sas *et al.* 2001a; Veneklaas *et al.* 2003). This suggests that other P acquisition strategies are used in cotton plants. These strategies may include changes in the root-to-shoot ratios and excretion of phosphatases which are discussed below.

3.4.1. Root and shoot growth

Only cotton exhibited an increase in root-to-shoot ratio under P deficiency in this study. The level of increase (15%) coincided with the values reported by Maqsood *et al.* (2005) for this species. However, irrespective of P addition, the root-to-shoot ratio of wheat was approximately 3 times greater than both cotton and white lupin. Bhadoria *et al.* (2001) reported that P acquisition efficiency of wheat was mainly based on its high root-to-shoot growth ratio. The greater allocation of assimilates to root growth due to P stress by cotton may not confer as a significant advantage in P acquisition by cotton when compared with white lupin and wheat.

Phosphorus deficiency symptoms, in terms of shoot biomass reduction, was observed 10 days earlier in wheat than cotton and white lupin, presumably because of its smaller seed size and thus lower P reserves. The cotyledons of white lupin were removed so the effect of the seed P reserves was minimized, whereas the growth of cotton seedlings could have benefited considerably from its seed P reserves. Considering that P concentration of cotton seed is 0.7% (Marcuswyner and Rains 1982), cotton seedlings will have nearly 4 mg P pot^{-1} from the seed reserves (seed size 0.12 ± 0.02 g $seed^{-1}$). The work of Thomson *et a*l. (1993) and Tang *et al.* (2007) illustrated the importance of seed P in P nutrition of lupin and soybean, respectively.

3.4.2. Rhizosphere pH

In general, pH decreases when N is supplied as ammonium and increases when supplied as nitrate due to the effect of N form on the cation/anion uptake ratio (Curtin and Wen 2004; Tang *et al.* 1999). However, enhanced net excretion of H^+ ions under nitrate-fed plants was observed in rape suffering from P deficiency (Hedley *et al.* 1982a; Moorby *et al.* 1985; Moorby *et al.* 1988) or cotton under Zn deficiency (Cakmak and Marschner 1990). In this experiment, nitrate was the N source. The pH in the rhizosphere of all species decreased under P stress, but remained unchanged with adequate P supply, indicating that all species have similar responses to rape plants in terms of acidifying the rhizosphere under P deficiency.

Soil acidification is not only related to H^+ release as a consequence of excess uptake of cation over anion (Hinsinger *et al.* 2003). In white lupin, proton extrusion would also be associated with the release of carboxylates (Hoffland 1992; Hoffland *et al.* 1989). Root respiration might also have contributed a substantial proportion of the pH decrease in the rhizosphere of all plant species (Hinsinger *et al.* 2003). The three species acidified the rhizosphere to a similar extent under P deficiency. A depression in NO_3^- uptake and a corresponding increase in the cation-anion uptake ratio might be the main cause of acidification in this study as has been observed previously (Kirk and Le Van Du 1997; Neumann and Römheld 1999). In another study, greater rhizosphere acidification of cotton was reported by Hylander *et al.* (1999), when compared with maize and soybean.

3.4.3. Phosphatase activity and depletion of NaOH-P_o

Crop species might alter the potential phosphatase activity in the rhizosphere, as the degree of phosphatase activity has been shown to vary among species and with severity of P starvation. In my study, acid phosphatase activity was greatly enhanced in the rhizosphere of wheat and cotton under P deficiency. However, an increased alkaline phosphatase activity relative to the control was only detected in the rhizosphere of cotton irrespective of P supply. George *et al.* (2002b) also reported species variation in phosphatase activity, and showed that agroforestry species enhanced the activity of acid phosphatase, while maize enhanced the activity of alkaline phosphatase in response to pH increases. Differential phosphatase activity associated with each species may indicate their differing potentials for obtaining P from organic pools. In Australia, cotton is normally grown in neutral to alkaline soils. The enhanced activity of alkaline phosphatases by cotton roots would be beneficial in mineralizing P_o and supplying P to plants in these soil types.

The release of phosphatases induced by P deficiency can be clearly seen by comparing between the +P and -P treatments in this present study. The maximum secretion of acid phosphatase due to P deficiency was observed for cotton, followed by wheat. Alkaline phosphatases however did not respond to P deficiency. This means that P-deficient cotton and wheat plants can potentially excrete significant amounts of acid phosphatases over an acid to neutral pH range, and these phosphatases would be efficient as they are accompanied by a fall in pH in the rhizosphere. Secretion of acid phosphatase from roots is a common response to P deficiency (George *et al.* 2006; Gilbert *et al.* 1999). However, these authors did not measure alkaline phosphatase activity under P deficiency. Previous studies also showed that a greater acid phosphatase activity was associated with P-deficient white lupin roots and the increase in phosphatase acitivity was most pronounced near the proteoid regions (Gilbert *et al.* 1999; Li and Tadano 1997). The lack of acid phosphatase activity increase in the soil-root interface of white lupin in my study could be attributed to its relatively less root mat, compared with cotton and wheat, and few cluster roots formed on it.

Wheat and cotton, but not white lupin, depleted NaOH-P_o to a significant level irrespective of P application. In accordance with other studies (Chen *et al.* 2002; George *et al.* 2002a; George *et al.* 2006), depletion of organic P was accompanied with a concurrent increase in phosphatase activity in the rhizosphere soil. While wheat excreted acid phosphatase into the rhizosphere with and without added P, cotton also excreted alkaline phosphatases irrespective of P supply. In addition, cotton excreted acid phosphatase under P deficiency. In contrast, white lupin did not excrete phosphatases, which is probably related to its high efficiency of mobilizing inorganic P through exudation of carboxylates and rhizosphere acidification. The greater depletion of NaOH-P_o in the rhizosphere of P-fed than P-deficient plants could be explained by the relatively higher root-soil contact (root mat coverage) under P-sufficient conditions (Table 3.1). The enhanced acid phosphatase activity associated with P deficiency, as well as excretion of alkaline phopsphatases of cotton plants irrespective of P supply, could confer a potential advantage in utilization of P from organic pools over other species.

3.4.4. Depletion of inorganic P

Cotton, wheat and white lupin differed in their ability to absorb P from different P pools. The depletion of inorganic P fractions by cotton was the smallest. Proton release did not facilitate the mobilization of P from HCl-P pools for cotton, possibly due to the slow depletion of soil solution P by cotton root. Apparently, cotton is less physiologically efficient in absorbing either labile Pi or sparingly soluble Pi compounds than the other two species. This inefficiency in Pi acquisition might be related to the negligible exudation of carboxylates when compared with white lupin. The higher efficiency of wheat plants over cotton plants in Pi acquisition might be related more to root morphological traits e.g. root hair development. Although I did not quantify their length, root hairs were dense, longer and clearly visible through the root mat of wheat. This was further supported by the greater depletion and wider depletion zone of various P pools in the rhizosphere of wheat than of the other species. According to Lamont *et al.* (1984) and Pessarakli (1999), average root hair length of wheat (about 1.5 mm) is 5 times longer than that of cotton and proteoid rootlets of white lupin (about 0.3 mm). A similar study suggested that the difference in P depletion intensity among white lupin, field pea and wheat was due to the variation in root hair density (Nuruzzaman *et al.* 2006). In comparison, P-deficient white lupin was very efficient in the mobilization of stable soil P pools through root exudates as demonstrated in this and other studies (Braum and Helmke 1995; Watt and Evans 2003).

Under P deficiency, while white lupin and wheat could access all P_i pools including the largest HCl-P pool, cotton could only access $NaHCO_3$-P_i and NaOH-P_i in a very narrow zone of the rhizosphere (Figure 3.5). Another study observed that wheat was also able to deplete the residual P in a Calcarosol that received applications of P fertilizers for 65 years (Vu *et al.* 2008). In addition, this present study illustrated that wheat had the greatest capacity among the species tested to absorb labile P from the soil and applied P, and thus showed the greatest response to P application in terms of biomass production and P concentration in plants. About 50% depletion in the $NaHCO_3$-P_i pool by both P-fed and P-deficient wheat plants had been detected. In contrast, the P applied in the upper compartment under cotton appeared to diffuse down to the lower soil compartment and be transformed into NaOH-P_i pool which is evidenced by the increase of the NaOH-P_i fraction in the cotton rhizosphere relative to the bulk soil. This apparent lack of use of labile P by cotton could be related to its poor root interception. Ultimately, root architecture and volume of soil exploration might partly account for the differences in P acquisition between the three species.

3.5. Conclusion

This chapter demonstrated that cotton is less efficient in P acquisition than both white lupin and wheat from rhizosphere soil. Carboxylates such as malic and citric acids were not detected in the root exudates of cotton under the current experimental conditions. However, the enhanced activity of both acid and alkaline phosphatases by cotton roots conferred a potential advantage for cotton to acquire P from organic pools. The low responsiveness of cotton crop to surface application of P fertilizers in the field may not only be related to its physiology of P acquisition, but also to its internal P requirement, root architecture and use of subsoil P pools. Studies into these aspects are underway.

Chapter 4: Cotton, wheat and white lupin differ in phosphorus acquisition from sparingly soluble P sources

4.1. Introduction

Inorganic P in the soil is mainly bound to either calcium (Ca) in calcareous soils or iron (Fe) and aluminium (Al) in acid soils, reducing availability to plants (Hinsinger 2001). Application of soluble P fertilizers is frequently required to achieve optimal yield. However, soluble P once applied would quickly be fixed by Ca, Fe or Al in the soil, mainly as precipitates of low solubility and low plant-availability. Thus, a large proportion of soil P is in stable and unavailable forms. When P supply is inadequate, the replenishing of soil solution P from these stable pools would mainly depend on plant-induced P solubilisation.

Chemical processes in the rhizosphere that are responsible for P solubilising-activity include proton or hydroxyl release and exudation of organic anions (Hinsinger 2001). Diversity in root responses to P deficiency is reflected in differing abilities of plant species to access P from sparingly soluble P forms. For example, Zhang *et al.* (1997) found that P-starved radish utilized P from Al phosphate efficiently when tartaric acid was detected as the major form of organic acid exuded. Buckwheat and rape were well recognized for their high capacity in proton release and thus were efficient in taking up P from alkaline soils (Mclachlan 1976). Ae *et al.* (1990a) also reported that pigeon pea is more efficient in utilizing Fe phosphate than cereals and leguminous crops due to the release of piscidic acid from roots. Knowledge on species' variation in utilizing sparingly soluble P sources could provide an insight into the capacity of each species to use reserves of P that often considered recalcitrant in soils.

However, such information is lacking for the cotton plant, the major crop species in eastern-northern Australia farming system. Low responsiveness of cotton to P fertilizers on alkaline soils with low soil test P values (Bronson *et al.* 2001; Dorahy *et al.* 2004) might indicate that cotton uses P mainly from stable pools rather than from soluble P sources. Further evidence is provided by the strong correlation between the soil Al-P and Fe-P fractions and P uptake of cotton plants at early flowering under field conditions (Dorahy *et al.* 2004). The previous rhizobox experiment revealed that cotton plant, under P deficiency, accessed little P from stable P pools in the soil (Chapter 3). However, the stable P pool in this soil mainly represents P bonded with Ca (HCl-extractable P), and the relative availability of P precipitated with Al and Fe to cotton still requires further investigation.

The aim of this chapter is to investigate the ability of cotton to access P from sparingly soluble P sources, such as $AlPO_4$ (Al-P), $FePO_4$ (Fe-P), and hydroxyaptite (Ca10-P), when compared with wheat and white lupin. Wheat is considered an inefficient P-acquiring species (Nuruzzaman *et al.* 2005a), whereas white lupin is an efficient species due to known organic acid release (Gardner *et al.* 1983). It has been hypothesized that the efficiency of cotton in acquiring sparingly soluble P sources lies between wheat and white lupin. Plant comparison is undertaken in washed sand to facilitate measurement of rhizosphere responses to P supplied in the different sparingly soluble forms.

4.2. Materials and Methods

4.2.1. Experiment design and plant culture

The experiment consisted of six P treatments, three crop species and three replicates. The six P treatments include zero P (P0) and five P sources supplied as KH_2PO_4 (K-P), $Ca(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P), and hydroxyapatite (Ca10-P). K-P and Ca-P are water-soluble P sources. Al-P, Fe-P and Ca10-P, which represent the main forms of sparingly soluble P in acid soils and alkaline soils, are commercial compounds obtained from Sigma-Aldrich Chemie GmbH.

Plants were grown in 3.5 kg of coarse river sand (<2 mm). The coarse river sand had the following chemical properties: pH 5.8 (0.01 M $CaCl_2$), total C 0.55 g kg^{-1}, total N 0.03 g kg^{-1}, available P (Olsen P) 2.6 mg kg^{-1} and exchangeable Al 0.12 mg kg^{-1} (1 M KCl). Basal nutrients were mixed with the river sand at the following rates (mg kg^{-1}): K_2SO_4, 140; $CaCl_2.2H_2O$, 150; $MgSO_4.7H_2O$, 20; $MnSO_4.H_2O$, 15; $ZnSO_4.7H_2O$, 9; $CuSO_4.5H_2O$, 2; H_3BO_3, 0.7; $Na_2MoO_4.2H_2O$, 0.2; FeEDTA, 5.5. Nitrogen was applied weekly at a rate of 30 mg N kg^{-1} as $Ca(NO_3)_2.4H_2O$ to each pot after planting. Phosphorus sources were added as powder at a rate of 40 mg P kg^{-1} soil to the designated treatment soils. Potassium was balanced for the treatment K-P. The soil was then mixed thoroughly with basal nutrients and P, and weighed into a PVC tube (10 cm diameter, 30 cm high) lined with plastic bags.

Cotton (*Gossypium hirsutum* cv. Sicala 60 BR), wheat (*Triticum aestivum* L. cv. Yitpi) and white lupin (*Lupinus albus* L. cv. Kiev) were planted in a glasshouse with a diurnal temperature range of 16-30°C. Uniform pre-germinated seeds were sown at a depth of 2 cm. One week after sowing, plants of wheat, cotton and white lupin were thinned to 6, 2 and 2 per pot, respectively, to keep similar biomass. The plants were watered to 80% of field capacity every 2 days for the first 3 weeks, and then daily for the final 3 weeks. In order to minimize the leaching effect during watering, water was supplied via a small tube (i.d. 6 mm) with outlet holes below the top 5 cm down to 30 cm.

4.2.2. Plant and rhizosphere sand measurement

Plants were harvested 40 days after sowing when the apparent treatment effect in terms of shoot biomass growth was visual. Before harvesting, the moisture of the coarse river sand was intentionally maintained at about 60% field capacity, the optimal moisture level tested for the collection of rhizosphere sand. After cutting the plastic bags into half, plants were lifted up and shaken gently to remove the bulk sand from the roots. Sand that remained adhered to the roots was considered rhizosphere sand and collected by shaking them off the roots.

Soil available P (Olsen P) and exchangeable Al (1 M KCl) was measured for both rhizosphere sand and bulk sand according to the method described by Olsen and Dean (1965) and Conyers *et al*. (1991), respectively. The activity of acid phosphatase was determined by measuring the release of para-nitrophenol from para-nitrophenyl phosphate following exposure to soil in a modified universal buffer (MUB) at pH 6.5 as described by Tabatabai and Bremner (1969). The pH was measured using a Thermo Orion 720 pH meter after extraction in 0.01 M $CaCl_2$ solution (1:5 w/v soil: solution ratio).

Shoots were cut off above the soil surface, washed and weighed. All roots were carefully collected, washed and weighed. Cluster roots on the white lupin were counted and recorded. Both roots and plant tissues were dried at 70°C for 48 h, weighed and ground. Subsamples were digested with concentrated nitric and percloric acid (4:1) for determination of P, K, Ca, Al and Fe using ICP-AES (Inductively Coupled Plasma Atomic Emission Spectrometry).

4.2.3 Statistical analysis

All data were subjected to one- or two-way ANOVA using R 2.3.0 software (R Development Core Team 2006), followed by the Duncan's multiple range test. Checks for normal distribution of the data, and for homogeneity of variance, found that no data transformations were required. Means are presented with standard errors.

4.3. Results

4.3.1. Plant growth

Application of soluble K-P and Ca-P increased shoot and root dry weight of wheat and cotton significantly when compared with either control or less soluble P treatments (Figure 4.1). Wheat obtained the maximum shoot dry weight with P supplied as K-P while cotton accumulated the most shoot biomass when supplied soluble Ca-P. Shoot and root dry weight of white lupin did not show significant response to any supplied P source.

Variation in species P acquisition from the sparingly soluble P sources can be expressed in relation to the response to soluble P sources. Wheat shoot growth showed the highest relative P efficiency when supplied Al-P (0.56), followed by cotton (0.21). However, relative P efficiency in accessing P from Fe-P and Ca10-P was low for both wheat (0.07, 0.07) and cotton (0.03, 0.11). Severe leaf senescence was observed 3 weeks after planting in wheat not supplied with P or supplied with Fe-P or Ca10-P. Phosphorus supplied as soluble P sources significantly suppressed cluster root formation in white lupin compared with no P supply, and most root clusters were produced in the treatments with P supplied as Fe-P and Ca10-P (Data not shown). Nodules on the white lupin were virtually absent due to regular N supply.

Regardless of P sources, the root/shoot ratio of wheat was 2 to 3 times higher than that of cotton and white lupin (Figure 4.1). The root/shoot ratio of both wheat and cotton was greatest when P was not supplied, decreased by 15% when supplied with sparingly soluble P forms, and 30% when supplied with soluble P. However, the roots/shoots ratio of white lupin was not affected by P treatment.

4.3.2. Plant P concentration and uptake

The concentration of P in the shoot and root, and total P uptake was highest in K-P and Ca-P treatments for all species (Figure 4.2). With application of any sparingly soluble P sources, cotton showed no significant increase in P concentrations compared with the control (P0). However, the addition of Al-P increased shoot P concentration of wheat and white lupin by 190% and 50%, respectively, compared with P0 ($P<0.05$). Shoot P concentration of wheat was not increased following supply of P as Fe-P or Ca10-P, in contrast to significant increase in root P concentration. When compared with P0, total P uptake from Al-P by wheat was approximately 9 times higher than cotton and 7 times higher than white lupin (Figure 4.2). Total P uptake by wheat from Fe-P and Ca10-P was less, though significant. Cotton and white lupin did not acquire P from either of these sources.

4.3.3. Concentrations of K, Ca, Fe and Al in plant

Concentrations of K and Fe in shoot and root of all species were not affected by P source. A dilution effect of biomass production on shoot Al concentration of cotton and root Al concentration of wheat was detected when P was applied as soluble K-P and Ca-P (Table 4.1). The concentration of K in shoot of wheat was much higher than cotton and white lupin, while the opposite was observed for the shoot Ca concentration ($P<0.05$). Application of Ca-P resulted in a significantly higher level of Ca accumulated in the shoot of cotton and white lupin, compared with other P sources. When averaged over the P treatments, Al concentration in roots of wheat was 2.5 and 4 times higher than that of cotton and white lupin, respectively ($P<0.05$).

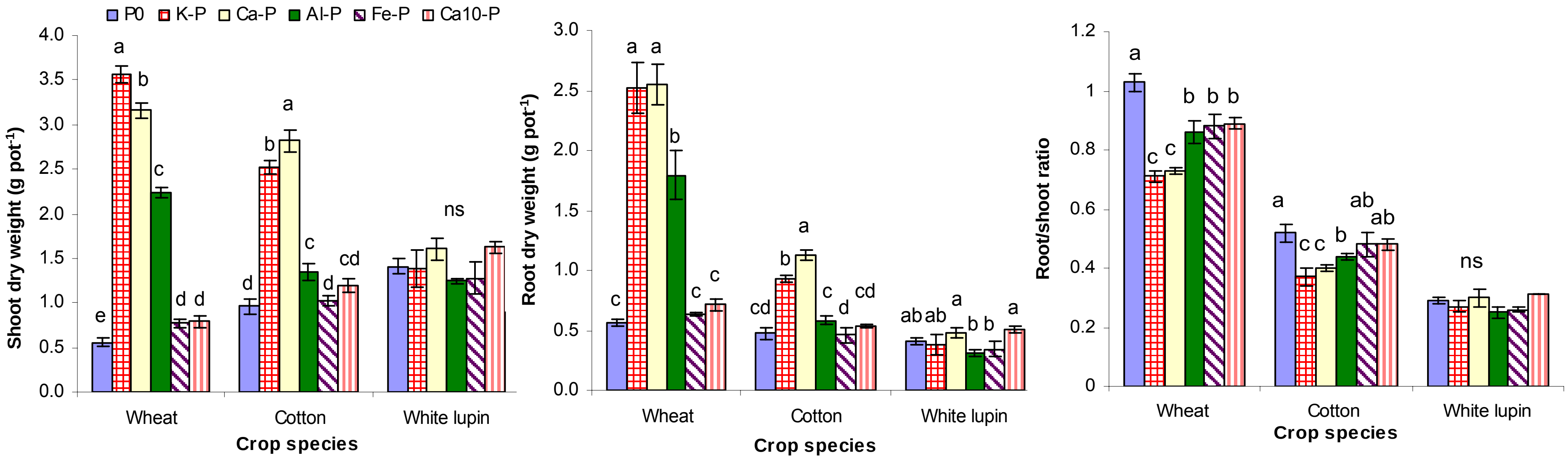


Figure 4. 1.Total shoot and root dry weight and root:shoot ratio of wheat, cotton and white lupin grown in 3.5 kg pots for 40 days with zero P (P0) or 40 mg kg^{-1} P as KH_2PO_4 (K-P), $Ca(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P), and hydroxyaptite (Ca10-P). Error bars indicate the standard error (n = 3). Values not sharing the same letter indicate significant differences between the P treatments within a species according to the Duncan's multiple range tests (*P* = 0.05). "ns" represents no significant difference.

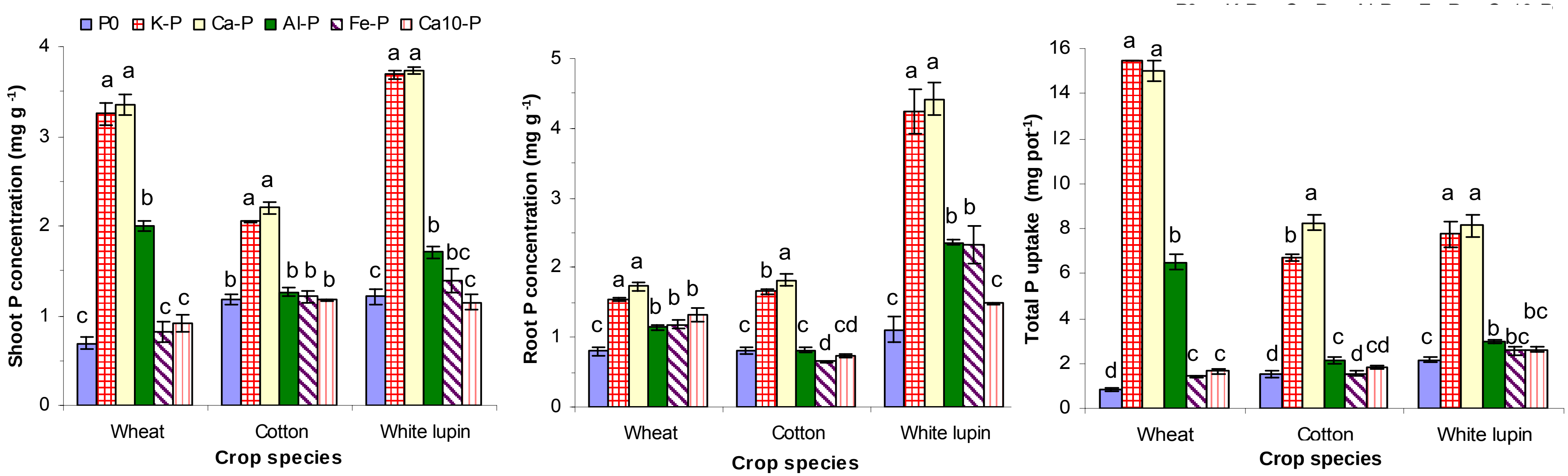


Figure 4. 2. The concentration of P in shoot and root, and total P uptake of wheat, cotton and white lupin supplied with zero P (P0), and 40 mg kg^{-1} P as KH_2PO_4 (K-P), $Ca(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P), and hydroxyaptite (Ca10-P). Error bars indicate the standard error (n = 3). The values not sharing the same letter indicate significant differences between the P treatments within a species according to the Duncan's multiple range tests (*P* = 0.05). "ns" represents no significant difference.

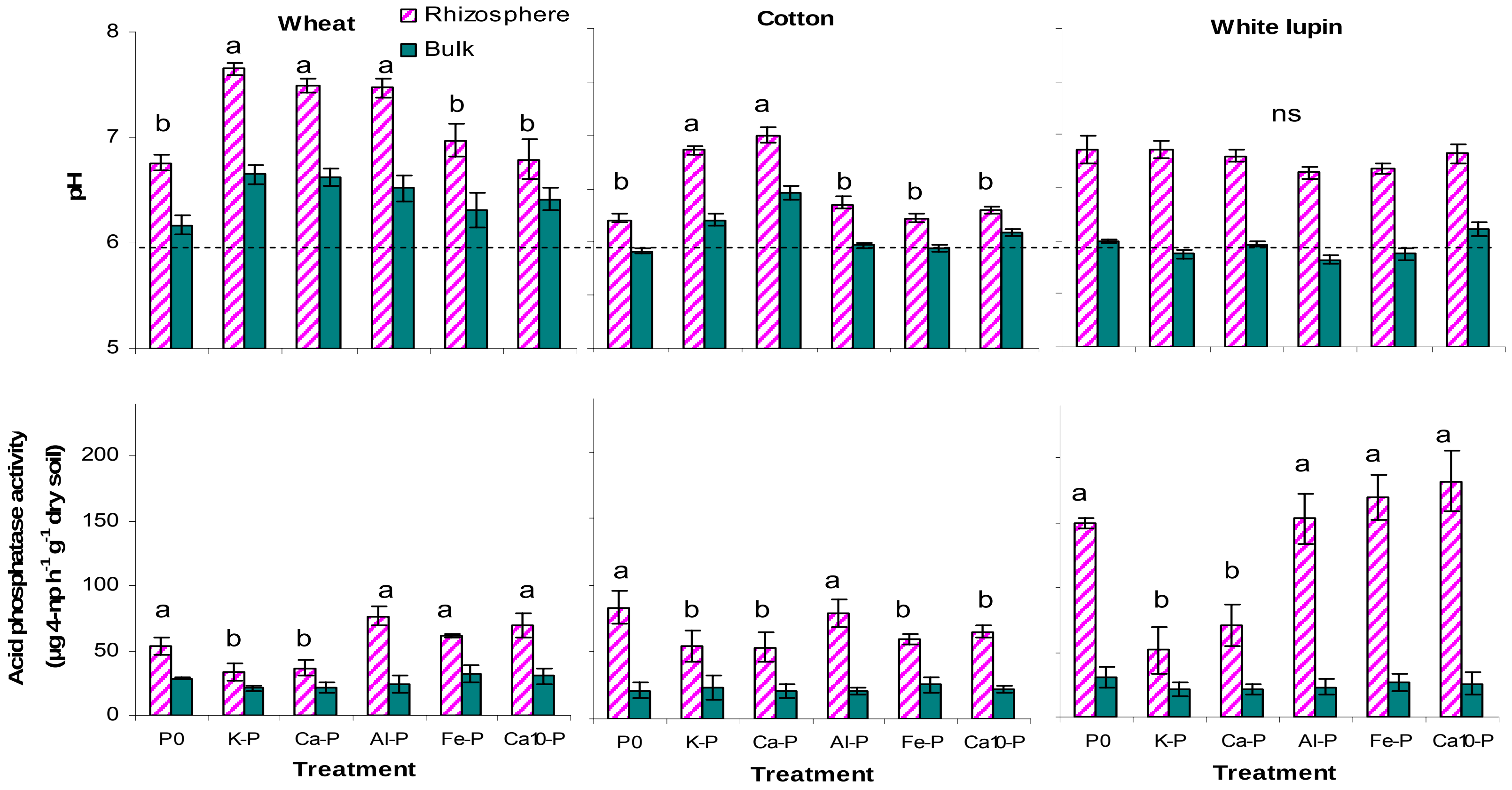


Figure 4. 3. The pH and acid phosphatase activity of rhizosphere and bulk sand of wheat, cotton and white lupin supplied with zero P (P0), and 40 mg kg^{-1} P as KH_2PO_4 (K-P), $Ca(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P), and hydroxyapatite (Ca10-P). Error bars indicate standard error (n = 3). The values not sharing the same letter indicate significant differences between the P treatments within rhizosphere of a species according to the Duncan's multiple range tests (*P* = 0.05). "ns" represents no significant difference. The dotted line represents the original pH of the sand medium.

Table 4. 1. Concentration of K, Ca, Fe and Al in shoot and root of wheat, cotton and white lupin supplied with zero P (P0), 40 mg kg^{-1} P as KH_2PO_4 (K-P), $Ca(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P), and hydroxyapatite (Ca10-P). The values not sharing the same letter indicate significant differences within the column according to the Duncan's multiple range tests (*P* = 0.05).

Crop species	Treatment	K concentration		Ca concentration		Fe concentration		Al concentration	
		Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
		(mg g^{-1})		(mg g^{-1})		(mg g^{-1})		(mg g^{-1})	
Wheat	P0	46.9 a	21.6 b	7.3 c	3.4 d	0.075 b	2.90 a	0.087 a	2.35 a
	K-P	50.1 a	19.4 b	6.4 c	7.7 b	0.089 b	3.20 a	0.060 ab	1.74 b
	Ca-P	49.3 a	18.8 b	6.9 c	9.2 b	0.087 b	2.77 a	0.068 ab	1.97 b
	Al-P	48.9 a	18.1 b	7.4 c	5.9 c	0.084 b	2.88 a	0.059 ab	2.48 a
	Fe-P	50.5 a	23.6 b	7.7 c	3.5 d	0.098 b	2.74 a	0.077 ab	2.56 a
	Ca10-P	50.8 a	22.2 b	7.6 c	4.0 d	0.082 b	2.65 a	0.069 ab	2.48 a
Cotton	P0	28.5 b	17.3 b	18.3 b	6.9 b	0.103 b	1.24 b	0.087 a	0.98 c
	K-P	37.0 b	20.6 b	20.6 b	7.6 b	0.092 b	1.27 b	0.056 b	0.61 c
	Ca-P	33.8 b	20.7 b	22.6 ab	9.1 b	0.087 b	1.20 b	0.056 b	0.58 c
	Al-P	32.6 b	18.9 b	18.7 b	6.6 b	0.101 b	1.02 b	0.080 ab	0.86 c
	Fe-P	30.4 b	18.3 b	18.0 b	6.5 b	0.131 b	1.12 b	0.071 ab	0.85 c
	Ca10-P	32.2 b	19.1 b	20.0 b	6.4 b	0.095 b	1.00 b	0.068 ab	0.81 c
White lupin	P0	31.6 b	35.8 a	17.2 b	13.0 a	0.373 a	2.48 a	0.091 a	0.58 c
	K-P	39.2 b	38.3 a	20.9 b	13.8 a	0.387 a	3.14 a	0.070 ab	0.63 c
	Ca-P	31.6 b	32.4 a	23.2 a	16.3 a	0.336 a	2.52 a	0.069 ab	0.63 c
	Al-P	32.4 b	33.9 a	18.7 b	15.4 a	0.388 a	2.64 a	0.068 ab	0.59 c
	Fe-P	33.3 b	36.2 a	17.6 b	16.1 a	0.356 a	2.92 a	0.072 ab	0.59 c
	Ca10-P	31.6 b	35.9 a	17.4 b	14.8 a	0.333 a	2.46 a	0.067 ab	0.52 c

4.3.4. Rhizosphere pH, acid phosphatase, exchangeable Al and available P

Irrespective of P source or plant species, pH was invariably higher in the rhizosphere than in the bulk sand (Figure 4.3). For wheat, rhizosphere pH was higher when supplied with soluble P sources and Al-P. Similarly, the rhizosphere pH of cotton was highest when supplied with soluble sources also. The increase in the rhizosphere pH did not vary among the P treatments for white lupin. Bulk soil pH was increased in all P sources for wheat and in soluble P sources for cotton.

The activity of acid phosphatases increased in the rhizosphere of all species when compared with the bulk soil (Figure 4.3). The activity was 20-30% higher in wheat, and 100-150% higher in white lupin when P was not applied or applied with the sparingly soluble P than when soluble P was applied. In comparison, only P0 and Al-P resulted in an increased acid phosphatase activity in the rhizosphere of cotton.

When P was applied as sparingly soluble P sources, available P in the rhizosphere did not vary among the treatments and species (Figure 4.4). Species supplied with soluble P sources had available P of more than 20 mg kg^{-1} in the rhizosphere at harvesting. There was no difference among the P treatments in exchangeable Al in the rhizosphere of cotton and white lupin (Figure 4.4). However, exchangeable Al in the rhizosphere sand of wheat increased from less than 0.1 mg kg^{-1} in the zero P treatment to 0.8, 0.6 and 1.1 mg kg^{-1} in the treatments of K-P, Ca-P and Al-P, respectively.

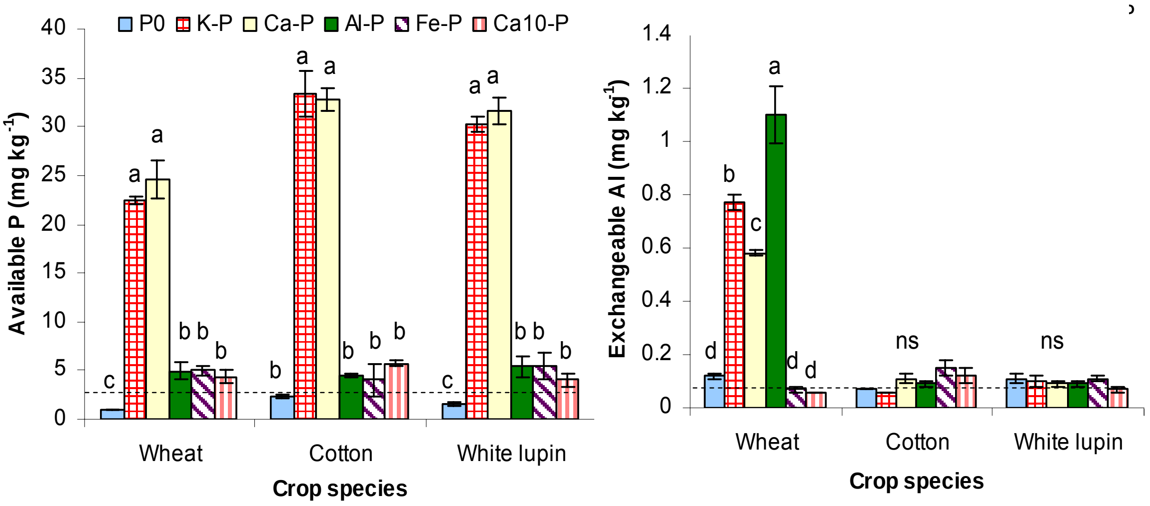


Figure 4. 4. Available P and exchangeable Al in the rhizosphere soil of wheat, cotton and white lupin supplied with zero P (P0), and 40 mg kg^{-1} P as KH_2PO_4 (K-P), $Ca(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P), and hydroxyapatite (Ca10-P). Error bars indicate the standard error (n = 3). The values not sharing the same letter indicate significant differences between the P treatments within a species according to the Duncan's multiple range tests (*P* = 0.05). "ns" represents no significant difference. The dotted line represents the original available P and exchangeable Al of the original sand medium.

4.4. Discussion

This study revealed, for the first time, that cotton is inefficient in accessing P from sparingly soluble P sources. Although wheat is considered to be a P-inefficient species, it was able to better utilize P from Al-P than was white lupin, a P-efficient species. The following discussion will focus on these differential species responses to sparingly soluble P sources and why these results were observed.

Among all the P sources tested, cotton accessed little P from Al-P and was completely unable to use Fe-P and Ca10-P. Physiological traits such as release of carboxylates which would allow plants to access sparingly soluble forms of P were not detected for the cotton plant under P-deficient conditions (Chapter 3). The less availability of Ca10-P to cotton was confirmed by the negligible depletion of acid-extractable P (mainly P bound with Ca) pool in the rhizosphere soil of cotton (Chapter 3). Nevertheless, Dorahy *et al.* (2004) found that P uptake of cotton at early flowering was more strongly correlated with the Al- and Fe-P fractions than Ca-P fraction in alkaline soils in eastern Australia**.** It is possible that Al-P and Fe-P fractions in this case mainly represent P being weakly adsorbed by Al and Fe-oxides. Precipitation of P with Al and Fe rarely occurs in alkaline soils (Lindsay *et al.* 1962). Thus, under field condition, low responsiveness of cotton to P fertilizers was not likely to be attributed to the efficient P utilization from stable inorganic P pools in the soils. Other factors, such as surface-drought induced decrease in P availability, or subsoil root P exploration, might account for the less utilization of P fertilizer by cotton.

The observed pH increase in the rhizosphere, when compared with bulk sand, could be due to the excess uptake of anions, mainly NO_3^-, over cations and subsequently release of OH^- ions from roots (Hinsinger *et al.* 2003). Higher rhizosphere pH was invariably detected for the treatments that achieved higher biomass response. This is attributed to the more NO_3^- assimilation under conditions of better shoot and root growth with sufficient P supply. Since the mobilization of P from Ca10-P was generally associated with the acidification of the rhizosphere (Bertrand *et al.* 1999; Hinsinger and Gilkes 1996), dissolution of P from Ca10-P could be greatly hampered by the release of OH^- ions and thus was less effective to all species in this study. Nevertheless, the increased rhizosphere pH here contradicts with the previously detected decrease in the rhizosphere pH using a rhizobox technique (Chapter 3). Since both NO_3-N and soil N acted as the main N sources in the previous rhizobox experiment, decreased pH possibly indicate less contribution of NO_3-N source to the total plant N uptake. However, the dramatic increase in rhizosphere pH in this study is attributed to the poor pH buffering of coarse river sand, and should not be used as an indication under field conditions.

The exceptional ability of wheat in accessing P from Al-P has been observed previously. Pearse *et al.* (2007; 2006b) demonstrated that wheat was more efficient than white lupin at accessing P from Al-P. When compared with both Fe-P and Ca10-P, Otani and Ae (1996) and Valizadeh *et al.* (2002) also observed greater P uptake from Al-P by wheat. Nevertheless, mechanisms responsible for this efficient Al-P utilization remain unclear and subject to speculation.

For Al-P to be utilized by wheat, it must be dissolved from the solid phase first. In this case, release of OH^- following NO_3^- assimilation would facilitate Al-P ($Ksp=6.3 \times 10^{-19}$) dissolution due to the formation of less soluble $Al(OH)_3$ ($Ksp = 4.6 \times 10^{-33}$). However, if this is the case, cotton and white lupin should be similarly efficient in using Al-P. In addition, drastic pH increase in the rhizosphere of wheat fed with Al-P might indicate that OH^- consumed for Al-P dissolution was less significant. I speculate that mechanisms involved in the efficiency of wheat in utilizing Al-P are independent of increased rhizosphere pH.

On the other hand, the removal of Al^{3+} by complexation reactions with organic acids would promote the dissolution of Al-P. Several studies reported that Al, the counter-ion, added with P could induce a release of di- and tri-carboxylates, e.g citrate or malate, from wheat root due to activation of an anion channel by Al (Lambers *et al.* 2002; Ryan *et al.* 1997). The Al-dependent secretion of carboxylates is widely considered as an important mechanism for Al detoxification (Ryan *et al.* 2001). Nevertheless, Al toxicity is less likely to occur in this study due to the relatively higher rhizosphere pH. Thus, significant carboxylate release from the root of wheat fed with Al-P is doubted. In a similar study, Pearse *et al.* (2006b) found that wheat was superior in using Al-P despite releasing fewer carboxylates into its rhizosphere. Although total carboxylate concentration in the rhizosphere of wheat did not vary among various P sources, a clear shift in malate-citrate composition had been observed with increasingly applied Al-P (Pearse *et al.* 2006b). Thus, the role of carboxylate release from wheat root in mobilizing Al-P is worth further investigation, and may not be disregarded due to its low exudation rate.

The higher accumulation of Al in the root of wheat might also contribute to the dissolution of Al-P. The principle here is similar to that suggested by many other studies with respect to higher uptake of Ca and stimulation of the dissolution of Ca-P sources e.g. rock phosphate (Johnston and Olsen 1972; van Ray and van Diest 1979). At pH above 6.3, soluble Al species in the rhizosphere of wheat exists almost exclusively as $Al(OH)_4^-$ (Davis and Hem 1989; Ma *et al.* 2003). Directly or indirectly, $Al(OH)_4^-$ has to be taken up by wheat to account for the high root Al concentrations measured. Solubility of $Al(OH)_4^-$ increase towards more alkaline conditions (Ma *et al.* 2003), which subsequently explains why increased exchangeable Al was only detected in the rhizosphere of wheat fed with K-P, Ca-P and Al-P which resulted in the highest rhizosphere pH.

Surprisingly, white lupin was not efficient in accessing any sparingly soluble P source in this study. For example, P uptake from Al-P by white lupin was 7 times lower than that for wheat, and Fe-P or Ca10-P was barely utilized, even though observed tissue P concentrations were below the critical values reported for white lupin (2.0 mg g^{-1}) (Li *et al.* 2008). Pearse *et al.* (2007; 2006b) also demonstrated a poor performance of white lupin in accessing sparingly soluble P sources. These authors attributed its inefficiency to the reduced chelating ability of carboxylates by the low rhizosphere pH detected (~pH 4.5) in their study (Pearse *et al.* 2006b). However, in this study, the rhizosphere pH was high (~pH 7) and still white lupin was inefficient in its use of P from sparingly soluble P sources. Possibly, the large seed size of white lupin delayed the onset of P deficiency and so the P acquisition strategies were not displayed due to the short duration of the experiment (Tang *et al.* 2007; Thomson and Bolger 1993). The elevated rhizosphere pH relative to bulk soil, in contrast to normally reported rhizosphere acidification by white lupin (Hinsinger and Gilkes 1995; Dinkelaker *et al.* 1989), might indicate that carboxylates exuded from root of white lupin in this study are extremely low or negligible. In addition, the increasing soil pH could hinder the normal functioning of the white lupin which is sensitive to alkaline pH (Tang *et al.* 1993).

The large increase in acid phosphatase activity in the rhizosphere of white lupin supplied with less soluble sources indicates that part of the efficiency of white lupin in acquiring P under field conditions is related to the mobilization of organic P sources. The potential benefit of increased acid phosphatase in the rhizosphere of all species in acquiring soil organic P could be substantial (George *et al.* 2006), although its direct effect could not be evaluated in this study due to low organic P in the sand medium.

In conclusion, species differed greatly in their ability to take up P from sparingly soluble P forms Al-P, Fe-P and Ca10-P. Cotton was inefficient in acquiring P from all of these P sources. Therefore, under field conditions, P acquisition from stable P pools was not likely to be responsible for the low responsiveness of cotton to P fertilizer. Wheat displayed an outstanding ability to utilize P from Al-P while the reported high P acquisition efficiency of white lupin was not detected in this study. Further work is necessary to identify possible mechanisms involved into efficient Al-P utilization by wheat roots.

Chapter 5: Phosphorus dynamic and fate of different P sources in three contrasting soil types as determined by P fractionation and isotopic labeling techniques

5.1. Introduction

The phosphorus (P) availability in acid and alkaline soil is predominantly controlled by mineral components such as aluminium (Al) and iron (Fe) oxides and calcium (Ca) carbonates (Chang and Jackson 1958; Matar *et al.* 1992). Freshly applied fertilizer P can react with these components and form less soluble mineral P compounds (Lindsay *et al.* 1962). Attention has been drawn to the potential role of these less soluble mineral P compounds on plant P nutrition due to observations that some plant species demonstrate specific ability to mobilize these P pools in soil (Ae *et al.* 1990a; 1990b; van Ray and van Diest 1979).

Many studies have sought to identify differences in the ability of species in utilizing sparingly soluble P sources by using sand culture and synthetic chemical P compounds such as $AlPO_4$, $FePO_4$ and hydroxyapatite to mimic soil P minerals (Pearse *et al.* 2007; Pearse *et al.* 2006b; Tang *et al.* 2007; Zhang *et al.* 1997; Chapter 4). However, sand varies significantly from soil, and may result in different root growth and root structure, and perhaps rhizosphere processes in plants. The use of soil culture in such studies is likely to result in a more accurate evaluation of the availability of P sources to plant. In this respect, understanding the fate of these sparingly soluble P sources in the soil would provide an insight into their relatively availability to different species in the following study (Chapter 6).

Sequential chemical extraction procedures are widely used to assess P availability and P dynamics in the soil. The underlying assumption is that readily available soil P is removed first with mild extractants while stable P can only be extracted with stronger alkalis and acids. The fate of different P sources in the soil can be indicated by the net change in each soil P pool, although net change cannot differentiate between applied P sources- and soil-derived P. Zapata (1990) suggests that the use of ^{32}P or ^{33}P labeled P source would be a more accurate and sensitive method to determine the fate of these P sources in the soil. However, the labeling of sparingly soluble P sources, such as hydroxyapatite by direct thermal neutron irradiation (Mackenzie and Borland 1952) is difficult. An alternative approach would be to label the soil P with ^{32}P and ^{33}P, and use the indirect reverse dilution techniques (Zapata and Axmann 1995). The combination of isotopic labeling techniques and sequential fractionation schemes should provide comprehensive information on the fate of these sparingly soluble P sources in the soil. This chapter aims to identify the fate of different sparingly soluble P sources, including $AlPO_4$, $FePO_4$ and hydroxyapatite, in three soil types with differing soil texture and chemical properties.

5.2. Materials and methods

5.2.1. Soils

Soils used in this study were classified as a Red Vertosol, Red Ferrosol and Calcic Calcarosol (Isbell 2002). The Red Vertosol was collected along the fence line of a long-term experimental site at Horsham, Victoria, Australia (36° 40’ S, 142° 18’ E). The Red Ferrosol was collected from bush land at Kinglake, Victoria, Australia (37° 31’ S, 145° 20’ E). The Calcarosol was sampled from a virgin native mallee scrub at the Mallee Research Station, Walpeup, Victoria, Australia (35° 08’ S, 142° 01’ E). All soils were sampled at depth 5-15 cm (Table 5.1). Olsen P (0.5 M $NaHCO_3$) and exchangeable Al (1 M KCl) was measured for all soil types according to the method described by Olsen and Dean (1965) and Conyers *et al.* (1991), respectively. The pH was measured using a Thermo Orion 720 pH meter after extraction in 0.01 *M* $CaCl_2$ solution (1:5 w/v soil: solution ratio) by shaking for 17 h on an end-over-end shaker. Soil organic carbon (OC) and total nitrogen (N) were analyzed using an Elemetar Vario EL III (Elementar Analysensysteme GmbH, Germany).

5.2.2. Soil labeling

The soils were labeled by weighing out 0.1 kg of sieved soil (<2 mm) and spreading it on a plastic container. A 10 ml solution containing 0.6 MBq ^{32}P (6 MBq kg^{-1} soil) was distributed evenly onto the soil surface which were then mixed thoroughly with a spatula. After labeling, additional water was added to soil to achieve 70% of field capacity. All containers were then sealed and incubated in the growth cabinet at 25 °C for three weeks to allow the ^{32}P to equilibrate with the native soil P.

5.2.3. Soil incubation

Five P treatments including zero P (P0), four P sources as $Ca(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P), and hydroxyaptite (Ca10-P) were applied to each of the three soils at the rate of 150 mg P kg^{-1}. These high rates, matching the total soil P values for these soils, were used to ensure that any dilution of soil P fractions could be detected easily. There were three replicates for each treatment. All containers were unsealed and air-dried for one day following three weeks incubation. Phosphorus sources were applied in the soil as a 10 ml suspension to enable complete mixing whilst minimizing dust generation. All trays were raised to field capacity using deionised water, sealed and incubated in a growth cabinet at constant 25 °C and sub-sampled at days 1, 14 and 42.

5.2.4. Soil P fractionation

A modified version of the Hedley's P fractionation scheme was used to sequentially fraction soil (Guppy *et al.* 2000). Briefly, 1.0 g of air-dried soil was weighed and extracted with 30 ml of 0.5 M $NaHCO_3$, 0.1 M NaOH and 1 M HCl sequentially. Residual P was analyzed using a concentrated H_2SO_4:$HClO_4$ (20:1) acid digestion. The inorganic P concentration in the extracts was determined colorimetrically using malachite green (Motomizu *et al.* 1980). Aliquots of the bicarbonate and hydroxide extracts were digested in an autoclave at 103 kPa, 120°C for 1 h using acid potassium persulfate (Rowland and Haygarth 1997) and analyzed for total P colorimetrically. Organic P was then calculated as the difference between total and inorganic P in both fractions.

The radioactivity in each fraction was determined with a liquid scintillation counter (Tri-card 2000 CA) by adding 10 ml of scintillation cocktail (ULTIMA Gold XR) to 2 ml of extracts. The values were corrected for decay back to the day of first sampling (Day 1). The quenching effect was corrected for each soil type and fraction by adding defined ^{32}P spikes. A simple separation of $^{32}P_i$ and $^{32}P_o$ in the NaOH-extract was undertaken with an acidification-centrifugation step. It is possible that some P_i may precipitate along with organic matter, while some organic materials (e.g. fulvic acids) remain in the supernatant (Tiessen and Moir 1993).

5.2.5. Extraction of microbial P

In order to identify the role of microbial activity in the changes in NaOH-P_o pool following the addition of different P sources, a second set of unlabelled treatment soils was established. These soils were subsampled immediately after mixing with P sources and after one day (24 hours) of incubation. Both microbial P and NaOH-P_o were determined for these samples. Microbial P was extracted using a chloroform/bicarbonate technique (Hedley and Stewart 1982), followed by NaOH extraction and NaOH-P_o determination on non-fumigated samples.

5.2.6. Calculation and statistical analysis

The ^{32}P reverse dilution technique requires a standard treatment (P0) as a reference. Thus, the change in each soil P fraction originating from P sources will be determined by comparing the isotopic composition of each fraction in the presence of P sources with that in the absence of P sources (P0) (Zapata and Axmann 1995).

$$\text{PdfS \%} = 100[1-(\frac{specific\ activity\ of\ each\ fraction\ in\ presence\ of\ P\ sources}{specific\ activity\ of\ each\ fraction\ in\ absence\ of\ P\ sources})]$$

$$\text{PrecF \%} = 100\left(\frac{P\ concentration\ in\ each\ \ fraction\,(mg/kg)*PdfS\%}{150\,(mg\,P/kg)}\right)$$

Where PdfS % is the percentage of P in the each fraction derived from the unlabelled P sources, and PrecF % is the recovery of P sources into each soil P fractions.

5.3. Results

5.3.1. Soil properties and P sources fractionation

The three soil types differed greatly in pH, exchangeable Al and texture (Table 5.1). The Red Ferrosol was acidic (pH-4.3) whilst the Vertosol (pH-7.2) and Calcarosol (pH-7.0) were closer to neutral. Exchangeable Al in the Ferrosol was 103 mg kg^{-1}, in contrast to only 0.20 and 0.14 mg kg^{-1} in Vertosol and Calcarosol, respectively. The Vertosol and Ferrosol had clay contents up to 45%, whilst the clay content of the Calcarosol was only 8%. Total soil P was highest on Calcarosol (187 mg kg^{-1}), followed by Ferrosol (167 mg kg^{-1}) and Vertosol (98 mg kg^{-1}). Soil P fractions extracted by the first three extractants, e.g. $NaHCO_3$, NaOH and HCl, were less than 12 mg kg^{-1} on all soils (Table 5.1). The r/R values represent the fraction of the total radioactivity added that remains in the soil solution after 21 days of isotopic exchange, which provided an indication for the P-sorption capacity of the three soils. The Ferrosol had the highest capacity for P sorption, followed by the Vertosol, and Calcarosol.

Fractionation of all P sources, including soluble and sparingly soluble P, showed that 80% of Ca-P was extracted into the $NaHCO_3$-P fraction while the rest (~ 20%) was extracted by 1M HCl (Table 5.2). Most of the Fe-P (96%) and Ca10-P (98%) was recovered in the NaOH-P and HCl-P fractions, respectively. About 87% of the Al-P was extractable by 0.1 M NaOH while nearly 10% remained and was extracted in the residual pool. Negligible (< 2.5%) amounts of P from sparingly soluble P sources were recovered in the labile, $NaHCO_3$-P pool.

5.3.2. Change in soil P fractions following addition of different P sources

One day after addition of soluble Ca-P, the $NaHCO_3$-extractable inorganic P ($NaHCO_3$-P_i) pool increased by ~85 and ~110 mg kg^{-1} for the Vertosol and Calcarosol respectively (Figure 5.1). The NaOH-extractable inorganic P (NaOH-P_i) acted as the second major sink for Ca-P added into Vertosol and Calcarosol. For both soils, the HCl-extractable P (HCl-P) was less affected by the addition of soluble Ca-P. The Ca10-P was recovered mainly in the HCl-P, in contrast to the increase in NaOH-P_i following the addition of Al-P on both Vertosol and Calcarosol. The residual-P pools in the Vertosol and Calcarosol were increased by about 70 and 55 mg kg^{-1}, and 40 and 45 mg kg^{-1} due to the addition of Ca10-P and Al-P respectively. For the Ferrosol, both

$NaOH\text{-}P_i$ and residual-P acted as the main sinks for all P sources except Fe-P (Figure 5.1). The Fe-P was invariably recovered into the $NaOH\text{-}P_i$ pool on all soils. With prolonged incubation, Ca-P recovered into $NaHCO_3\text{-}P_i$ pool decreased, in contrast to an increased residual-P in the Vertosol and Ferrosol, and $NaOH\text{-}P_i$ and HCl-P in the calcarosol. Unlike other P sources, Al-P extracted into soil $NaHCO_3\text{-}P_i$ pool increased from 4.4 to 24 mg kg^{-1} in the Vertosol, and from 2.0 to 16 mg kg^{-1} in the Calcarosol after 42 days incubation.

The concentration of $NaHCO_3$-extractable organic P ($NaHCO_3\text{-}P_o$) showed little difference among the treatments (data not shown). However, a significant increase in NaOH-extractable organic P ($NaOH\text{-}P_o$) pool (10~15 mg kg^{-1}) was detected in all soils following the addition of Ca-P and Al-P (Figure 5.1), which was further confirmed by a second experiment without ^{32}P labeling irrespective of whether the soil was sampled immediately after mixing or had been incubated 24 hours with P sources (Table 5.3). For all soils, $NaOH\text{-}P_o$ pool decreased gradually with prolonged incubation (Figure 5.1). The total microbial P content extracted in 0.5 M $NaHCO_3$ was not affected by addition of any sparingly soluble P source, but increased from 4.2, 1.8 and 3.3 mg kg^{-1} to 9.5, 6.0 and 8.5 mg kg^{-1} for Vertosol, Ferrosol and Calcarosol, respectively, following immediate addition of Ca-P (Table 5.3). With 24 hours incubation, microbial P increased by a further ~80% only in the Vertosol.

5.3.3. Recovery of P sources into each soil P fraction (PrecF %)

In the Vertosol and Calcarosol, Ca-P mainly recovered in the $NaHCO_3$-P and $NaOH\text{-}P_i$ pool and made up to nearly 90% and 80% of total recovery, respectively at day 1 (Table 5.4). $NaOH\text{-}P_i$ was the main sink for both Al-P (48~64%) and Fe-P (~95%), although some of the Al-P may have been extracted subsequently in the residual pool (~40% for Vertosol and ~30% for Calcarosol). About 50% and 65% of Ca10-P was recovered in HCl-P pool on Vertosol and Calcarosol, respectively; the remainder was extracted in the residual pool. In Ferrosol, all P sources were predominantly recovered into $NaOH\text{-}P_i$ pool and the maximum recovery was from Fe-P (nearly 100%), followed by Al-P (~65%), Ca-P (~60%) and Ca10-P (~60%). At day 1, the Ca-P and Ca10-P showed ~8% of recovery into the $NaHCO_3$-P pool in Ferrosol. Around 7-9% of the Ca-P and Al-P was recovered in the $NaOH\text{-}P_o$ pool on all soil types on day 1 (Table 5.4).

With prolonged incubation, the recovery of Ca-P into $NaHCO_3$-P decreased from 58% to 48% in the Vertosol, from 9% to 0% in the Ferrosol and from 66% to 60% in the Calcarosol. Increased recovery of Ca-P into $NaOH\text{-}P_i$ and HCl-P pool was observed on Calcarosol. Ca-P and Al-P recovered into $NaOH\text{-}P_o$ also decreased gradually from day 1 to day 42. However, the recovery of Al-P into $NaHCO_3\text{-}P_i$ pool increased from 2% to 14% in Vertosol and 4% to 10% in Calcarosol (Table 5.4). Fe-P and Ca10-P recovered into $NaOH\text{-}P_i$ and HCl-P pool of Vertosol and Calcarosol

Table 5. 1. Phosphorus fractions and selected properties of three soil types used in the experiment.

	Soil P fractions					Total P	Total C	Total N	pH	Exchangeable Al	Clay	$r_{3\ weeks}/R$*
Soil types	NaHCO$_3$		NaOH		HCl-P							
	P_i	P_o	P_i	P_o								
	mg kg^{-1}						g kg^{-1}	g kg^{-1}	(0.01 M CaCl$_2$)	mg kg^{-1}	(%)	
Vertosol	5.6	4.8	4.1	6.3	4.2	98	8.4	0.82	7.2	0.20	45	0.25
Ferrosol	4.4	2.2	11.1	3.0	2.0	167	2.9	0.33	4.3	103	45	0.03
Calcarosol	7.7	5.5	6.0	4.4	8.9	187	5.1	0.42	7.0	0.14	8	0.40

* The fraction of total radioactivity added (R) that remained in the soil solution (r) after 3 weeks of isotopic exchange.

Table 5. 2. The recovery of P source into each P fraction. Four P sources include $Ca(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P), and hydroxyapatite (Ca10-P). Standard errors are shown in parentheses.

	P recovery into each fraction (%)				
P sources	NaHCO$_3$-P$_i$	NaOH-P$_i$	HCl-P	Residual	Sum
Ca-P	79.6 (0.7)	0.9 (0.1)	21.9 (0.3)	0	101.0
Al-P	0.2 (0)	87.4 (0.5)	0.2 (0.1)	10.6 (0)	98.4
Fe-P	1.8 (0)	96.4 (0.7)	2.9 (0)	0	100.1
Ca10-P	2.1 (0)	0.1 (0)	97.8 (2.0)	0	102.4

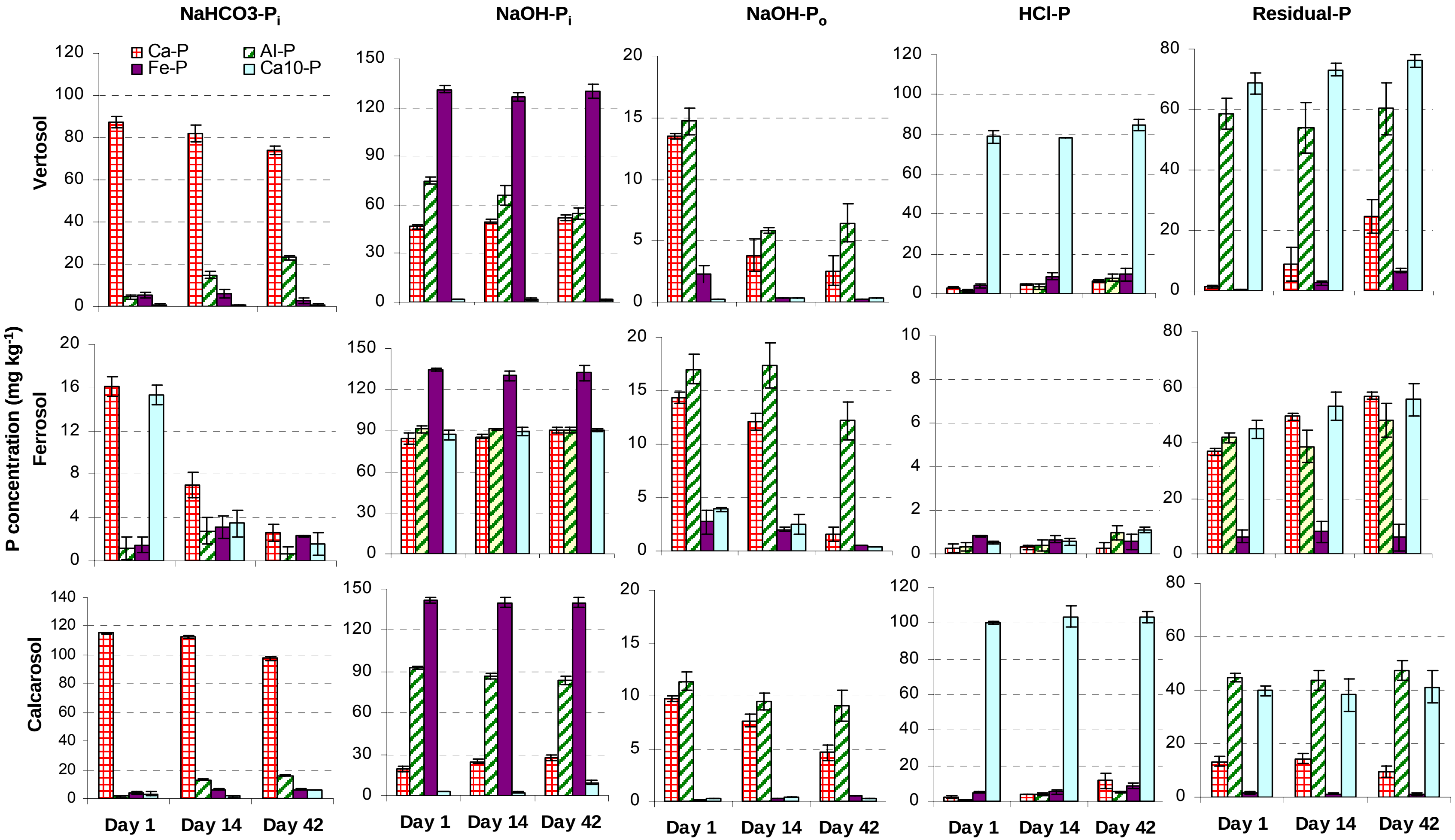


Figure 5. 1. Net increase in each P fraction in Vertosol, Ferrosol and Calcarosol after 1, 14 and 42 days of incubation with P sources as $Ca(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P), and hydroxyapatite (Ca10-P). Vertical bars represent the standard error of the means of 3 replicates.

Table 5. 3. Changes in the microbial P and NaOH-Po in Vertosol, Ferrosol and Calcarosol incubated with zero P (P0), and four P sources as $Ca(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P), and hydroxyapatite (Ca10-P) for 0 and 24 hours. Within the rows, means not sharing the same letter indicate significant difference according to the Duncan's multiple range test (P = 0.05).

Treatment		P0		Ca-P		Al-P		Fe-P		Ca10-P	
Incubation time (hour)		0	24	0	24	0	24	0	24	0	24
		mg P kg^{-1} soil									
Vertosol	Microbial P*	4.2c	3.0c	9.5b	17.5a	3.8c	3.5c	3.8c	3.0c	3.0c	2.4c
	NaOH-P_o	6.6b	7.2b	18.5a	19.4a	23.5a	20.6a	6.2b	8.8b	5.2b	6.9b
Ferrosol	Microbial P	1.8b	1.5b	6.0a	6.5a	2.2b	2.0b	1.5b	1.3b	1.8b	2.0b
	NaOH-P_o	5.2b	5.6b	18.0a	18.0a	21.5a	21.0a	5.5b	4.7b	4.5b	8.9b
Calcarosol	Microbial P	3.3b	2.3b	8.5a	11.0a	3.3b	3.0b	2.5b	2.3b	3.3b	2.8b
	NaOH-P_o	9.6b	8.0b	20.6a	19.3a	23.7a	22.8a	10.8b	8.8b	11.0b	8.2b

* Total microbial P content is calculated using a correction factor of 0.4 (Gijsman *et al*., 1997; Lukito *et al*., 1998).

Table 5. 4. The recovery of applied P sources as % of total added P (150 mg kg^{-1}) (PrecF %) in each P fraction of Vertosol, Ferrosol and Calcarosol after 1 day, 14 days and 42 days' incubation. Four P sources include $Ca(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P), and hydroxyapatite (Ca10-P). Standard errors are shown in parentheses.

		P recovery into each fraction (%)														
		Day 1					Day 14					Day 42				
Soil types	Treatment	$NaHCO_3$-P	NaOH-P		HCl-P	Sum	$NaHCO_3$-P	NaOH-P		HCl-P	Sum	$NaHCO_3$-P	NaOH-P		HCl-P	Sum
			Pi	Po				Pi	Po				Pi	Po		
Vertosol	Ca-P	58 (3)	30 (1)	8 (2)	3 (0)	99	50 (2)	34 (1)	3 (0)	3 (0)	90	48 (1)	32 (2)	2 (0)	4 (0)	84
	Al-P	2 (0)	48 (2)	9 (1)	1 (0)	60	12 (1)	45 (3)	4 (1)	1 (0)	62	14 (0)	36 (2)	3 (0)	5 (1)	58
	Fe-P	1 (0)	95 (7)	0 (0)	3 (0)	99	1 (0)	96 (2)	0 (0)	3 (1)	100	0 (0)	96 (2)	0 (0)	3 (2)	99
	Ca10-P	0 (0)	1 (0)	0 (0)	51 (0)	52	0 (0)	2 (0)	0 (0)	50 (1)	52	0 (0)	0 (0)	0 (0)	53 (3)	53
Ferrosol	Ca-P	9 (0)	59 (2)	7 (2)	0 (0)	75	2 (0)	58 (1)	5 (0)	0 (0)	65	0 (0)	62 (1)	2 (0)	0 (0)	64
	Al-P	0 (0)	64 (1)	8 (3)	0 (0)	72	0 (0)	65 (3)	6 (2)	0 (0)	71	0 (0)	66 (3)	4 (2)	0 (0)	70
	Fe-P	0 (0)	99 (1)	0 (0)	0 (0)	99	0 (0)	98 (2)	0 (0)	0 (0)	98	0 (0)	101 (1)	0 (0)	0 (0)	101
	Ca10-P	8 (0)	60 (2)	0 (0)	0 (0)	68	0 (0)	61 (2)	0 (0)	0 (0)	61	0 (0)	61 (0)	0 (0)	0 (0)	61
Calcarosol	Ca-P	66 (0)	17 (0)	7 (1)	2 (0)	92	64 (1)	22 (0)	6 (1)	2 (0)	94	60 (1)	22 (1)	3 (0)	8 (2)	93
	Al-P	4 (0)	59 (1)	8 (0)	1 (0)	72	9 (1)	57 (1)	6 (1)	3 (0)	75	10 (0)	54 (2)	4 (1)	4 (0)	72
	Fe-P	2 (0)	95 (2)	0 (0)	3 (0)	100	3 (0)	94 (2)	0 (0)	3 (0)	100	2 (1)	96 (1)	0 (0)	3 (1)	101
	Ca10-P	4 (0)	2 (0)	0 (0)	64 (1)	70	2 (0)	3 (0)	0 (0)	65 (3)	70	2 (0)	3 (0)	0 (0)	66 (3)	71

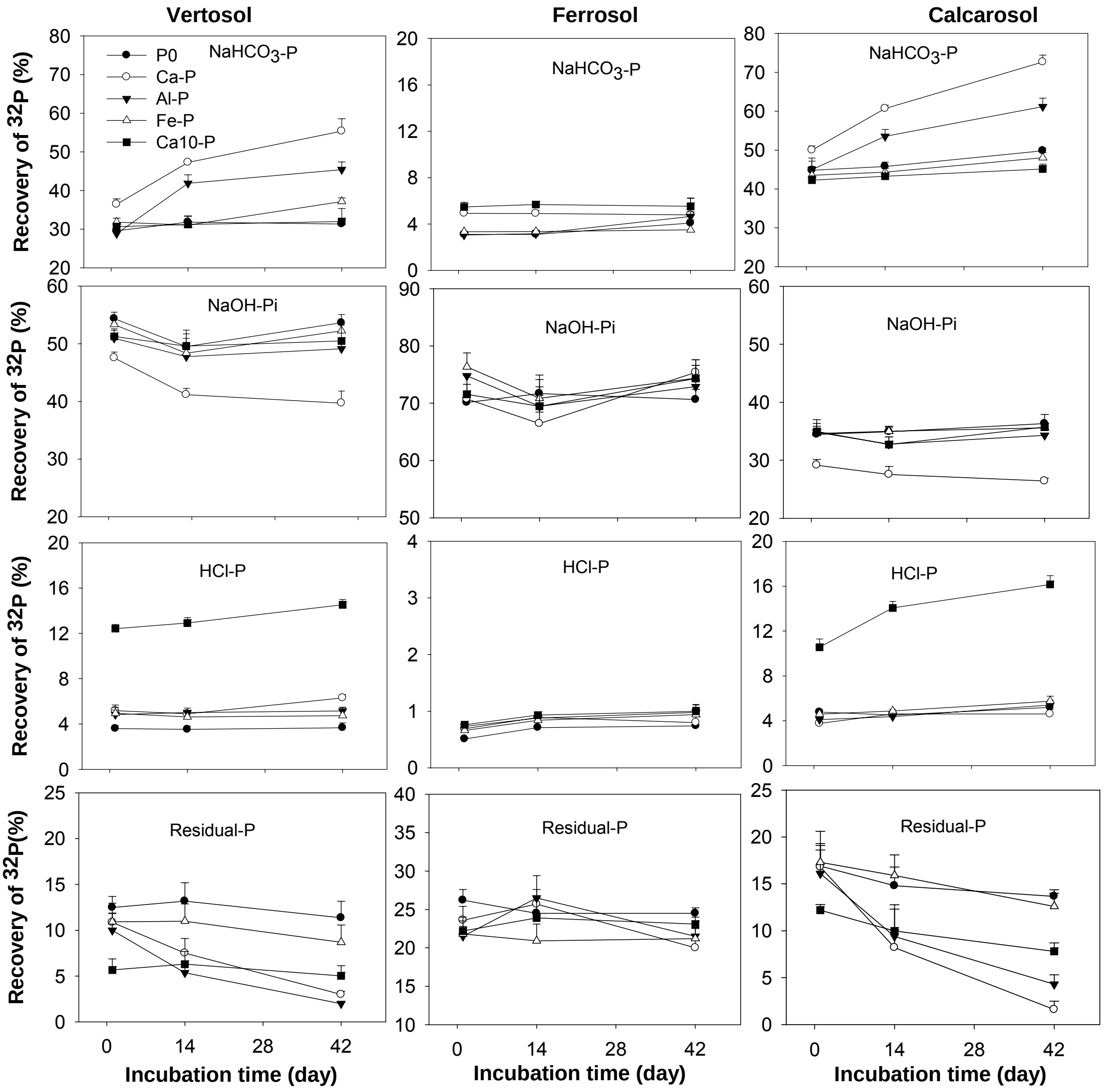


Figure 5. 2. The recovery of ^{32}P into soil P fractions of Vertosol, Ferrosol and Calcarosol after 1 day, 14 days and 42 days incubation. P0 represent zero P addition. Four P sources include $Ca(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P), $FePO_4$ (Fe-P), and hydroxyapatite (Ca10-P). Vertical bars represent the standard error of the means of 3 replicates. Note: recovery of ^{32}P in residual pool was calculated as the difference between total applied ^{32}P (100%) and sum of ^{32}P recovered into $NaHCO_3$-P, NaOH-P_i, NaOH-P_o and HCl-P pools.

remained constant during the whole incubation period. The recovery of P sources in the different pools in the Ferrosol was less influenced by the prolonged incubation time.

5.3.4. Recovery of ^{32}P into each fraction

The recovery of ^{32}P into each soil fraction varied greatly among the soil types (Figure 5.2). ^{32}P recovered into residual pool was calculated as the difference between total applied ^{32}P (100%) and the sum of ^{32}P recovered into $NaHCO_3$-P, NaOH-P_i, NaOH-P_o and HCl-P pools. At day 1, about

30% of ^{32}P applied to the Vertosol was extracted in the NaHCO$_3$-P pool while 50% in the NaOH-P$_i$ pool, when averaged over the P treatments. The opposite occurred in the Calcarosol. With the Ferrosol, about 70% and 20% of ^{32}P was recovered in NaOH-P$_i$ and the residual pools, respectively. The recovery of ^{32}P in the HCl-P pool was generally less than 4%, but increased up to 12% when Ca10-P was applied in the Vertosol and Calcarosol.

Following the addition of Ca-P or Al-P, ^{32}P recovered in the NaHCO$_3$-P fraction in both the Vertosol and Calcarosol increased from day 1 to day 42, in contrast to the decreased ^{32}P recovery in the residual-P pools and/or NaOH-P$_i$ (Figure 5.2). In the Ferrosol, the recovery of ^{32}P into each fraction was less affected by both P treatments and incubation time. The movement of ^{32}P into NaOH-P$_o$ pool was less than 1% in all soils and the difference between the treatments was barely detectable (Data not shown).

5.4. Discussion

5.4.1. Fate of P sources on different soil types

The direct fractionation of all P sources supports theoretical discrimination of soil P pools by different extractants (Table 5.2). For example, NaOH extract P mainly bound with Al and Fe while HCl- released P mainly precipitated with Ca. Considering that HPO_4^{2-} was the major species at pH above 7.2 (Lindsay 1979), less soluble P species than CaHPO$_4$ might be formed during NaHCO$_3$ extraction (pH = 8.5). This accounts for the full recovery of applied soluble Ca-P source not occurring until a stronger acid extractant (1M HCl) was used (Table 5.2).

The lower P adsorption capacity of Vertosol and Calcarosol (Table 5.1), compared with Ferrosol, resulted in soluble Ca-P being predominantly recovered in the NaHCO$_3$-P$_i$ pool. The elevated NaOH-P$_i$ pools following Ca-P addition suggest that part of soluble Ca-P was either precipitated with free Al and Fe or adsorbed by metal oxides. Adsorption by Al and Fe oxides should be the dominant mechanism controlling P dynamics in these two soils considering that exchangeable Al in the original soils was too low to form precipitates with P. This is in agreement with previous findings that even small amount of Al- and Fe-oxides contributed to the apparent P sorption behaviours of a range of neutral-alkaline soils, including Vertosol and Calcarosol (Beauchemin and Simard 1999; Bertrand *et al.* 2003; Tran and Giroux 1987). By using X-Ray Absorption Near-Edge Spectroscopy, Beauchemin *et al.* (2003) provided spectroscopic evidence for the occurrence of P species adsorbed on Al- and Fe-oxides surface for either high clay soil or soils developed on calcareous material. For sparingly soluble P sources, the prediction of their reaction with the soil is difficult. It is likely that Fe-P sources applied to the Vertosol and Calcarosol, with relatively lower P fixing capacity, were extracted from the soil in the same form as that applied. In contrast to Fe-P, Al-P and Ca10-P might be more reactive and partly reacted with the soil matrix and subsequently recovered into the residual-P pool. Free Ca $^{2+}$ present in Calcarosol can induce further transformation of Ca10-P to more inaccessible fractions.

The fate of P source in the Ferrosol was distinct in that NaOH-P_i pool acted as the main sink for all P sources, including Ca10-P, regardless of their solubility. The Ferrosol are high in Fe and Al oxides which have a high affinity for P (Sanchez 1976). In addition, the low pH of the soil encouraged soluble P applied to the soil solution to react quickly with soluble Al or Fe and form precipitates (Lee *et al.* 1990). Therefore, the low recovery of Ca-P into $NaHCO_3$-P_i pools in Ferrosol was not surprising. It is possible that an adsorption-driven P dissolution mechanism was mainly responsible for the transformation of acid-soluble Ca10-P source into NaOH-P_i pool. The low pH of Ferrosol undoubtedly further facilitated this process by either promoting dissolution or increasing adsorption strength (Giesler *et al.* 2005). Norrish and Rosser (1983) and Smyth and Sanchez (1982) also observed the maximum dissolution of phosphate rock (mainly Ca bond P) in soils with the highest P-sorption capacities. By adding goethite into soil, Kanabo and Gilkes (1987) demonstrated that an enhanced dissolution of phosphate rock was due to the capacity of goethite to absorb phosphate ions from the soil solution. Therefore, it is less likely that Ca-bonded P, e.g. phosphate rock, will be an effective P sources in acid soils with high P-sorption capacity, since the adverse effect of P retention may exceed the beneficial effect of increased dissolution. The dominant solid- phase P species in the Ferrosol, almost without exception, was P associated with Al and Fe oxides.

Since NaOH-P_o was determined by the difference between total NaOH extractable P and NaOH-P_i, the increased NaOH-P_o following Ca-P and Al-P addition might originate from underestimation of NaOH-P_i due to P_i precipitation with organic matter during acidification-centrifugation if P_i concentration in the extracts was higher for these treatments (Magid and Nielsen 1992; Tiessen and Moir 1993). However the high recovery of applied Fe-P sources in the NaOH extract in all soil types, and lack of a corresponding increase in NaOH-P_o suggests change in NaOH-P_o are unlikely to be an experimental artifact.

The increased NaOH-P_o following Ca-P addition was concomitant with an elevated value of microbial P, which suggests rapid incorporation of Ca-P into microbial cell debris that is extractable by NaOH (Waara *et al.* 1993). Rapid microbial P assimilation has been observed in many studies. For example, Oehl *et al.* (2001) found that 2-6% of tracer ^{33}P initially applied to the soil was recovered into microbial P during 5 days. Oberson *et al.* (2001) also recovered 25% of added ^{33}P from the microbial P pool within 2 days. Wetting-drying cycles and mixing processes possibly stimulated the microbial uptake of P_i (McLaughlin and Alston 1986; Walbridge and Vitousek 1987), and thus significant biological transformation of soluble P sources into microbial

P was detected within hours in this study.

The recovery of Al-P in the NaOH-P_o pool could also be attributed to microbial assimilation, although, unlike Ca-P, an increase in the microbial P was not detected in any soil type following Al-P addition. Considering that some microorganisms are highly resistant to chloroform fumigation (Eberhardt *et al.* 1996), it is possible that microbes that assimilated the Al-P may survive fumigation or, even if killed, were still extracted as NaOH-P_o. In addition, immobilization of P could also occur during fumigation by microbial cell P fixation (Hedley and Stewart 1982; Myers *et al.* 1999).

5.4.2. P transformation during incubation

The transformation of P in the soil with prolonged incubation varied among the soil types and P sources. The recovery of Ca-P in $NaHCO_3$-P_i fraction declined gradually from day 1 to day 42. The corresponding P increase was observed in the NaOH-P_i, HCl-P or residual pool. Soil properties, such as pH, clay content, $CaCO_3$ and active Fe and Al possibly drive the differences in the final sink for the soluble P sources among soil types. For example, the slow movement of P into HCl-P pool on Calcarosol, as observed from 2 to 6 weeks, confirms earlier work demonstrating that loss of available P over time in calcareous soils is associated with sorption/precipitation reactions with $CaCO_3$ (Samadi and Gilkes 1998; Soils and Torrent 1989), in addition to a significant initial retention by Fe and Al oxides. In contrast, NaOH-P_i and residual P pool acted as the final sink in Ferrosol due to its high Fe and Al oxides and low pH.

In the absence of rhizosphere processes, P-solubilising bacterial and fungal species were more likely to be responsible for the gradually increased availability of Al-P from day 1 to day 42 in the Vertosol and Calcarosol. The occurrence of P-solubilising organisms can be as much as 26-39% of biomass in soils (Kucey *et al.* 1989). In thirteen Brazilian soils, Nahas *et al.* (1994) found that the number of P-solubilizing microorganisms could represent up to 58% of the total soil microorganisms. In addition, some solubilizing microorganism, e.g. fungus *Aspergillus niger*, are more active in dissolving Al-P than Fe-P and Ca10-P (Barroso and Nahas 2003). The ability of P-solubilising microorganisms to solubilise Al-P is often related to the excretion of organic acids (Illmer *et al.* 1995; Kucey *et al.* 1989). In this study, it is possible that insoluble inorganic Al-P was possibly assimilated by microorganisms following soil mixing, as suggested by the increased NaOH-P_o pool, and turned into soluble P through the action of acids within the microorganisms. This soluble P was ultimately liberated during microbial turnover. Nevertheless, further study is needed to verify the proposed mechanism.

5.4.3. ^{32}P dynamics following the addition of P sources

The ^{32}P recovery data suggest that all soil P pools underwent exchange with ^{32}P, although the degree of exchanges with each pool varied greatly among the soil types. The essential role of NaOH-P_i pool on P dynamic of Ferrosol was further demonstrated by the highest recovery of ^{32}P into this pool, when compared to both $NaHCO_3$-P and NaOH-P_i pool which acted as the major sinks for ^{32}P applied into Vertosol and Calcarosol.

The recovery of ^{32}P in the residual pool was much less than that into $NaHCO_3$-P and NaOH-Pi pool, but was significant for all soil types (Figure 5.2), suggesting that part of this pool is exchangeable and potentially available to plants. Actually, it is very hard to distinguish truly exchangeable ^{32}P from that irreversibly adsorbed ^{32}P since the specific adsorption of ^{32}P onto the surface of the soil particles cannot be excluded (Barrow 1991). Nevertheless, the increasing ^{32}P recovery in the $NaHCO_3$-P_i pool over 42 das following Ca-P addition at the expense of NaOH-P_i and residual-P pool in both Vertosol and Calcarosol (Figure 5.2) indicates that adsorption in this case was exchangeable rather than non-exchangeable. It is well documented that P extracted into NaOH pools in the Vertosol and Calcarosol are weakly adsorbed on the Al and Fe oxide and are relatively plant-available (Dorahy *et al.* 2004; Holford and Mattingly 1975; Soils and Torrent 1989). In contrast, ^{32}P recovery in the NaOH-P_i and residual pools in the Ferrosol was not affected by any of the P sources added, which demonstrates the limited availability of these pools in Ferrosols. Hence the nature and availability of soil P pools separated by the same extractant differed greatly among soil types, which is consistent with many studies (Guo *et al.* 2000; McKenzie *et al.* 1992a; 1992b; Tiessen *et al.* 1992). The fact that residual P pools represent significant amounts of the exchangeable P in both Vertosol and Calcarosol raises questions about the supposed stability and relative lability of this fraction as determined using sequential extraction methods. Non-isotopic evidence is accumulating supporting the contention that residual P in slightly weathered soils is partly plant-available due to the fact that it declined substantially following continuous plant P uptake, irrespective of P fertilizer application (Guo and Yost 1998; Hedley *et al.* 1982c; McKenzie *et al.* 1992b; Vu *et al.* 2008).

The increased Al-P solubility, in the Vertosol and Calcalrosol, with prolonged incubation could contribute to the increased ^{32}P recovery into $NaHCO_3$-P pool. Fe-P was shown to be relatively stable, less reactive and had negligible effect on the ^{32}P recovery into each P pool. The increased ^{32}P recovery into the HCl fraction following Ca10-P addition in Vertosol and Calcarosol, but not Ferrosol, could possibly be attributed to the fact that ^{32}P was unexpectedly adsorbed on the surface of Ca10-P and subsequently extracted into the HCl fraction. For the Ferrosol, the bonding energy of Al and Fe oxides might be more competitive for ^{32}P ions than the surface of Ca10-P.

5.5. Conclusion

The fate of P sources varied greatly among soil types, as demonstrated by both the fractionation scheme and ^{32}P labeling technique. Sparingly soluble Al-P exhibited increased availability in Vertosol and Calcarosol, but not Ferrosol. Acid-soluble Ca10-P was completely transformed into P associated with Al and Fe oxides in Ferrosol. The NaOH-P_i and residual pools, which acted as the main sink for all P sources in the Ferrosol, had been proved of low exchangeability with labile P sources. However, these pools consisted of significant amounts of reversely exchangeable P in Vertosol and Calcarosol and would be potentially available to plants. Therefore, a reasonable interpretation of the availability of different P sources to plants must consider their fate in various soils. The significance of biological P transformation for both soluble and insoluble P sources requires further investigation.

Chapter 6: Availability of sparingly soluble phosphorus sources to cotton (*Gossypium hirsutum* L.), wheat (*Triticum aestivum* L.) and white lupin (*Lupinus albus* L.) supplied with different forms of nitrogen as evaluated by a ^{32}P isotopic dilution technique

6.1. Introduction

The form of nitrogen (N) supply can affect the availability of sparingly soluble P compounds such as Ca phosphate, Al and Fe phosphate (Apthorp *et al.* 1987; Gahoonia and Nielsen 1992). The effect is attributed to the acidification or alkalization of the rhizosphere soil following assimilation of NH_4^+ (NH_4-N) and NO_3^- (NO_3-N), respectively (Gahoonia *et al.* 1992; Troelstra *et al.* 1985). For example, application of NH_4-N increased the availability and uptake of sparingly soluble P bound with Ca (Hinsinger and Gilkes 1996; Thomson *et al.* 1993). Bertrand *et al.* (1999) detected a two-fold increase in the depletion of NaOH-extractable inorganic P (NaOH-P_i), or P associated mainly with Al and Fe oxides, by maize when N was supplied as NH_4-N in contrast with NO_3-N. However, Zoysa *et al.* (1998) showed that depletion of NaOH-P_i by tea (*Camellia sinensis*) plants was higher when NO_3-N rather than NH_4-N acted as the sole N source. Further research is still required to clarify the effect of the assimilation of different N sources on the availability of less labile P sources to different plant species.

Previous sand culture experiment revealed that cotton plant lacked the ability in utilizing sparingly soluble P sources including $AlPO_4$, $FePO_4$ and hydroxyapatite (Chapter 4). However, sand varies significantly from soil, and may result in different root growth or even rhizosphere process. Hence renewed investigation into the availability of sparingly soluble P sources under soil conditions is warranted. Normally, biological evaluation of the availability of P sources to plants is subject to the invalid assumption that total P uptake by a plant from the soil in the presence of P sources is identical to that in the absence of P sources (i.e. it assumes no priming effect occurs from the addition of P sources) (Morel and Fardeau 1989; 1990). By labeling the fertilizer P with ^{32}P, fertilizer- and soil-derived P in the plant was distinguishable, and the proportion of P in the plant that originates from the P fertilizer can be calculated directly (Armstrong *et al.* 1993). However, the labeling of sparingly soluble P sources by neutron irradiation is relatively costly and can sometimes lead to the labeling of accompanied cations (Armstrong *et al.* 1993). A ^{32}P reverse dilution technique which involves labeling the bioavailable soil P instead of P sources overcomes this problem (Zapata and Axmann 1995). This technique involves the measurement of P uptake from ^{32}P labeled soil in the presence and absence of the unlabeled P sources.

By adopting the ^{32}P reverse dilution technique, this chapter aims to quantitatively determine the availability of sparingly soluble P sources such as $AlPO_4$ and hydroxyapatite to cotton, wheat and white lupin possessing contrasting root P acquisition strategies. The effect of N form on mobilization of these sparingly soluble P sources will be examined using two N sources: NH_4-N and NO_3-N. Both of these N sources (source-N) had been labeled with ^{15}N in order to evaluate their relative contributions to total N uptake by the plant species.

6.2. Materials and Methods

6.2.1. Soil and experiment design

Soils used in this study were classified as Red Vertosol (Isbell 2002) and were collected at a depth of 10-30 cm along the fence line of a long-term experimental site at Horsham, Victoria, Australia (36° 40' S, 142° 18' E). The soil had the following properties: clay content 40%, total carbon 0.84%, total N 0.082%, pH 7.2 (0.01 M $CaCl_2$), and available Colwell P of 6 mg kg^{-1} (Colwell, 1963).

The experiment consisted of a completely randomized design with factorial combinations of 4 P treatments, 3 species (wheat, cotton and white lupin) and 2 N sources (NH_4-N and NO_3-N). The four P treatments include zero P (P0) and P applied as Ca $(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P) and hydroxyapatite (Ca10-P) at the rate of 150 mg kg^{-1}. The higher rate of P application was applied to ensure a significant dilution of radioisotope content in the plant. All treatments were replicated 3 times. Treatments without addition of P were used as controls and to reflect the P derived from the soil. Nitrogen source was applied as Ca $(^{15}NO_3)_2$ and $(^{15}NH_4)_2SO_4$ with 2% ^{15}N atom at a rate of 30 mg N kg^{-1} every week from week 2. NH_4-N was applied with nitrification inhibitor dicyandiamide (DCD) (8 mg kg^{-1}) to stop or slow down the conversion of NH_4-N to NO_3-N (Di and Cameron 2004).

6.2.2. Labeling soil with ^{32}P

The soils were labeled by weighing 0.7 kg of the soil in a polythene bags. Basal nutrients were thoroughly mixed with the soil at the following rates (mg kg^{-1}): K_2SO_4, 140; $CaCl_2.2H_2O$, 150; $MgSO_4.7H_2O$, 20; $MnSO_4.H_2O$, 15; $ZnSO_4.7H_2O$, 9; $CuSO_4.5H_2O$, 2; H_3BO_3, 0.7; $Na_2MoO_4.2H_2O$, 0.2; FeEDTA, 5.5. After mixing, ^{32}P was added in 100 ml aliquots containing 4.2 MBq ^{32}P (6 MBq ^{32}P kg^{-1} soil) with 0.7 mg P as a carrier in order to reduce adsorption of ^{32}P on glassware (Olsen and Dean 1965). Complete mixing of ^{32}P and soil was aided by frequently tumbling the bags by hands behind a thick Perspex shield. Additional water was added to make it up to 70% of field capacity (48% w/w). The soils were then sealed and incubated in a growth cabinet at 28°C for three weeks.

6.2.3. Plant culture

After three weeks, all bags were unsealed and air-dried for one day. Phosphorus sources were applied to the soil as 80 ml suspensions and mixed thoroughly with a spatula to ensure maximal mixing but minimal dust production. One day after the addition of P sources, uniform, germinated seeds were planted in each pot. Wheat and white lupin were grown in a growth cabinet with a temperature regime of 25 °C day/18°C night. Cotton was grown in a separate growth cabinet with temperatures set at 28 °C day/20°C night. Light was provided with lamps as 14 h day/10 h night. One week after sowing, seedlings were thinned to 12 for wheat, 6 for cotton and 6 for white lupin pot $^{-1}$. Pots were arranged in a completely randomized design and re-randomized every two weeks. Soil moisture was adjusted to 80% of field capacity every 2 days for the first 3 weeks, and then daily for the final 3 weeks.

6.2.4. Harvest and plant analysis

Eight plants of wheat, 4 cotton and 4 white lupin plants were removed at 2 cm above soil surface from each pot after 30 days growth (Day 30). The remaining plants were harvested 45 days after sowing (Day 45). The harvested plant material was dried in an oven at 70 °C and shoot dry weight was recorded. After grinding, subsamples of shoot were digested with concentrated nitric and perchloric acid (4:1), and P in digests was determined colorimetrically (Motomizu *et al.* 1980). The radioactivity in the digest was determined with a liquid scintillation counter (Tri-card 2000 CA) by adding 10 ml of scintillation cocktail (ULTIMA Gold XR) to 2 ml of extracts. Total plant N and ^{15}N abundance were measured with a magnetic-deflection mass spectrometer (Sira 10, VG Isogas, Middlewich, UK).

6.2.5. Determination of the seed P contribution to the total P uptake in the shoot

Seeds of each species were ground and P content was determined as 5.3, 4.0 and 3.8 mg P g^{-1} for cotton, wheat and white lupin respectively. The amount of seed P which was translocated to the shoot was determined by growing each species in coarse river sand mixed with basic nutrients except P. After 45 days, both shoots and roots were harvested and analyzed for P content. The contribution of seed P to the plant shoot, calculated as the ratio of shoot P content to the total P uptake, was 74%, 40% and 70% for cotton, wheat and white lupin respectively. Thus, total amount of P that could be derived from seed ($^{31}P_{dfseed}$) was estimated as 0.52, 0.16 and 0.76 mg P plant^{-1} for cotton, wheat and white lupin respectively.

6.2.6. Calculation and statistical analysis

Specific activity (SA) in the plant was determined as radioactivity (^{32}P) per unit of P (^{31}P) (Becquerel μg^{-1} P). The proportion of P (Pdff %) and the amount of P (Pdff mg) in the plant derived from different P sources were calculated by using the isotope dilution formula described by Zapata and Axmann (1995).

$$\text{Pdff}\,\% = 100[1-(\frac{\text{specific activity of plants in presence of P sources}}{\text{specific activity of plants in absence of P sources}})]$$

$$\textit{Specific activity of plants in presence or absence of P sources} = \frac{^{32}P_{shoot}}{^{31}P_{shoot} - {}^{31}P_{dfseed}}$$

Here, seed P contribution ($^{31}P_{dfseed}$) had been subtracted from the total shoot P uptake ($^{31}P_{shoot}$) in the determination of plant SA.

Pdff mg = [Pdff% × Total P uptake]/100

The proportion of N (Ndff %) and total amount of N (Ndff mg) in the plant derived from two N sources were calculated as:

$$\text{Ndff}\,\% = 100(\frac{Atom\ \%\ {}^{15}N_{plant\ sample} - 0.37}{Atom\ \%\ {}^{15}N_{labelled\ chemical}})$$

Ndff mg = [Ndff% × Total N uptake]/100

Where 0.37 is the natural abundance of atom % ^{15}N

For each harvest, a three-way analysis of variance involving the P treatment × N form × species was performed for shoot and root dry weights, P concentration and P uptake. Isotopic parameters such as SA, Pdff%, P recovery and Ndff% were subjected to a two-way analysis of variance involving the P treatment × N form for each species. Comparison of treatment means was done using the Duncan's Multiple Range tests after performing analysis of variance using R 2.3.0 software (R Development Core Team 2006).

6.3. Results

6.3.1. Plant growth

Shoot biomass production was not affected by N source in any species (Figure 6.1). After 45 days, all three species were responsive to soluble P application ($P<0.05$) in this soil (Figure 6.1) and none of these plants exhibited P deficiency symptoms. Wheat was more responsive (130% increase) than either cotton (50%) or white lupins (25%) (Figure 6.1). No biomass increases were observed in response to sparingly soluble P sources in either cotton or white lupin (Figure 6.1). However, application of Al-P resulted in 45% and 130% increase in shoot dry weight of wheat after 30 and 45 days growth, respectively (Figure 6.1). There was no significant interaction between P source, N sources and species in shoot biomass production (Table 6.1).

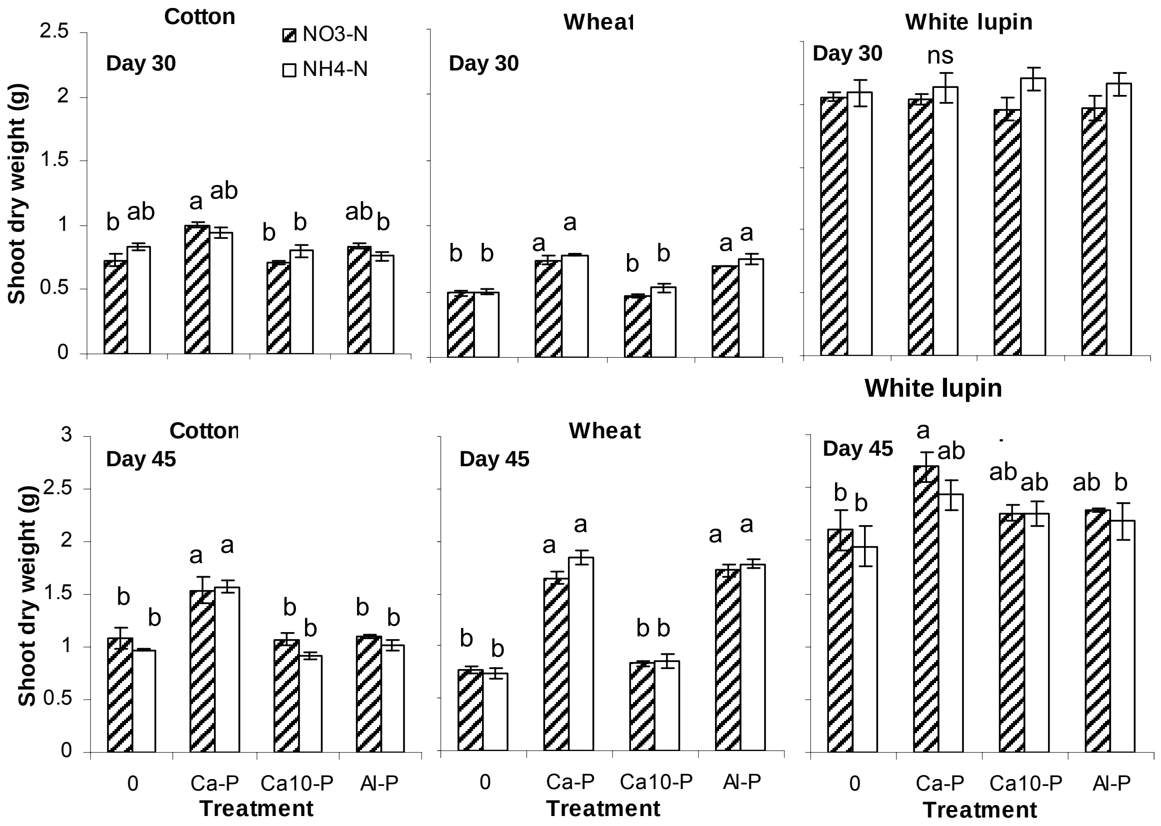


Figure 6. 1. Shoot dry weights of cotton, wheat and white lupin grown for 30 (day 30) and 45 days (day 45) without (P0) and with P supplied as Ca $(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P) and hydroxyapatite (Ca10-P) under different N forms (NO_3-N and NH_4-N). Error bars indicate standard error (n = 3). Values not sharing the same letter indicate significant differences between the P treatments within a species according to the Duncan's multiple range tests (*P* ≤ 0.05). "ns" represents no significant difference.

6.3.2. Shoot P concentration and P uptake

Application of soluble Ca-P with NH_4-N gave the highest shoot P concentration and P uptake for all species after 45 days growth (Figure 6.2, Figure 6.3). When compared with control, wheat showed the most P uptake following application of sparingly soluble Al-P, giving ~75% of the amounts provided by soluble Ca-P (Figure 6.3). Shoot P concentration of wheat supplied with Al-P was above the critical value (3.0 mg g^{-1}) normally reported for wheat (Rashid *et al.* 2005).

Significant increase in P uptake from Al-P and Ca10-P over the control by cotton and white lupin, respectively, was not detected until 45 days growth. The Al-P source was more available to both cotton and white lupin when N was supplied as NH_4-N (Figure 6.3). Nevertheless, the shoot P concentration of cotton and white lupin supplied with sparingly soluble P sources was below the critical values reported for both species (2.0 to 3.1 mg g^{-1} for cotton and 2.0 mg g^{-1} for white lupin) (Cox and Barnes 2002; Crozier *et al.* 2004; Li *et al.* 2008; Sas *et al.* 2001a).

Table 6. 1. Significance levels of main effects and interactions of P, N source and species on shoot dry weight, shoot P concentration and shoot P uptake for each harvest.

	Shoot dry weight	Shoot P concentration	Shoot P uptake
Harvest I			
P source	**	***	***
N source	n.s.	n.s.	n.s.
Species	***	***	***
P source × N source	n.s.	**	n.s.
P source × species	*	***	***
N source × species	n.s.	n.s.	n.s.
P source × N source × species	n.s.	n.s.	n.s.
Harvest II			
P source	***	***	***
N source	n.s.	***	***
Species	***	***	***
P source × N source	n.s.	***	***
P source × species	***	***	***
N source× species	n.s.	n.s.	n.s.
P source× N source × species	n.s.	n.s.	n.s.

*, $P \leq 0.05$; **, $P < 0.01$; ***, $P < 0.001$; n.s., not significant

6.3.3. Shoot specific activity (SA), P derived from P sources (Pdff%) and P sources recovery

Only data from the second harvest (45 days growth) was used for the calculation of isotopic parameters as unexpectedly low isotope counts in both cotton and white lupin after 30 days growth precluded the calculation of P availability. As expected, application of P sources decreased the specific activity (SA) of P in all species regardless of N source after 45 days growth (Table 6.2). The lower the SA of the shoot, the higher the amount of available P supplied by the P sources tested. Similarly, this was reflected by the calculated Pdff % value, i.e, the proportion of P in the plant derived from the applied P sources, and the P sources recovery. For example, P sources applied as soluble Ca-P, when compared with the sparingly soluble P sources, had the lowest SA and highest Pdff% value and P source recovery for all species (Table 6.2, Table 6.3).

The availability of sparingly soluble Al-P and Ca-P varied among species. The proportion of P sourced from Al-P was ~89% for wheat, but only 53% and 18% for cotton and white lupin, respectively, when averaged over N sources (Table 6.3). Correspondingly, the recovery of P sources from Al-P by wheat was 9 times higher than cotton, and 30 times higher than white lupin.

However, Pdff% value, when supplied Ca10-P, was ~75% for white lupin, in contrast to less than 50% and 20% for wheat and cotton respectively. Thus, the recovery of P from Ca10-P was greatest for white lupin (0.71%), followed by wheat (0.50%), and cotton (0.07%). Nitrogen applied as NH_4-N increased Pdff% value and the recovery of P, for all the sparingly soluble P sources, except in the case of wheat fed with Al-P (Table 6.3).

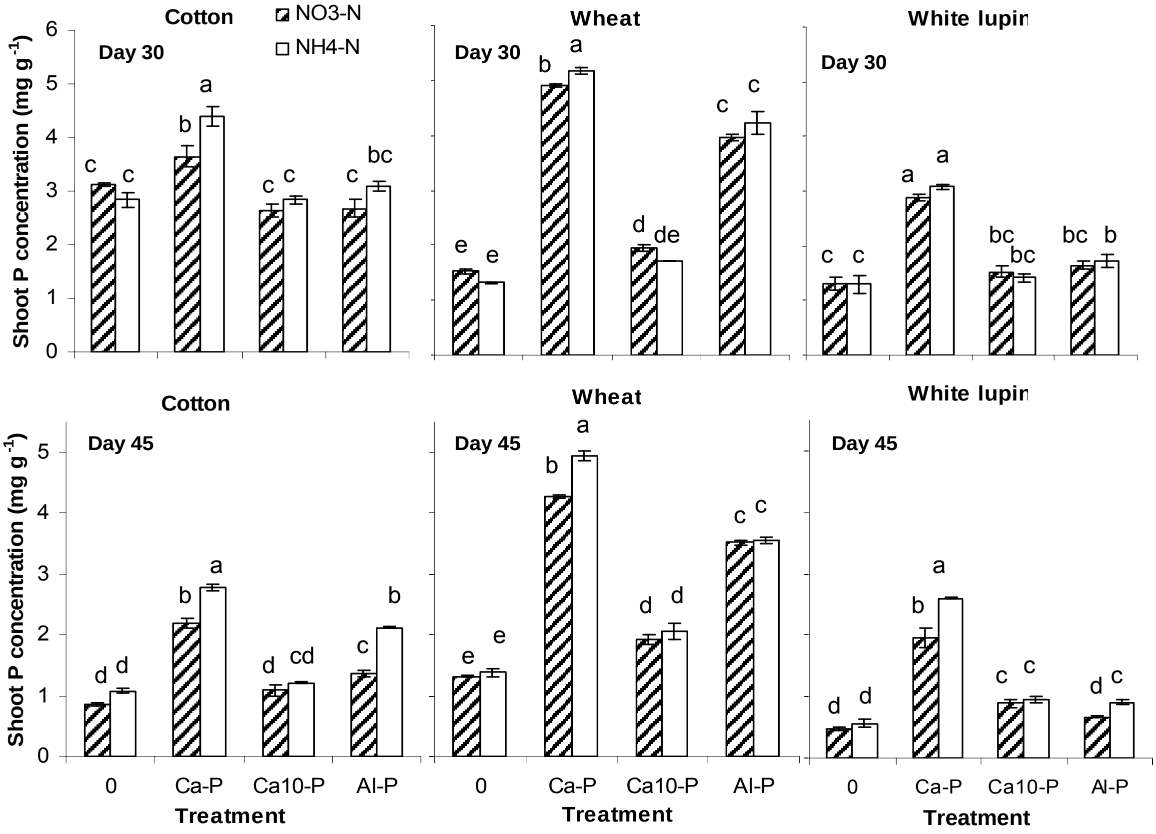


Figure 6. 2. Shoot P concentration (mg g^{-1}) of cotton, wheat and white lupin grown for 30 (day 30) and 45 days (day 45) without (P0) and with P supplied as Ca $(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P) and hydroxyapatite (Ca10-P) under different N forms (NO_3-N and NH_4-N). Error bars indicate standard error (n = 3). Values not sharing the same letter indicate significant differences between the P treatments within a species according to the Duncan's multiple range tests ($P \leq 0.05$).

6.3.4. N derived from N sources

The proportion of N derived from the N sources was highest for wheat and lowest for white lupin (Table 6.4). As expected, supplied N sources were used more where soluble P was supplied. White lupin used 20-40% more N when supplied as NO_3 than as NH_4 (Table 6.4). In the case of P applied as Ca10-P, total N in the plant derived from NH_4-N was the highest for the wheat (2.18 mmols pot^{-1}), followed by white lupin (1.42 mmols pot^{-1}) and cotton (1.14 mmols pot^{-1}).

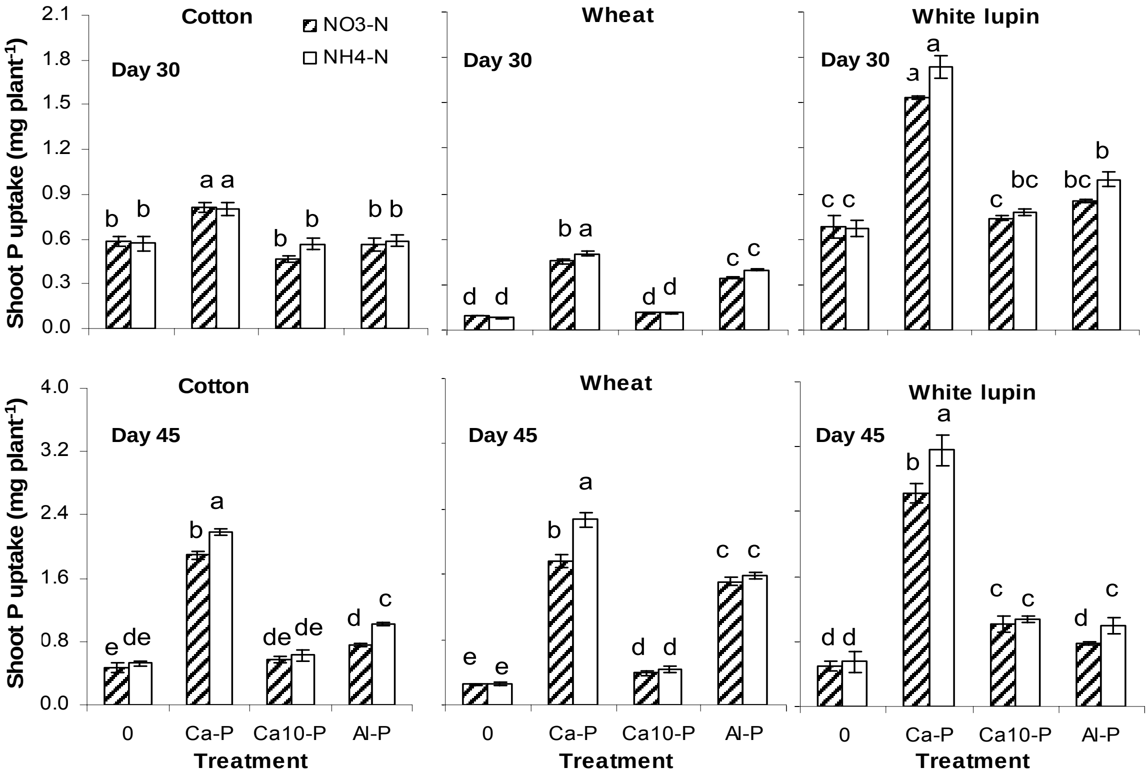


Figure 6. 3. Shoot P uptake (mg plant^{-1}) of cotton, wheat and white lupin grown for 30 (day 30) and 45 days (day 45) without (P0), and with P supplied as Ca $(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P) and hydroxyapatite (Ca10-P) under different N forms (NO_3-N and NH_4-N). Error bars indicate standard error (n = 3). Values not sharing the same letter indicate significant differences between the P treatments within a species according to the Duncan's multiple range tests ($P \leq 0.05$).

Table 6. 2. Specific activity (Bq ^{32}P/µg P) in the shoot of cotton, wheat and white lupin grown for 30 (day 30) and 45 days (day 45) without (P0) and with P supplied as Ca $(H_2PO_4)_2$ (Ca-P), $AlPO_4$ (Al-P) and hydroxyapatite (Ca10-P) under different N forms (NO_3-N and NH_4-N). Values not sharing the same letter indicate significant differences between the treatments within a column according to Duncan's multiple range tests ($P \leq 0.05$).

N form	P treatment	Cotton		Wheat		White lupin	
		Day 30	Day 45	Day 30	Day 45	Day 30	Day 45
NO_3-N	P0	0.04c	0.46a	3.40a	4.83a	0.12b	0.30ab
	Ca-P	0.21a	0.22c	0.73d	0.36e	0.27a	0.14c
	Al-P	0.13b	0.30b	1.19c	0.60d	0.26a	0.28b
	Ca10-P	0.07c	0.43a	3.46a	4.01b	0.12b	0.15c
NH_4-N	P0	0.06c	0.40a	3.80a	4.52a	0.13b	0.35a
	Ca-P	0.21a	0.20c	0.88d	0.41de	0.27a	0.16c
	Al-P	0.15b	0.23c	1.14c	0.69d	0.28a	0.27b
	Ca10-P	0.06c	0.30b	2.88b	2.80c	0.14b	0.14c

Table 6. 3. Pdff (%, mg pot^{-1}) and P recovery (%) for the cotton, wheat and white lupin grown for 45 days with P supplied as Ca (H_2PO_4)$_2$ (Ca-P), $AlPO_4$ (Al-P) and hydroxyapatite (Ca10-P) under different N forms (NO_3-N and NH_4-N). Values not sharing the same letter indicate significant differences between the treatments within a column according to Duncan's multiple range tests ($P \leq 0.05$).

N form	P treatment	Cotton			Wheat			White lupin		
		Pdff[A] (%)	Pdff[B] (mg pot^{-1})	P recovery[C] (%)	Pdff (%)	Pdff (mg pot^{-1})	P recovery (%)	Pdff (%)	Pdff (mg pot^{-1})	P recovery (%)
NO_3-N	Ca-P	77a	2.37b	2.26b	94a	6.41b	6.10b	72b	3.06b	2.91b
	Al-P	50c	0.41d	0.39d	90b	5.20c	4.95c	9d	0.05f	0.05f
	Ca10-P	6e	0.03f	0.03f	26d	0.35e	0.33e	71b	0.58d	0.55d
NH_4-N	Ca-P	76a	2.85a	2.71a	93a	8.22a	7.83a	74ab	3.99a	3.80a
	Al-P	57b	0.76c	0.72c	88b	5.35c	5.10c	28c	0.27e	0.26e
	Ca10-P	18d	0.10e	0.10e	46c	0.69d	0.66d	79a	0.91c	0.87c

[A] Proportion P in the plant derived from different P sources.

[B] Total amount of P in the plant derived from different P sources

[C] Percentage of P sources (150 mg P kg^{-1}) recovered in the plant shoot

Table 6. 4. Ndff (%, mmols pot^{-1}) value for the cotton, wheat and white lupin grown for 45 days without (P0) and with P supplied as Ca (H_2PO_4)$_2$ (Ca-P), $AlPO_4$ (Al-P) and hydroxyapatite (Ca10-P) under different forms of ^{15}N labeled N sources (NO_3-N and NH_4-N). Values not sharing the same letter indicate significant differences between the treatments within a column according to Duncan's multiple range tests ($P \leq 0.05$).

N form	Treatment	Cotton		Wheat		White lupin	
		Ndff[A] (mmols pot^{-1})	Ndff[B] (%)	Ndff[A] (mmols pot^{-1})	Ndff[B] (%)	Ndff[A] (mmols pot^{-1})	Ndff[B] (%)
NO_3-N	P0	1.19c	66d	1.54c	93c	1.79c	48c
	Ca-P	3.61a	92a	4.47a	100a	3.05a	70a
	Al-P	1.72b	82b	4.93a	100a	2.04b	58b
	Ca10-P	1.17c	67d	1.67c	96b	2.01b	54b
NH_4-N	P0	0.98c	64d	1.41c	93c	1.44d	42d
	Ca-P	3.30a	93a	4.96a	100a	2.13b	54b
	Al-P	1.70b	74c	4.56a	100a	1.44d	38d
	Ca10-P	1.14c	66d	2.18b	98ab	1.42d	41d

[A] Total amount of N in the plant derived from N sources

[B] Proportion of N in the plant derived from N sources.

6.4. Discussion

6.4.1. Species' variation in utilizing different P sources

Cotton absorbed little P from Ca10-P, but did demonstrate slightly higher capacity to acquire Al-P relative to white lupin. The sparingly soluble Al-P source used in this study was increasingly available with time, e.g. nearly 10% of applied Al-P (10 mg P pot^{-1}) in this red Vertosol was recovered into the labile P pools over 45 days (Chapter 5). Thus, cotton with relative higher root length density than white lupin (0.56 cm cm^{-3} vs 0.28 cm cm^{-3}) would be better equipped to acquire this progressively available P source. The low availability of P from Ca10-P for cotton was consistent with the previous results (Chapter 3, Chapter 4).

The availability of Al-P to wheat was 9 and 30 times higher than cotton and white lupin, respectively. This confirmed my previous findings that wheat was better able to access Al-P than both cotton and white lupin (Chapter 4). However, the specific mechanism by which wheat is able to use this sparingly soluble P source still remains unclear. Aluminium toxicity-induced organic acid release has been suggested as one potential driver of Al-P acquisition (Lambers *et al.* 2002; Pearse *et al.* 2006b). Nevertheless, the quantity of organic acids, such as malate and citrate, exuded from wheat roots, even in the presence of Al-P, is reported to be below that required for significant mobilization of Al-P (Nuruzzaman *et al.* 2005a; Pearse *et al.* 2006b; Veneklaas *et al.* 2003). Morphologically, the high root length density of wheat could increase potential uptake of P from the soil solution, and P uptake from unamended soil (total ^{32}P radioactivity) was indeed significantly higher than both cotton and white lupin (Data not shown). Armstrong *et al.* (1993) also suggested that the availability of the less soluble Al-P to several tropical crop species was attributed to their ability to locate this P rather than to mobilize less soluble P.

In contrast, white lupin accessed more P from Ca10-P than Al-P (Table 6.3). The proportion of P derived from Ca10-P (Pdff %) was comparable to that of soluble Ca-P. This is consistent with the ability of white lupin roots to acidify the rhizosphere and subsequently dissolve P bound with Ca (Dinkelaker *et al.* 1989; Hinsinger and Gilkes 1995). Even when supplied with NO_3-N, under P-deficient conditions, white lupin had been observed to acidify its rhizosphere due to either decreased uptake of NO_3-N or increased citrate exudation (Hinsinger *et al.* 2003). However, the potential ability of white lupin in solubilizing Ca10-P did not result in a considerable, although significant, uptake of P in the shoot, suggesting that inadequate mobilization of this P source to fully meet demands remained. It is likely that the rate of Ca10-P dissolution induced by rhizosphere acidification of white lupin is much slower than that from the rapid dissolution of water soluble Ca-P in the moist soil. Thus, even when soil- and P sources-derived P was taken up at a similar proportion by white lupin fed with Ca-P and Ca10-P, net P uptake from Ca10-P remained smaller due to the low P intensity in the soil. Considering that significant P uptake from Ca10-P by white lupin had not started until the second harvest, I suggested that utilization of Ca10-P by white lupin was also delayed by its relatively larger seed P reserve.

6.4.2. Effect of N form on the availability of P sources

Although total P uptake from Ca10-P by all species did not vary between N forms, the availability of Ca10-P was enhanced when NH_4-N was applied, as indicated by the Pdff% and P sources recovery value (Table 6.3). This demonstrates that isotope-derived parameters are more sensitive in detecting the availability of various P sources to plants than that of yield-dependent parameters. In addition, the total N uptake from NH_4-N would act as an indicator of corresponding proton release (Table 6.4). Thus, cotton fed with Ca10-P would have the least capacity in acidifying its rhizosphere due to the least N uptake as NH_4-N, and consistently the least ability in utilizing Ca10-P, when compared with wheat and white lupin. The effect of rhizosphere acidification accompanying NH_4-N assimilation in elevating the availability of P bound with Ca had been well documented (Gahoonia *et al.* 1992; Hinsinger and Gilkes 1996; Zoysa *et al.* 1998). However, with less NH_4-N assimilation than wheat, white lupin showed a better Ca10-P utilization, as indicated by the greatest recovery of P from Ca10-P with NH_4-N supply (Table 6.3). This is probably due to the additional acidification originated from either N_2 fixation (Raven *et al.* 1990) or increased citrate exudation under P deficiency (Neumann and Römheld 1999). The lower Ndff% values of white lupin than wheat and cotton might indicate that biological N_2 fixation still occurred despite the high rate of N sources applied (Talbe 6.4).

Aluminum-P also exhibites pH dependent solubility (Bertrand *et al.* 1999; Cumming and Weinstein 1990; Shane *et al.* 2008), and elevated rhizosphere acidification following NH_4-N application possibly led to an enhanced Al-P dissolution. Another possibility is that the availability of Al-P in the soil is mainly dependant on the solubilizing-ability of microorganisms (Chapter 5). If this is the case, more P would be solubilized from Al-P in the presence of NH_4-N since the activity of P-solubilizing microorganisms is generally higher when NH_4-N rather than NO_3-N was the sole N source (Cerezine *et al.* 1988; Whitelaw *et al.* 1999). Nevertheless, total P uptake from Al-P, relative to control, in the case of NH_4-N assimilation by both cotton and white lupin was far less (6 times) than that by wheat. The effectiveness of Al-P to wheat was not affected by the forms of N applied. This indicates that the main mechanism involved in the efficiency of wheat in Al-P utilization is independent of rhizosphere pH.

Total P uptake from soluble Ca-P by all species at day 45 was significantly elevated by the addition of NH_4-N. This stimulatory effect of NH_4-N on P uptake was related not only to the charge of different N form, but also to the acidification of rhizosphere soil (Cox and Reisenauer 1973; Rayar and Hai 1977; Sangakkara and Cho 1987). The uptake of anions such as P is likely to be accelerated by absorption of cations such as NH_4^+, in comparison to the competitive effect from NO_3^-. Prominent assimilation of NH_4-N by plants fed with Ca-P could result in a significant proton efflux from roots, which increase the uptake rate of P as $H_2PO_4^-$ ions (Tisdale *et al.* 1985).

6.4.3. ^{32}P isotopic dilution technique

The lower SA in the shoots of cotton and white lupin relative to wheat is attributed to the large contribution of seed P to the total P uptake rather than more efficient P uptake from unlabelled soil P or added P sources. Therefore, caution should be exercised when using the reverse dilution technique to identify a species' ability in acquiring stable P fractions. Without considering the seed P reserves, the conclusion that white lupin with relatively lower SA than soybean was more efficient in utilizing non-labile soil P from ^{32}P labeled soil than soybean (Braum and Helmke 1995) appears less convincing.

For cotton and white lupin, the Pdff% value was greater with seed P being subtracted from the total P content than without the subtraction (data not shown). The role of seed P reserves in assessing availability of P sources should not be neglected as higher reserves may delay P uptake from either P sources or soil (Bolland and Brennan 2008). To effectively use reverse dilution techniques on species with large seed size, researchers should i) grow species for prolonged periods or remove cotyledons, ii) consider only marginally P responsive soils (those with higher, but not adequate, P supplying capacity) to ensure a significant ^{32}P uptake from the soil or iii) quantify the contribution of seed P to each treatment in order to accurately estimate the P contribution from the P sources.

Theoretically, the SA in the plant shoot in the presence of P sources should be lower than that in the absence of P sources, due to the extra P supplied from the unlabelled P source. Unexpectedly, the SA in the shoots of cotton and white lupin supplied with Ca-P and Al-P was higher than that of the control at the first harvest (Day 30). It has been proposed that desorption of ^{32}P from the soil solid phase occurred following addition of Ca-P and Al-P at higher rate, as also demonstrated by a incubation experiment (Chapter 5), which subsequently resulted in a increased ^{32}P uptake and SA in the shoot. This effect would be prominent when the contribution of unlabelled P sources to the total P uptake was relatively less significant, e.g at first harvest. With prolonged growth, applied P sources became the dominant P source and shoot SA decreased correspondingly due to the dilution effect of unlabelled P sources on ^{32}P content in the plant. The addition of more soluble P sources, such as Ca-P and Al-P, at higher rates would be expected to cause a large disturbance to the soil P equilibrium between solution, adsorbed and sparingly soluble P pools. Thus, for assessing the effectiveness of more soluble P sources to plants, direct labeling of P sources is recommended.

6.5. Conclusion

The addition of N as NH_4-N elevated total plant P uptake from all P sources except in the case of wheat fed with Al-P. The superiority of wheat in utilizing Al-P was independent of the form of N supply. When compared with wheat and white lupin, cotton was inferior in acquiring P from all of the sparingly soluble P sources applied in the Vertosol, which confirmed the previous study conducted in a sand-culture system. The role of root carboxylate release and root morphological traits on the utilization of Al-P by wheat warrants further investigation. On the other hand, the poor use of Al-P and Fe-P by white lupin does not reflect its exceptional capacity to acquire P from acid soils with low available P. Further study on verifying this is also needed.

Chapter 7: The role of hydraulic lift and subsoil P placement in P uptake of cotton (*Gossypium hirsutum* L.)

7.1. Introduction

Cotton is normally cultivated in areas that exhibit high evaporation and frequent soil surface drought. As soil phosphorus (P) is strongly stratified in surface layers, rapidly drying shallow soil after irrigation or rain could result in decreased P availability and root nutrient uptake capacity (Jupp and Newman 1987; Kaspar and Timmons 1989; Pan and Hopkins 1991; Radersma *et al.* 2005; Simpson and Pinkerton 1989). During dry cycle, cotton could rely on the relatively deep moist soil layer for water. However, the opportunity for P uptake by cotton from dried top soil remains unknown (Norrish *et al.* 2003; Singh *et al.* 2005), and under field conditions, the inconsistent P response of cotton to P application had been reported on either alkaline soils (Bronson *et al.* 2001; Dorahy *et al.* 2004; Funderburg *et al.* 1996) or acidic and more coarsely texture soils with low soil test P levels (Cope 1981; Le Mare 1974).

Redistribution of water from wet subsoil layers into drier topsoil through plant root systems, or hydraulic lift, may enhance shallow root survival and increase P acquisition in the topsoil (Bauerle *et al.* 2008; Huang 1999; Matzner and Richards 1996). Cotton is generally considered a drought-tolerant species (Leidi *et al.* 1999; Pace *et al.* 1999). One potential mechanism for drought tolerance may be hydraulic lift, as demonstrated for drought-tolerant maize (Wan *et al.* 2000). The occurrence of hydraulic lift has been documented for trees, shrubs and rangeland grasses (Caldwell *et al.* 1998; Emerman and Dawson 1996; Jonathan and Stephen 1998), although water efflux from roots of plant species, such as wheat, maize and canola, into rhizosphere soil has also been observed (Rose *et al.* 2008; Valizadeh *et al.* 2003; Wan *et al.* 2000). By using gamma densitometry, Baker and van Bavel (1988) detected an overnight movement of water from wet to dry soil through the cotton root system, contrasting strongly with the results of Molz and Peterson (1976) who observed that cotton roots are largely resistant to reverse flow. Therefore, the occurrence of hydraulic lift in cotton plants and its role in P availability of dry topsoil remains unclear.

The importance of subsoil P nutrition is increased where the availability of P in the topsoil is limited by surface dryness. Deep banding of P fertilizer (10-15 cm) in soil layers with higher moisture significantly increased the yield of wheat (by 30-43%) and cotton (17-67%) plants when compared with conventional P application (depth 5-10 cm) (Singh *et al.* 2005). In Australia, cotton production occurs on relatively fertile, deep, dark clay soils. The P resource in the subsoil can be considerable in some areas (Norrish *et al.* 2003). Norrish *et al.* (2003) reported a positive correlation between the P concentration in wheat shoots and available P in the deeper 10-20 cm soil layer of Vertosol soils in the northern grain zone of Australia. This suggests that high root proliferation in the subsoil induced by surface water stress promoted P uptake from the subsoil.

Understanding the role of hydraulic lift and subsoil P resource on P nutrition of cotton under surface-drought conditions might render more predictable P response of cotton to fertilizers. Therefore, this study aimed at identifying (1) whether cotton can hydraulically lift water; (2) whether this hydraulically lifted water facilitates P acquisition from a dry topsoil; and (3) the role of subsoil P placement on cotton growth and P uptake. Because soil texture has a strong impact on both P availability and diffusion in soil, which may in turn affect the capacity to detect benefits from hydraulic lift in P nutrition, two soils with contrasting texture were used.

7.2. Materials and Methods

7.2.1. Soils

A P-deficient soil was collected at a depth of 10-30 cm along the fence-line of a long-term experimental site at Horsham, Victoria, Australia (36° 42′36″ S, 142°11′23″ E). The soil is classified as Red Vertosol (Isbell 2002) and had the following properties: clay content (40%), total carbon 0.84%, total N 0.082%, pH 7.2 (0.01 M $CaCl_2$), and available Colwell P of 6 mg kg^{-1}. This soil shrinks and swells during drying and wetting. The coarser-textured ‘sand’ soil was made by mixing 20% of this Red Vertosol with 80% of coarse river sand (w/w) and had a Colwell P of 2.6 mg kg^{-1}. The water content of the sandy soil and clay soil at wilting point (-1500 kPa) was 4% and 18% (w/w), respectively, and at field capacity (-10 kPa) was 16% and 48% (w/w), respectively.

7.2.2. Soil columns

Specially designed soil column systems were constructed for this study (Figure 7.1). Each column was formed by two halves of a PVC cylinder (60 cm long, 10 cm in diameter) that was split vertically and held together with adhesive tape. Three soil layers were placed in each column: these comprised 10-cm topsoil layer overlying a 6-cm buffer layer, which in turn overlayed a 32-cm subsoil layer. The buffer layer consisted of a 2-cm soil layer sandwiched by two 2-cm layers of plastic beads, with a circular layer of waxy baking paper (from Coles supermarket, Australia) being positioned above each beads layer to prevent any mixing of topsoil or subsoil into the beads. The waxy paper had little influence on root penetration into the subsoil; a preliminary experiment revealed that cotton roots readily passed through the paper which began to decay after 2 weeks. The whole soil column was free-draining with a 10-cm layer of coarse gravel placed below the subsoil, and held above a retaining nylon net attached to the base of the plastic column with adhesive tape.

The topsoil volume was divided into an inner, middle and outer compartment (Figure 7.1) by placing of a plastic-sheet-rolled tube (10 cm high with a diameter of 4 cm) on top of the upper waxy paper. A second larger circular tube 10 cm high with a diameter of 8 cm was then placed to the outside of the inner tube, on the waxy paper. The intention of including two compartments (inner and outer) for each pot was to maximize the chance of contact between roots and P applied at the topsoil layer after the treatments commenced.

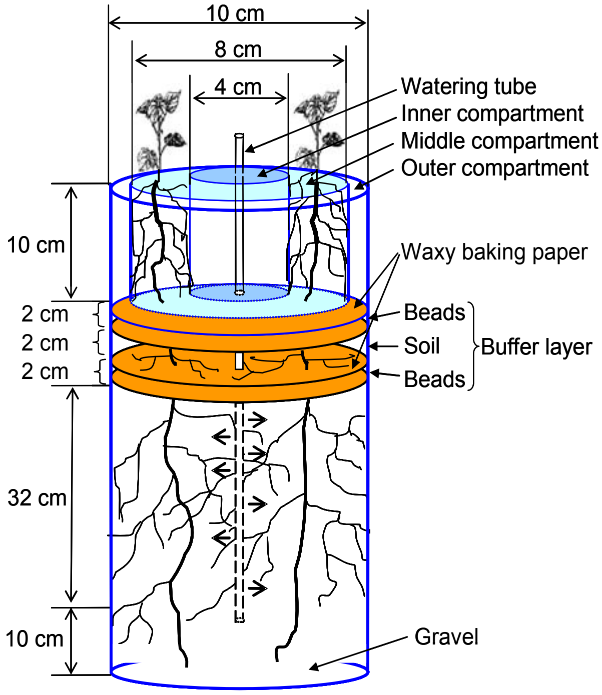


Figure 7. 1. Diagram of the specially designed soil column used in this study. The soil column consisted of three compartments of different diameters in the top 10-cm. The top soil and subsoil were separated by a buffer layer (hydraulic barrier) which was made of a 2-cm soil layer sandwiched by two layers of plastic beads. The subsoil was watered through a PVC tube in the middle of the column.

7.2.3. Experimental design and treatments

The experiment consisted of 3 P treatments, 2 watering treatments and 2 soils, and replicated 3 times (36 in total), in a completely randomised design. The 3 P treatments were topsoil +P, subsoil +P, and whole column -P. The 2 watering treatments were watering (+W) and watering withhold (-W) from the topsoil; all the treatments received adequate water in the subsoil. Two soil textures were clay and sand. The six P and watering combined treatments for each soil type are illustrated in Figure 7.2. Columns without plants were also included as control and repeated 3 times for both soil textures. Additional 12 columns (four treatment combinations of subsoil ±P and two soil types with 3 replicates) were set up with plants being harvested when the topsoil watering was withheld, in order to provide baseline data of shoot and root growth and P uptake.

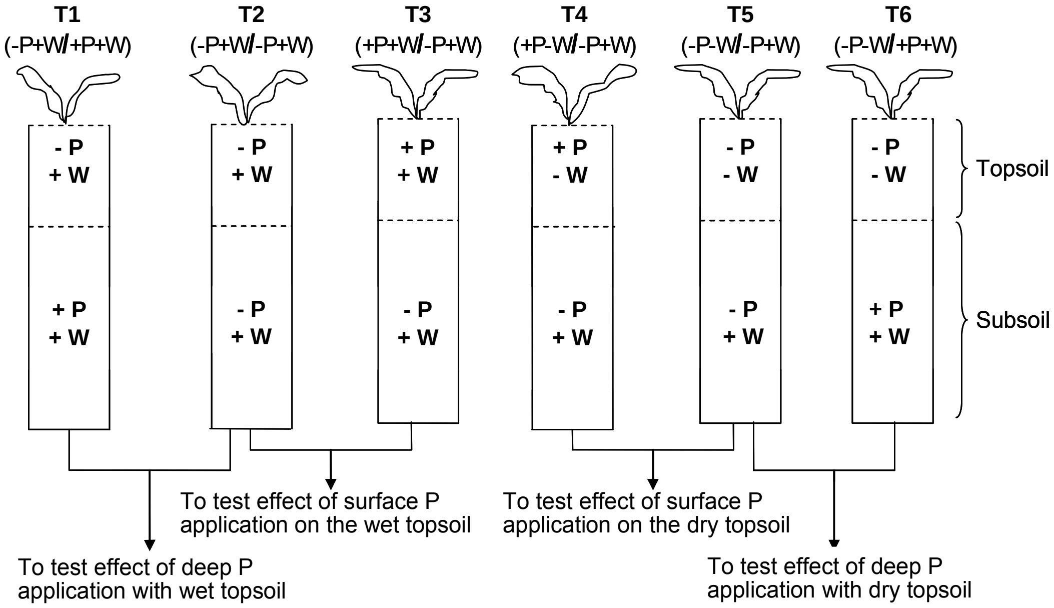


Figure 7. 2. Illustration of P and watering treatments applied to the columns of both clay and sandy soil. "+P" means P applied as Ca $(H_2PO_4)_2$ at the rate of 120 mg P per column into either topsoil (0-10 cm) at the same time as the initiation of drought treatment or into the subsoil (16-48 cm) at the beginning of the experiment. "–P" refers to no P applied in the topsoil or subsoil. "–W" means water was withhold from the surface soil after roots had penetrated into the subsoil. "+W" represents that water was frequently supplied to the topsoil or subsoil to maintain the soil at 80% of field capacity. Treatment codes T1 to T6 represent the six treatments that were applied to each of the two soil types.

7.2.4. Procedures for plant growth

The soil columns were constructed by placing soil, with basal nutrients and with P added according to Figure 7.2, into the bottom 16-48 cm subsoil layer. This was followed by the buffer layer. Soil with basal nutrients was then added to the middle compartment of the topsoil layer. Basal nutrients were applied to all the soil in the column in the following compositions (mg kg^{-1}): K_2SO_4, 140; $CaCl_2.2H_2O$, 150; $MgSO_4.7H_2O$, 20; $MnSO_4.H_2O$, 15; $ZnSO_4.7H_2O$, 9; $CuSO_4.5H_2O$, 2; H_3BO_3, 0.7; $Na_2MoO_4.2H_2O$, 0.2; FeEDTA, 5.5. These were mixed thoroughly with soil. Phosphorus was applied as $Ca(H_2PO_4)_2$ to the 0-10 cm layer for the "topsoil + P" treatment and to 16-48 cm layer for the "subsoil + P" treatment, to give a total rate of 120 mg P per column. Nitrogen was applied in a $Ca(NO_3)_2$ solution, at the rate of 30 mg N kg^{-1} weekly to each column after seedling establishment.

Cotton plants (*Gossypium hirsutum* L. cv. Sicala 60 BR) were grown in a greenhouse with a diurnal temperature range of 20-35 ºC. Six uniform, germinated seeds of cotton were sown into the middle compartment of topsoil and thinned to two plants per column one week after sowing. During the first 4-5 weeks no water stress was imposed in order to establish the plants. Once roots had penetrated through the buffer layer into the subsoil (4 weeks for clay soil and 5 weeks for the sandy soil) as seen visually through an additional set of transparent plastic columns (wrapped with black plastic sheet to prevent light), watering treatments of the topsoil commenced. Soil with (+P) or without P (-P), according to Figure 7.2, was moistened to 50% of field capacity (24 % w/w and 8% w/w for clay and sandy soil, respectively) and placed in the inner and outer compartments of the topsoil layer. This level of soil moisture aided root penetration but minimized the effect of the water in this added soil, on topsoil dryness. The transparent plastic tubes separating the compartments were then removed. The subsoil in all treatments was frequently watered through a narrow plastic tube with outlet holes below the buffer layer (Figure 7.1) until harvest. Because plant roots penetrated to the subsoil one week later in the sandy soil than in the clay soil, the treatments were imposed in the topsoil layer (0-10 cm) of the sandy soil for 6 days less than they were imposed for the clay soil.

7.2.5. Soil moisture monitoring

Hydraulic lift occurs when there is an overnight increase in soil water content in the dry surface soil. The water content in the topsoil of the middle compartment was therefore monitored with a Theta probe soil moisture sensor (Measurement Engineering Australia) in the late afternoon (7:00 pm) and in the early morning (6:00 am) for all the treatments where water was withheld from the topsoil layer, during the second half of the experiment. During this measurement, four stainless steel rods of the Theta probe were inserted vertically into the middle compartment area of topsoil. Volumetric soil water content obtained with Theta probe was calibrated to gravimetric soil water content for each soil type according to the User Manual (ML2x-UM-1.21). Soil specific calibrations make the measurement accurate to ± 1%. The amount of water released by the roots in the topsoil was calculated from the change in the gravimetric soil water content between 7:00 pm, and 6:00 am the following morning.

7.2.6. Plant measurement

Plants were harvested 14 and 20 days after the topsoil water treatment were imposed for the sandy and clay soil respectively. Shoots were cut off at the soil surface. Roots were collected at depths of 0-10, 10-16, 16-32 and 32-48 cm. Roots from each layer were carefully washed with tap water, and scanned for root length and diameter on a EPSON EU-35 scanner (Seiko Epson Corp., Japan) using the Mac Rhizo Pro version 2003b Programme. All washed roots and plant shoots were dried at 70°C for 48 h, and weighed. After grinding, subsamples of shoot and root were digested with concentrated nitric and percloric acid (4:1), and P in digests was determined colorimetrically (Motomizu *et al*. 1980). Dead roots were separated from live roots in the topsoil before drying for the dry topsoil treatments, and root mortality was measured as dry weight of dead root, expressed as a % of the total root dry weight.

7.2.7. Statistical analysis

Different measurement data, for different treatments, were subjected to a series of analyses of variance using R 2.3.0 software (R Development Core Team 2006). Checks for normal distribution of the data, and for homogeneity of variance, found that no data transformations were required.

The first analyses involved a one-way analysis of variance of gravimetric water content data in the topsoil layer for the three P treatments of individual soils where watering was withheld (Figure 7.3). An additional two-way analysis of variance was undertaken for the gravimetric water content data for these three P treatments and time intervals after withholding watering (Figure 7.3). Changes in water contents between the early morning and preceding evening were used as an indication of hydraulic lift, and were subjected to a one-way analysis of variance for each day for six days after watering was withheld (Table 7.1).

Table 7. 1. Overnight increase in soil water content at the top 10-cm of the clay and sandy soil for continuous 6 nights after water being withheld.

Soil type	Treatment	Overnight increase in soil water content (g H_2O kg^{-1} soil)					
		Night 1	Night 2	Night 3	Night 4	Night 5	Night 6
Clay soil	Control (no plant)	-1.1	0.4	-3.0	-0.9	-0.9	-3.5
	T4 (+P-W/-P+W)	19.4	35.8	20.9	2.8	3.2	9.6
	T5 (-P-W/-P+W)	20.8	33.1	17.2	7.9	7.6	3.8
	T6 (-P-W/+P+W)	48.3	22.0	19.0	3.2	0.9	6.5
LSD (P=0.05)		9.1	6.4	2.0	n.s	n.s	7.5
Sandy soil	Control (no plant)	-5.9	-2.9	-1.8	-3.0	-0.8	-3.0
	T4 (+P-W/-P+W)	-12.9	2.6	2.7	1.0	0.9	-0.7
	T5 (-P-W/-P+W)	-12.9	1.9	-4.2	3.8	1.0	-2.1
	T6 (-P-W/+P+W)	22.2	3.6	2.2	2.2	1.7	1.2
LSD (P=0.05)		6.1	2.6	n.s	2.5	n.s	n.s

Negative values indicate the net water loss from either surface evaporation or night-time water uptake by plants. n.s, not significant *P*=0.05.

For each soil, two-way analyses of variance involving 3 P × 2 topsoil water treatments were performed for root length density at individual depths, shoot and root dry weights, P concentration and P uptake (Table 7.2). In addition, root length density measurements were compared for the four soil depths, for the four treatments of topsoil and four treatments where subsoil P addition and topsoil watering were varied. These data were subjected to a three-way analysis of variance involving the soil depth × P × watering combinations (Figure 7.4).

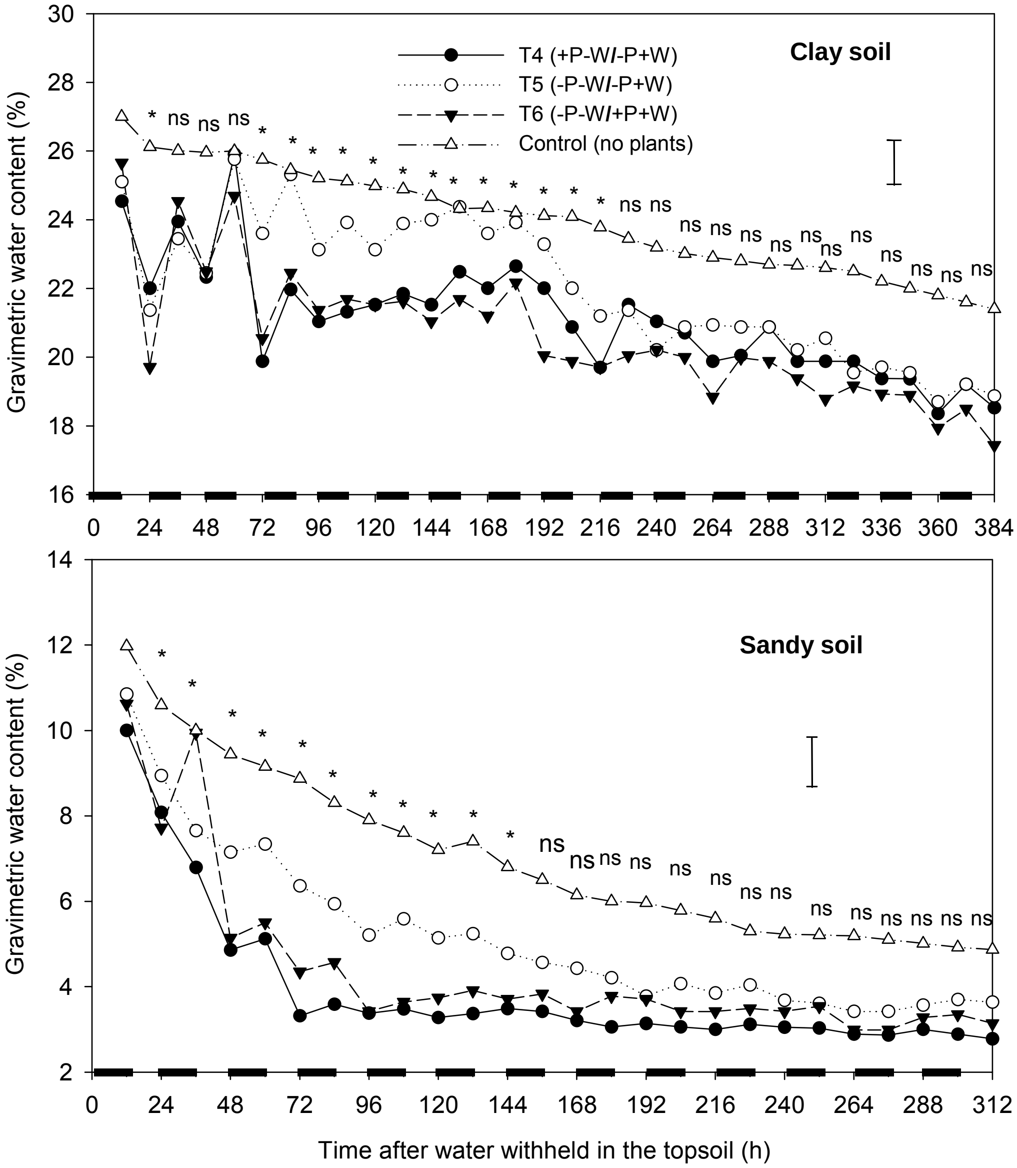


Figure 7. 3. Change in gravimetric soil water content in the top 10-cm layer of the clay and sandy soils after water was withheld. The black bars on the x-axis represent the night time. The vertical bar indicates the LSD (*P*=0.05) for P treatment × time interaction (including the no-plants control), whereas ns and * represent the probability of >0.05 and ≤ 0.05, respectively (excluding the no-plants control). See Fig 7. 2. for treatment codes.

7.3. Results

7.3.1 Change of soil water content in topsoil

The double-bead layer provided an effective barrier to water movement between topsoil and subsoil, as indicated by consistent, evaporation-driven, fall in surface soil moisture in the control pots after watering ceased (Figure 7.3). Significant overnight increases in soil water content occurred for the first three days for the clay soil in amounts ranging from 17.2 to 48.3 g water/kg soil (Figure 7.3, Table 7.1). The maximum increase occurred in the clay topsoil during the first night, in the columns with subsoil P application (Table 7.1). The marked diurnal changes ended after day 3 where soil water content remained constant for four days before steadily declining in line with, but below the control pots without plants. In contrast, soil water content in the sandy soil decreased rapidly over the first 3 days and remained at 4% (about wilting point) thereafter (Figure 7.3). A significant overnight increase in soil water in the sandy topsoil was only detected for one day for the treatment with subsoil P application (22 g water/ kg soil). The water content of the topsoil remained relatively higher where there was no P input than where P was added to either topsoil or subsoil, for both soil types ($P<0.05$) due to less water being transpired by the P-stressed plants (Figure 7.3). However, it declined to the same level as other treatments (20% w/w for the clay soil and 4% for the sandy soil) when surface evaporation became the main cause of moisture loss from the topsoil (9 days on the clay soil and 6 days on the sandy soil). As no change in soil water content was detected after harvest, the possibility that increased soil water content measured by Theta probe might simply represent the rehydration of root tissue overnight can be rejected.

7.3.2. Root length density

Root length density decreased with depth in both soils regardless of surface P application (Figure 7.4). A significant interaction between P and watering treatment on the root length density was only shown for the clay soil at depth 0-10 cm (Table 7.2). Surface-applied P with watering increased the root length density by 110% in the top 10 cm in the clay soil ($P<0.05$) and by 40% in the sandy soil ($P<0.05$), compared to the topsoil with no P and no watering. In contrast, surface-applied P in the absence of water had no effect on root length density in the clay soil, but increased surface root length density by 20% ($P<0.05$) in the sandy soil. Severe surface desiccation after 20 days of drought resulted in >90% root mortality in the clay topsoil, contributing to the observed decrease in root length density. In contrast, root mortality was < 5% in the sandy soil due to water withholding for 14 days. The root diameter, averaged over depths and treatments, was smaller in the clay soil (0.45 ± 0.02 mm) than in the sandy soil (0.55 ± 0.01 mm).

Table 7. 2. Significance levels of main effects and interactions of P and watering treatments on root length density of individual depths, shoot and root dry weight (g column^{-1}), shoot and root P concentration (mg g^{-1}), and total P uptake (mg column^{-1}) of cotton plants grown in soil columns.

	Root length density				Dry weight		P concentration		Total
	0-10 cm	10-16 cm	16-32 cm	32-48 cm	Shoot	Root	Shoot	Root	P uptake
Clay soil									
P	**	ns	**	***	***	***	***	**	***
Watering	***	ns	**	*	ns	ns	***	**	***
P × Watering	*	ns	ns	ns	ns	ns	***	**	ns
Sandy soil									
P	*	*	ns	**	***	***	***	***	***
Watering	ns	ns	ns	**	ns	ns	ns	ns	**
P × Watering	ns	ns	ns	ns	ns	ns	***	ns	***

*, $P \leq 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant

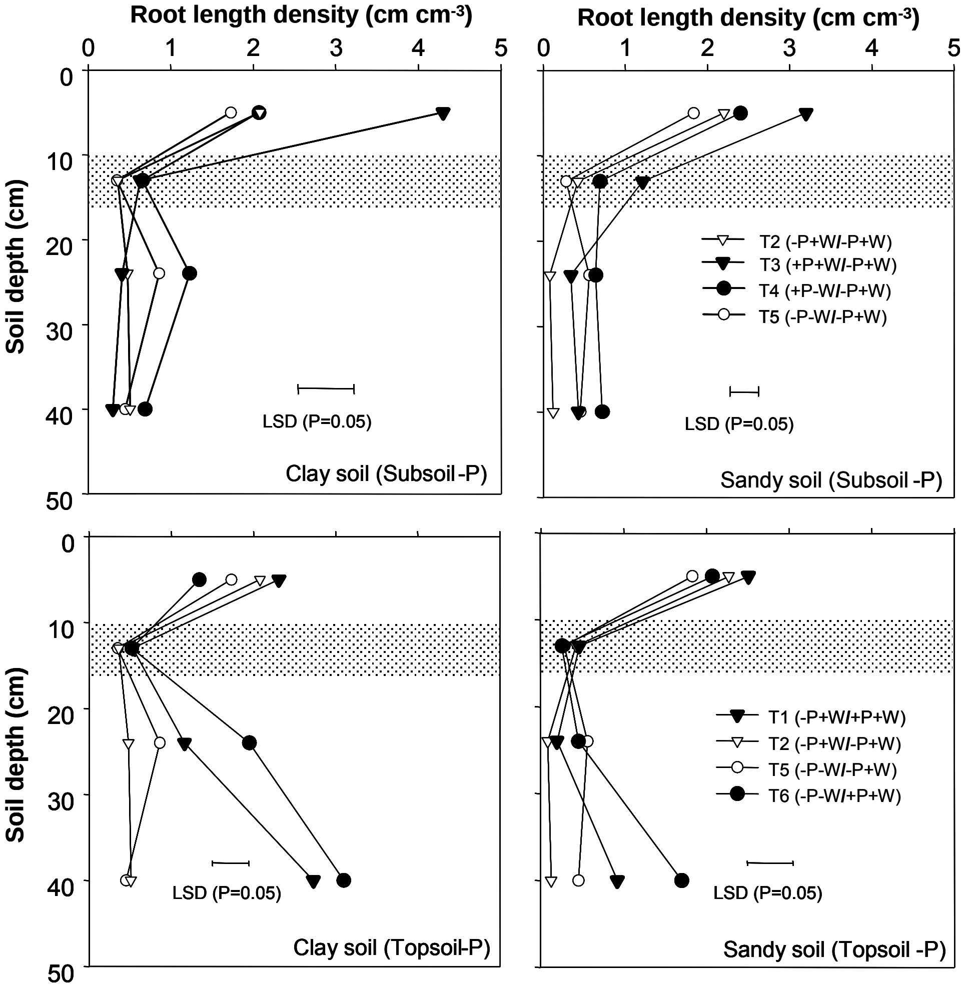


Figure 7. 4. Distribution of root length density along the soil depth of cotton plants grown without (-W) and with (+W) topsoil watering, and without (-P) and with (+P) addition of $Ca(H_2PO_4)_2$ at the rate of 120 mg P column^{-1} into the topsoil (0-10 cm) and subsoil (16-48 cm). Horizontal shaded bars show the position of the buffer layer in soil columns. Error bars represent LSD (*P*=0.05) for comparison between any two means. See Figure 7. 2. for treatment codes.

Irrespective of P treatment and soil types, the plants grown in columns with surface water withhold tended to have a higher proportion of roots in the subsoil than plants grown in columns with continuously watered topsoil. The highest root length density in the subsoil was observed for the treatments with drought stress in the surface soil and with subsoil P application (Figure 7.4). Subsoil P application increased root length density ($P<0.05$) throughout the whole subsoil in the clay soil, but only in the lower subsoil in the sandy soil, compared with no subsoil P application (Figure 7.4).

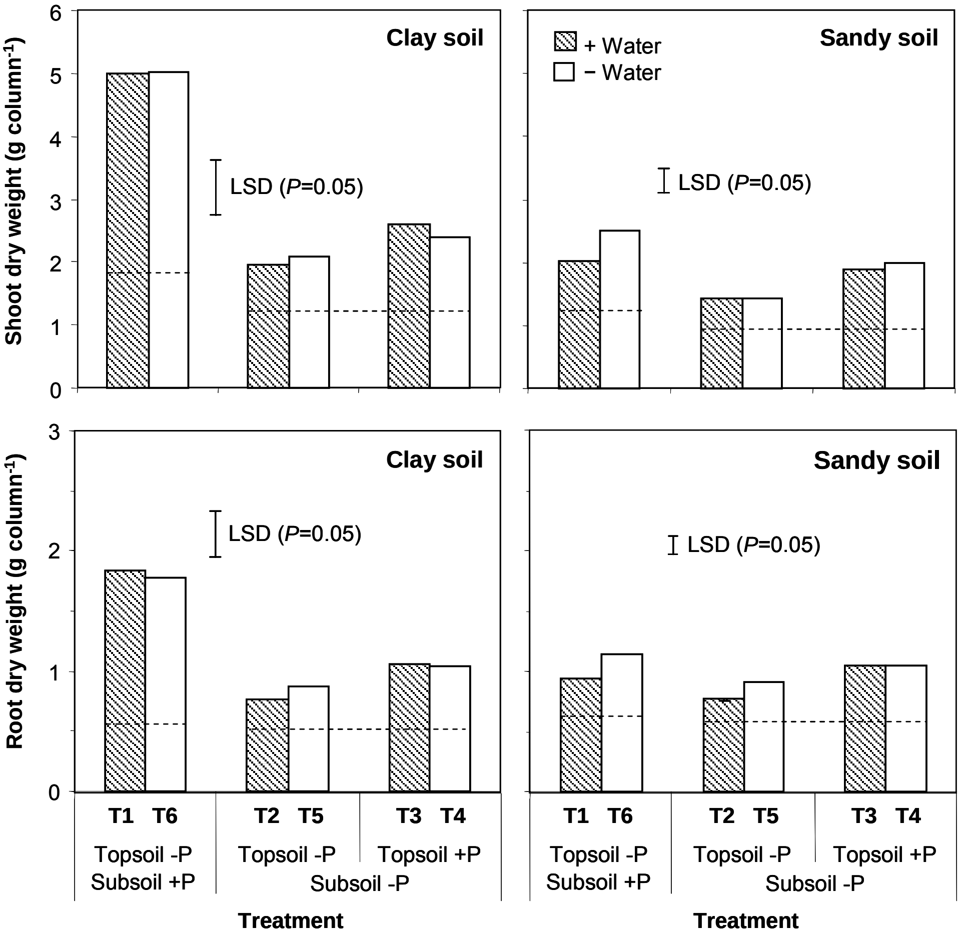


Figure 7. 5. Dry weights of shoots and roots of cotton plants grown without (-W) and with (+W) topsoil watering, and without (-P) and with (+P) addition of Ca $(H_2PO_4)_2$ at the rate of 120 mg P column^{-1} into the topsoil (0-10 cm) and subsoil (16-48 cm). The dotted lines represent the value at the commencement of the treatment. The error bars are LSD for any two means. See Figure 7. 2. for treatment codes.

7.3.3. Shoot and root growth and P uptake

The plants harvested prior to withholding water provided a baseline for comparison between the effects of deep and surface P application (Figure 7.5). Regardless of watering treatment, deep P placement (T1 and T6) in the clay soil increased biomass production of shoot and roots threefold

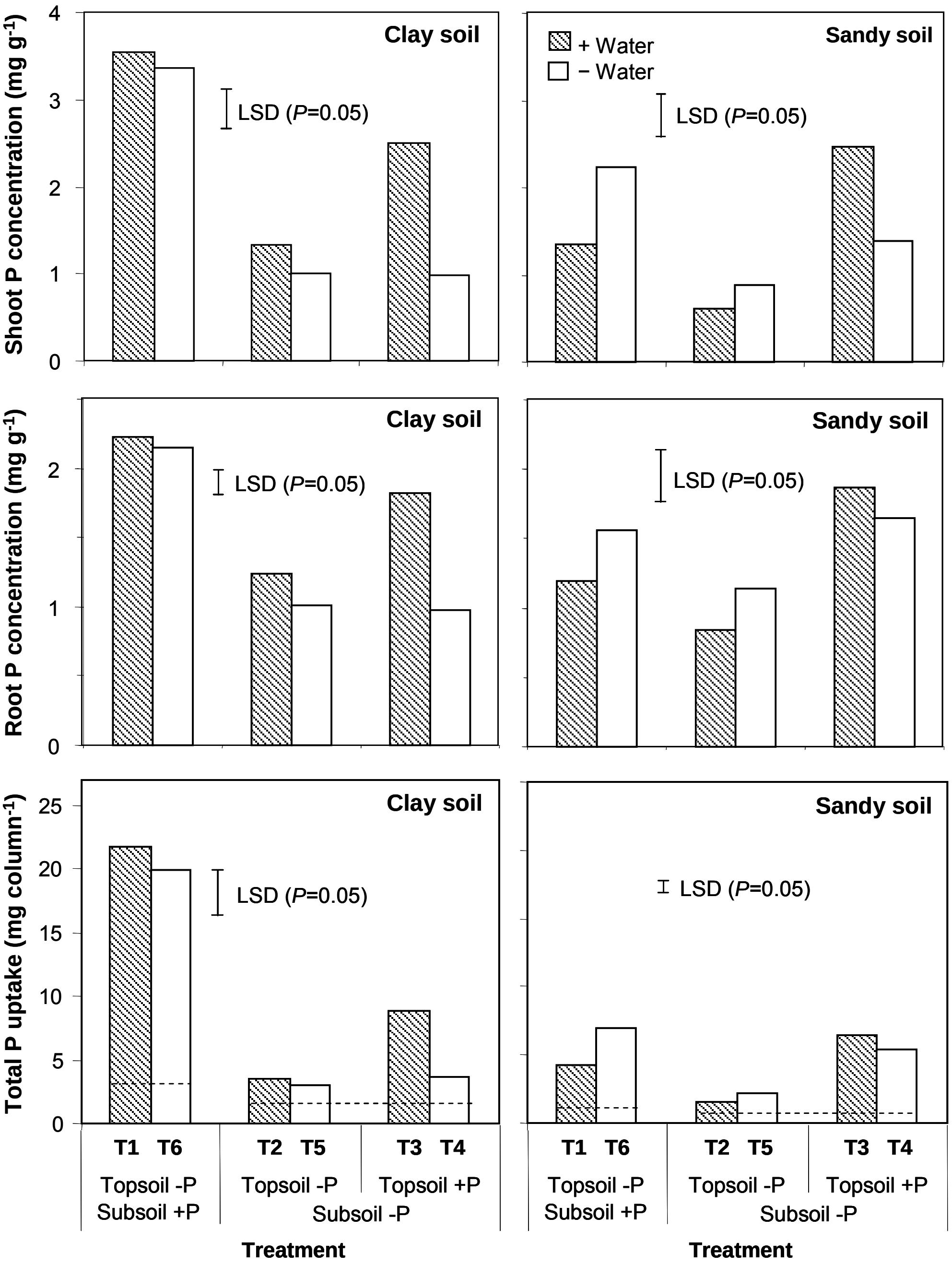


Figure 7. 6. Concentrations of P in shoots and roots, and total P uptake of cotton plants grown without (-W) and with (+W) topsoil watering, and without (-P) and with (+P) addition of Ca $(H_2PO_4)_2$ at the rate of 120 mg P column^{-1} into the topsoil (0-10 cm) and subsoil (16-48 cm). The dotted lines represent the values at the commencement of P and water treatment. The error bars are LSD for the water × P interaction. See Figure 7. 2. for treatment codes.

(from the baseline), compared to the treatment where no P was applied to either the topsoil or subsoil (T2 and T5). By comparison, surface P application (T3 and T4) did not significantly affect biomass production. For the sandy soil, P application increased shoot and root growth (from the baseline) by approximately 95% irrespective of the soil layer to which the P was applied (Figure

7.5). Water-deficiency stress in topsoil did not significantly affect biomass production (Figure 7.5, Table 7.2).

In the clay soil, the application of P in the subsoil (T1 and T6) increased P concentrations in shoots and roots and total P uptake to a greater extent than P application in the topsoil (T3 and T4), relative to the treatments without P supply (T2 and T5) (Figure 7.6). When P was applied to the topsoil, P concentration in shoot and roots and total P uptake was more suppressed by the surface water-deficiency on the clay soil than that on the sandy soil (T3, T4) (Figure 7.6).

A key finding in this study is that in the sandy soil, P application to the surface soil increased total P uptake by the cotton plant, even when water was withhold from the surface soil (Figure 7.6). This did not occur in the clay soil. Similarly, P applied in the surface of the sandy soil increased P concentration in shoots and roots, irrespective of whether water was withhold from the surface layer. This was not the case for the clay soil.

7.4. Discussion

7.4.1. Hydraulic lift

Overnight increases in soil water content in the dry topsoil can be interpreted as water efflux from the roots (Baker and van Bavel 1988; Caldwell *et al.* 1998), although the quantity of water release might be underestimated due to the night-time evaporation and transpiration. Therefore, cotton plants hydraulically lifted water for at least the first three days in the clay soil. The relatively constant soil water content during the following four days could also be attributed to the buffering effect of hydraulic lift; otherwise, soil water content should have declined further as a result of both surface evaporation and root uptake. Furthermore, the lack of significant daytime reductions in soil moisture from day 4 to day 7 indicates that hydraulic lift was not always confined to the night time. Reduced transpiration from overcast conditions that occurred during this period might also create a water potential gradient between root and soil, driving day time hydraulic lift.

The magnitude of hydraulically-lifted water overnight, when averaged over time and treatment, was greater than that reported for canola (2~3 g kg^{-1} soil) (Rose *et al.* 2008), but close to pearl millet (27 g kg^{-1} soil) (Vetterlein and Marschner 1993) and wheat (~25 g kg^{-1} soil) (Valizadeh *et al.* 2003). By using a horizontal spit-root box, Baker and van Bavel (1988) detected that the amount of water transferred by the cotton root from the wet soil compartment to the dry soil compartment (~0.05 m^3) overnight ranged from 0.13 to 0.37 kg (2-6 g kg^{-1} soil) which were much less than what I had measured. However, if cotton taproots are mainly responsible for lifting water from the subsoil to the topsoil, then it is not surprising that less water was lifted in the study of

Baker and van Bavel (1988), as tap roots might be excised after seed germination in order to horizontally split the promoted lateral roots into wet and dry soil compartment. Nevertheless, the maximum specific water release by cotton plant overnight, calculated as 80 g H_2O g^{-1} dry roots, in this study, was lower than that reported for the wheat plant (~100 g H_2O g^{-1} root) (Valizadeh *et al.* 2003) which possesses a relatively large root surface area per unit root dry weight.

Hydraulic lift ceased after 7 days of surface drying in the clay soil. This is consistent with the findings of Vetterlein and Marschner (1993) that hydraulic lift by pearl millet occurred for only 8 days after the drought treatment. In contrast, hydraulic lift was observed for up to 28 days in canola (Rose *et al.* 2008). Since the occurrence of hydraulic lift largely depends on factors such as root hydraulic conductivity, root-soil contact and soil hydraulic conductivity (Nobel and Cui 1992; Passioura 1988), loss of hydraulic lift occurs where water stress adversely affects any of these factors. At harvest, the high root mortality in the clay soil suggests that either reduced root hydraulic conductivity or root soil contact may have limited continuing hydraulic lift.

For the sandy soil, soil water content showed a continuous depletion pattern and the detection of hydraulic lift was transient and only confined to the treatment with subsoil P application. In order to clarify the discrepancy between two soil textures, I suggest hydraulic lift occurs due to two independent processes. First, water is redistributed within the plant at night or during periods of minimal transpiration. The direction is normally upward towards the roots in the dry surface layer (Caldwell *et al.* 1998). The degree and rate of water redistribution within the plants depends on the root hydraulic conductivity and percentage of roots present in the moist soil. Apart from the increased root length density resulting from subsoil P application, the sandy soil had relatively lower root length density in the subsoil, compared with the clay soil, and thus less water might be lifted up to the roots in the topsoil.

Second, water is released from the roots to soil following establishment of a water potential gradient. Cotton grown on the sandy soil would have lower root-sand surface contact than the clay soil (Passioura 1991). The decreased contact between the root and sand particles would greatly increase the resistance of water movement. Hence, much of the water lifted by the deeper roots might not necessarily be released from the roots to the sandy soil, resulting in less overnight increase in soil water content. In addition, the rapid drying of the sandy soil would have decreased soil hydraulic conductivity (Nobel and Cui 1992) and hence reduced the amount of water that could potentially be released to a dry soil (Vetterlein and Marschner 1993). Therefore, apart from factors such as growth stage, severity and duration of drought (Caldwell *et al.* 1998), detection of hydraulic lift for a species also depends on subsoil root distribution and soil properties such as texture. Yoder and Nowak (1999) also observed negative correlations between the frequency of observed hydraulic lift and increasingly-coarse soil textures.

7.4.2. P uptake from the dry surface soil

Root P uptake from the dry topsoil of the clay was negligible (Figure 7.6) despite the fact that soil moisture was maintained by the hydraulic lift at the beginning of the drought. On the other hand, increased P uptake from surface-applied P on the dry sandy soil was unlikely to be attributed to hydraulic lift since overnight increase in soil water were not observed for these treatments. Therefore, the detected hydraulic lift in the clay soil did not facilitate P uptake from the dry surface soil by cotton. Earlier work is equivocal with respect to enhanced nutrient uptake arising from hydraulic lift. Some authors reject a significant increase in nutrient uptake (Crabtree *et al.* 1998; Grundon 1980; Simpson and Lipsett 1973; Vetterlein and Marschner 1993) whilst others observed a net increase in the uptake of immobile nutrients such as Zn and P (Matzner and Richards 1996; Nambiar 1976; Rose *et al.* 2008; Valizadeh *et al.* 2003). However, Nambiar (1976) found that Zn uptake from the dry surface soil by oats could be attributed to mucilage exudation. In addition, Rose *et al.* (2008) and Valizadeh *et al.* (2003) used a single layer of beads in their experiment to act as a water barrier but this could not fully prevent the movement of water vapor from the subsoil to the topsoil. Nevertheless, significant P uptake, if it was aided by the hydraulic lift in both of these studies (Rose *et al.* 2008; Valizadeh *et al.* 2003), would have occurred in a loamy sand soil. Thus I speculate that part of the discrepancy in detecting the benefit of hydraulic lift on nutrient uptake might also lie in the effect of soil texture.

In this study, difference in P uptake by cotton from the dry surface of the clay compared to the sandy soil could be explained by the kinetics of P diffusion, P availability, root production and mortality. According to an early report by Olsen and Watanabe (1963), differences in the P diffusion coefficients between the clay and sandy soils could make the uptake rate of P from a clay soil three times lower than that from a sandy soil, at similar solution P concentrations. In addition, P applied to the clay soil would be less available due to the higher P sorption capacity than that of the sandy soil. My study also demonstrated that P uptake from the clay soil was more suppressed by the water stress, than from the sandy soil. Therefore, roots in water-deficient clay topsoil would extract P at a much slower rate than from a sandy soil, and P uptake from the clay soil would be more dependent on new root extension to exploit soil P resources. Although new root extension in the dry topsoil of the clay soil was detected, its contribution to P uptake would be questioned due to the high mortality of roots with water stress. On the contrary, enhanced root proliferation and continuing root viability accounted for significant P uptake from the surface-dried sandy soil by the cotton plant. It has been suggested that root growth is more responsible for the P uptake than P diffusion rate under water-stressed conditions (Barber and Mackay 1985; Crabtree *et al.* 1998; Vetterlein and Marschner 1993).

7.4.3. P uptake from the moist subsoil

Compared to the sandy soil, the clay soil was relatively more fertile and held more available water. Irrespective of topsoil watering, higher root length density in the subsoil of the clay soil accounted for the pronounced response to deep P placement, when compared with either the sandy soil or surface P application. Consistent with many studies, surface drought induced more root proliferation on the subsoil (Benjamin and Nielsen 2006; Hoogenboom *et al.* 1987; Merrill *et al.* 2002). For the clay soil, increased root proliferation in the subsoil due to surface drought was possibly counter-balanced by net root loss in the dry topsoil, resulting in a similar total root dry weight to that with topsoil watering. The potential benefit of subsoil P sources and application has been demonstrated in many studies (Haak 1977; Kuhlmann and Baumgartel 1991; Norrish *et al.* 2003; Singh *et al.* 2005). Frequent surface drought decreases both root growth and function in the topsoil (Purohit and Mukherjee 1974) but increases the significance of subsoil P nutrition.

Although cotton growth was much better when P was placed in the subsoil rather than topsoil, even when both topsoil and subsoil were watered, extending the results to the field conditions is not warranted. The same amount of P was applied to each column, and P was more concentrated in the topsoil than in the subsoil (as occurs in the field). However, it should be noted that P was applied to the topsoil half way through the experiment, which inevitably delayed the P access and decreased the growth. In addition, the limited topsoil volume forced more roots to grow down to the subsoil. Further research into subsoil P placement in the field prone to surface drought is necessary.

7.5. Conclusions

Hydraulic lift was detected for the cotton plant, but its magnitude and longevity were greatly affected by the soil texture. Hydraulic lift detected in the clay soil did not aid P acquisition by cotton plants from the dry surface soil. Difference in P uptake between clay and sandy soil could be explained by the kinetics of P diffusion, P availability, root production and mortality. Since the contribution of hydraulic lift to P uptake from dry surface soil was less significant, low responsiveness of cotton to P fertilizers in the field conditions could partly be explained by the decreased P availability due to topsoil water stress, or by the subsoil root P exploration, providing available P sources are present in the subsoil.

Chapter 8: Changes in phosphorus fractions at various soil depths following long-term P fertilizer application on a Black Vertosol from southeastern Queensland

8.1. Introduction

Colwell soil test phosphorus (P) (sodium bicarbonate reagent) (Colwell and Esdaile 1968) is routinely used as an indicator of plant-available P in soils of northern New South Wales and southern Queensland. This method has a wider solution/soil ratio and longer extraction time when compared with other soil tests such as Olsen and Bray. Colwell (1963) reported that the 100:1 ratio gave more reproducible results for several reasons, one being more reproducible equilibrium conditions associated with the long shaking period, hence P could equilibrate from the relatively slowly available pools making it more closely related to the exchangeable P than P response (Holford 1997). Combined with the measurement of P buffer capacity, Colwell P provides an accurate prediction of plant-available P (Holford 1980; Holford and Mattingly 1979).

However, the application of the Colwell-P soil test as an indicator of P sustainability in Vertosols is questioned by recent studies conducted in northern-eastern Australian. For example, Dorahy *et al.* (2004) reported low responsiveness of cotton to P fertilizer applied on Vertosol even with low Colwell P value. On the other hand, Lester *et al.* (2003) showed an unchanged Colwell P value in the nil P treatments despite more than 200 kg P ha^{-1} being removed from the soil in grain. Although it is generally accepted that exchangeable P is the major source of plant-available P in neutral to alkaline soils (Holford and Mattingly 1976), these results suggest that exchangeable P or plant-available P in Vertosol is being replenished from other soil P pools.

Phosphorus pools that may buffer soil test P levels have been identified for various soil types through P fractionation methods (Beck and Sanchez 1994; Saleque *et al.* 2004; Sharpley 1996). Without P addition, P fractions considered non-labile may become increasingly available to plants, although the proportion of contribution depends on soil type and other environmental conditions (Guo *et al.* 2000; Schmidt *et al.* 1996). Beck and Sanchez (1994) reported that sodium-hydroxide-extractable inorganic P (NaOH-P_i) was the dominant fraction related to plant P availability in an 18-year continuously cultivated and fertilized cropping system on an Ultisol. In contrast, acid-extractable and residual-P fractions were found to act as a major buffering pool for labile P in slightly weathered soils (Hedley *et al.* 1982b; McKenzie *et al.* 1992a; McKenzie *et al.* 1992b). It seems that the main P buffering pools are closely associated with both soil type and the dominant P fractions. Application of P fertilizer can replenish the labile P pools and maintain or increase soil fertility. The sink of P fertilizer can be in the more labile fraction (Iyamuremye *et al.* 1996;

Oberson *et al.* 1999) or stable fractions such as acid-extractable or even residual pools (Daroub *et al.* 2000; Linquist *et al.* 1997; Zhang and MacKenzie 1997a). Investigating the source of P for plant uptake is problematic due to the confounding effect of continuous P fertilizer input.

To date, the majority of soil P research worldwide has focused on the topsoil. Little attention has been given to subsoil P pools which may act as an additional source of plant available P. In the northern region of eastern Australia where stored moisture is critical to successful crop production, the contribution of subsoil P resources needs to be quantified considering that the roots of grain or cotton crops may penetrate deeply into the soil profile, particularly later in the growing season. Norrish (2003) reported a positive correlation between grain yield and the mean concentration of Colwell-P to a depth of at least 60 cm in north-western New South Wales. Unchanged Colwell P value in the nil-P treatments at two long-term N × P experiment sites (Lester *et al.* 2003) demonstrated the necessity to investigate what was going on below the soil test depth of 0-10 cm. In this chapter, it has been hypothesized that crops could also access soil P either from subsoil layers (below 10 cm) or from surface soil P pools which are not extracted by the Colwell method. As part of this investigation, both topsoil and subsoil were collected from one of the long-term N × P fertilizer experiments and from an un-cropped reference site to study the impacts of long-term cropping with and without P fertilizer input on P fractions at various soil depths.

8.2. Materials and Methods

8.2.1. Experimental design

A long-term N × P fertilizer experiment was initiated in 1985 at the “Colonsay” property in the Norwin district of the central Darling Downs, south east Queensland. Full details of the experimental site and history are provided in Lester *et al.* (2008). Briefly, the soil at the field site is a Black Vertosol (Isbell 2002) (Table 8.1). The experiment consists of factorial combination of four N rates (0, 40, 80 or 120 kg N ha^{-1} crop^{-1}) at each of four P rates (0, 5, 10 or 20 kg P ha^{-1} crop^{-1}) in a randomised complete block design. The soil used in this study was collected in 1994 and 2003 from the plots receiving 80 kg N ha^{-1} with P application rates of 0 and 20 kg ha^{-1} at each of 3 blocks. Grain yield has been optimised at this N rate over the experiments’ history and hence has the highest grain P removal. Nitrogen was applied as urea away from the seed, and P applied as triple superphosphate (20.7% P) in the seed furrow at sowing. From 1985 to 2003, 18 crops were sown. These included 10 crops of grain sorghum (*Sorghum bicolour*), 4 crops of barley (*Hordeum vulgare*), 3 crops of wheat (*Triticum aestivum*), and chickpea (*Cicer arietinum*) once. All the plant residues were returned to the field.

Table 8. 1. Soil characteristics (0-60 cm) at Colonsay, Darling Downs, Queensland

	0-10 cm	10-30 cm	30-60 cm
pH $CaCl_2$	7.7	8.1	8.1
EC $_{\text{(saturated extract)}}$ (dS/m)	3.5	3.5	3.7
Organic Carbon (%)^A	1.2	0.9	-
Exchangeable Ca (cmol(+) kg^{-1})	36	36	29
Exchangeable Mg (cmol(+) kg^{-1})	25	25	29
Exchangeable K (cmol(+) kg^{-1})	1.4	1.4	1.1
Exchangeable Na (cmol(+) kg^{-1})	4.3	4.3	8.7
Bulk density (g cm^{-3})	1.0	1.1	1.1

^{A} = 1994 site data

8.2.2. Soil sampling and phosphorus determination

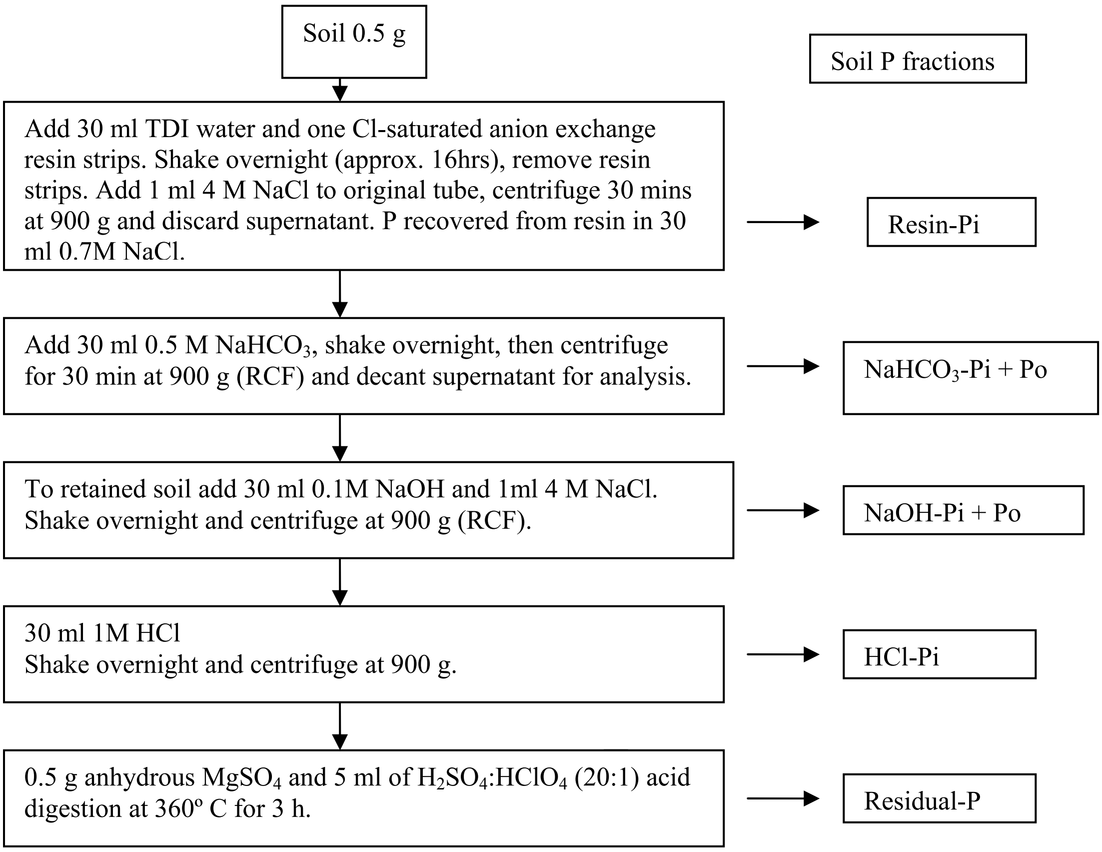
Soil samples in 2003 were collected at three depths: 0-10, 10-30 and 30-60 cm. In banded P fertilizer applications under minimal or zero-tillage, soil sampling is problematic due to the presence of residual P bands. To overcome variability in distribution and P residue differences due to varying row spacing configurations, soil samples were taken across the plot perpendicular to the line of application for 0-10 cm depth from all replicates at five distances down a row (Stecker and Brown 2001). Samples below 10 cm were collected using a hydraulic core sampler and 32 mm tube. An uncropped reference soil sample was taken from outside the planted area at the same depths. The experiment site was cropped for at least 44 years prior to establishment of the experiment. In addition, archived samples collected in 1994 from the N × P experiment were also fractionated for P determination.

Samples were dried at 40°C for 48 h, then ground to <2 mm. A subsample was further ground to ≤0.149 mm (100 mesh) for depths from 0-10 and 10-30 cm and ≤0.5 mm for depths from 30-60 cm. A modified version of the Hedley P fractionation scheme (Guppy *et al.* 2000) was used to sequentially fractionate soil P (Figure 8.1). Aliquots of the bicarbonate and hydroxide extracts were microwave digested at 120 ºC for 45 min using acid potassium persulfate (Turner *et al.* 2003a) and analyzed for total P colorimetrically. Organic P of these two fractions was calculated as the difference between total and inorganic P. Total soil P was analysed using concentrated H_2SO_4:$HClO_4$ (20:1) acid digestion to calculate the P recovery. Inorganic phosphate in the extracts was determined colorimetrically using malachite green (Motomizu *et al.* 1980). Each P pool value expressed in mg P kg^{-1} soil was converted to kg P ha^{-1} by using the measured bulk density shown in Table 8.1.

8.2.3. Calculations and statistical analysis

A P mass balance for the cropping system was generated using the following assumptions.

Phosphorus inputs were calculated based on the application of P fertilizers. Phosphorus outputs were calculated from grain yields and prior to 1992, estimated grain P concentration for sorghum (0.34%), and wheat and barley (0.28%). After 1992, grain P concentration in the fertilized treatments was measured as 0.37% for sorghum and 0.35% for wheat and barley, while in unfertilized plots, 0.33% for sorghum and 0.28% for wheat and barley. Net P balance was then calculated as the difference between P input from the fertilizer and outputs in the crop grain.



Soil 0.5 g

Add 30 ml TDI water and one Cl-saturated anion exchange resin strips. Shake overnight (approx. 16hrs), remove resin strips. Add 1 ml 4 M NaCl to original tube, centrifuge 30 mins at 900 g and discard supernatant. P recovered from resin in 30 ml 0.7M NaCl.

Add 30 ml 0.5 M $NaHCO_3$, shake overnight, then centrifuge for 30 min at 900 g (RCF) and decant supernatant for analysis.

To retained soil add 30 ml 0.1M NaOH and 1ml 4 M NaCl. Shake overnight and centrifuge at 900 g (RCF).

30 ml 1M HCl
Shake overnight and centrifuge at 900 g.

0.5 g anhydrous $MgSO_4$ and 5 ml of H_2SO_4:$HClO_4$ (20:1) acid digestion at 360° C for 3 h.

Figure 8. 1. Sequential soil P fractionation procedure modified from Guppy *et al.* (2000). Pi and Po indicate the inorganic and organic fractions, respectively, within a pool.

Although time-consuming, the sampling method was robust and variation within rows and treatments was minimal (data not shown). Variation from subsampling within rows of each treatment was included in random error terms (i.e. 9 replicates were used for soil sampled in 2003) to increase the power of the statistical analysis. Variance within each rows and the effect of P addition on distribution of P fractions along the soil depth were statistically analyzed by using a three-way analysis of variance for each fraction. The reference soil was taken at one location and three cores were bulked to a single sample. This limits the ability to perform statistical analysis on the differences in each fraction between reference site and experiment site. The long-term cropping effect between 1994 and 2003 were evaluated by a t-test between means. The statistical analyses were performed using the R software package (R 2.3.0).

8.3. Results

8.3.1. P removal in the grain from 1985 to 2003

Compared with nil P input, 20 kg ha^{-1} P fertilizer application significantly increased P uptake in grain in most years (Figure 8.2). Reductions in crop P removal in grains were frequently associated with the poor yields in these years, e.g. 1992 and 2002, irrespective of P application (Lester *et al.* 2008). On average, 12 kg P ha^{-1} had been removed from the unfertilized soil each year. No significant decreasing pattern in P removal could be detected within each crop species from 1985 to 2003. Additionally, the extent of P removal in response to P addition varied between crop species over 18 years. With 20 kg ha^{-1} P input, the averaged P uptake increased by 19% for sorghum and 55% for wheat and barley. Differential P response could be related to many factors, particularly variation in rainfall distribution and crop water use, or vesicular arbuscular mycorrhizae infection (Lester *et al.* 2008; Thompson 1987).

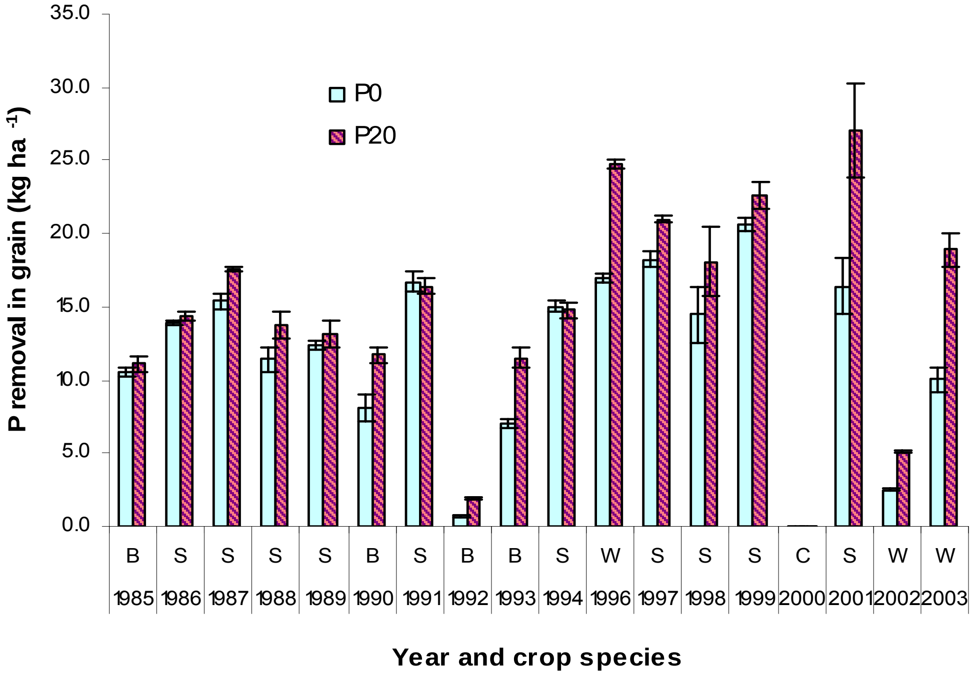


Figure 8. 2. P removal in response to P fertilizer application at Colonsay, Darling Downs, Queensland from 1985 to 2003. Presented data are selected from plots received 80 kg N ha^{-1} at two P rate: 0 and 20 kg ha^{-1}. Error bars represent standard error of means of 6 replicates. Note: B: Barley; S: Sorghum; W: Wheat; C: Chickpea.

8.3.2. Effect of fertiliser P addition on the distribution of P fractions along the soil depth

The fractionation scheme used in this study extracted more than 90% of the total P from the soil (Table 8.2). Acid-extractable P and residual-P were the dominant fractions and collectively constituted to at least 75% of the sum of all fractions. Less than 30% of the total P, irrespective of soil depth or treatment, was extracted from labile (Σ Resin + $NaHCO_3$ + NaOH) fractions (Table 8.2). Both total P and labile P pools decreased with depth, an effect more noticeable in the fertilized treatments (Table 8.2). In the surface 0-10 cm, the ratio of labile P to total P increased from approximately 5% without P application to 20% with P application (Table 8.2). The proportion of NaOH extracted inorganic P (NaOH-P_i) had also been raised from 4% to 8% ($P<0.05$) with P application. Minimal influence of P fertilization on soil P fractions was observed below 10 cm (Table 8.2). Only small increases in resin-P and NaOH-P_i ($P<0.05$) at 10-30 cm depth were observed. There were no differences in any pools within the 30-60 cm layer.

Bicarbonate-extracted organic P ($NaHCO_3$-P_o) is negligible (less than 1%) while hydroxide-extractable organic P (NaOH-P_o) accounted for less than 8% of the total extracted P at top soil (Table 8.2). Both of these organic pools decreased with soil depth as expected since under no-till systems, crop residues remained at the surface.

8.3.3. Cropping effects on soil P

The effect of conversion to cropping on soil P fractions was evaluated by comparing the reference site, which was assumed to represent the original soil condition, to the 2003 samples (Table 8.3). Without P fertilisation, there was a total P loss of 264 kg ha^{-1} from the soil profile, 55% of which came from the surface 10 cm, with 35% and 10% from 10-30 cm and 30-60 cm, respectively. Resin-P + $NaHCO_3$-P_i in the top 10 cm decreased by 70%, and accounted for 8% of the total P decrease. The NaOH-P_i pool declined by 60% and accounted for 5% of total P decrease. Acid and residual-P, theoretically the most stable pools, dropped by 50 and 20%, respectively, and accounted for about 19% and 15% of the total P decrease. Below 10 cm, very little P was removed by the first three extractants. Most of the P was present in the acid and residual fractions (Table 8.2). The acid and residual-P dropped by 30% and 12%, respectively, at 10-30 cm while 12% and 8% at 30-60 cm. By comparison with the reference soil, long-term application of P fertilizer caused the soil to accumulate P preferentially in the labile fractions (Resin/$NaHCO_3$/NaOH) in the surface 10 cm (Table 8.3). Resin-P and $NaHCO_3$-P_i doubled. NaOH-P_i and acid pool increased by 25% and 10%, respectively, whilst residual-P decreased by 14 %. Below 10 cm, acid and residual pools were the dominant fractions and their reduction was equivalent to that of unfertilized soil.

Change in $NaHCO_3$-P_o was undetectable due to its minimal proportion of total P (Table 8.3). However, NaOH-P_o pools from 0 to 60 cm decreased by about 30% regardless of P application. In the unfertilized soil, decrease of this pool accounted for nearly 10% of total P decrease from 0 to 60 cm.

Table 8. 2. Distribution of different soil P fractions (mg kg^{-1}) at soil depths of 0-10, 10-30 and 30-60 cm on a Black Vertosol collected from a long-term experiment site at Colonsay, Darling Downs, Queensland in 1994 and 2003. Reference represents a site which remained uncropped since the start of trial and was included for comparison. Standard errors are shown in parentheses.

			NaHCO$_3$		NaOH						
P addition (kg ha^{-1})	Soil depth (cm)	Resin-P	Pi	Po	Pi	Po	HCl-P	Residual-P	Sum	Total P	Recovery (%)
		………	………	………	………	mg kg^{-1} ………	………	………	………	………	
					2003						
0	0-10	5.5 (0.5)	5.0 (0.2)	1.8 (0.2)	10.7 (0.3)	21.4 (0.9)	61 (2)	154 (1)	259 93)	270 (4)	96
	10-30	0.8 (0.2)	2.2 (0.1)	0.5 (0.1)	5.1 (0.4)	9.9 (1.0)	57 (3)	139 (3)	214 (3)	223 (2)	95
	30-60	1.1 (0.1)	1.6 (0.1)	0.5 (0.1)	5.7 (0.4)	5.8 (1.2)	68 (6)	129 (3)	212 (5)	223 (3)	95
20	0-10	33.7 (1.4)	34.1 (1.4)	2.9 (0.6)	34.1 (1.1)	17.2 (1.6)	130 (4)	170 (1)	407 (4)	450 (5)	93
	10-30	2.5 (0.6)	2.7 (0.5)	0.3 (0.0)	7.3 (0.6)	7.8 (0.8)	59 (2)	139 (3)	218 (5)	228 (5)	96
	30-60	1.1 (0.1)	1.4 (0.1)	0.5 (0.1)	5.6 (0.2)	4.8 (0.3)	69 (6)	130 (3)	212 (6)	221 (3)	96
Reference	0-10	16.7	16.8	3.3	27.1	32.1	117	198	411	410	95
	10-30	1.7	3.0	1.0	9.0	13.7	82	157	267	267	100
	30-60	1.3	1.7	0.3	5.8	7.7	72	136	240	232	104
					1994						
0	0-10	7.8 (0.6)	5.0 (0.4)	2.1 (0.1)	11.9 (0.9)	22.8 (0.4)	80 (4)	179 (4)	309 (4)	316 (5)	97
	10-30	1.3 (0.4)	1.5 (0.7)	1.4 (0.0)	5.6 (1.2)	9.0 (0.7)	65 (4)	148 (4)	234 (6)	238 (4)	98
	30-60	1.6 (0.4)	2.0 (0.2)	0.4 (0.1)	5.4 (0.4)	5.4 (1.1)	70 (7)	132 (5)	218 (7)	226 (9)	94
20	0-10	28.9 (4.0)	20.1 (2.7)	3.0 (0.3)	23.5 (0.9)	16.5 (2.0)	121 (4)	180 (6)	386 (7)	419 (8)	94
	10-30	2.0 (0.9)	1.7 (0.8)	1.4 (0.3)	6.6 (1.5)	8.3 (0.5)	69 (6)	147 (4)	230 (5)	240 (6)	96
	30-60	1.6 (0.3)	2.0 (0.2)	0.1 (0.1)	5.2 (0.5)	4.2 (1.5)	70 (10)	132 (3)	219 (4)	226 (7)	97

Changes were also seen in soil P fractions from 1994 to 2003 (Table 8.3). In the nil P treatment there was a decline in profile total P of 88 kg ha^{-1}, with the proportions lost from each depth being remarkably similar to those for the comparison based on the reference soil (Table 8.3). Labile P pools in the surface soil (0-10 cm) remained relatively unchanged during these 10 years of continuous cropping without P input. However, acid and residual-P pools decreased greatly and explained almost all of the total P decrease in the surface soil. With P addition, labile pools acted as the main sink for P. The acid pool increased by 7% while the residual-P showed an obvious decrease at the top soil. At depth 10 to 30 cm, acid and residual pools were the dominant fractions and significantly decreased irrespective of fertilizer addition. Below 30 cm, no significant changes were detected for each fractions and total P. Unlike that based on the reference soil, NaOH-P$_o$ pools did not contribute significantly to the plant uptake during these 10 years continuous cropping with and without P input. Therefore, it is possible that the reference soil had increased this fraction over 18 years by deep grass roots pumping P up from below 60 cm.

Table 8. 3. Changes in different P fractions (kg P ha^{-1}) at depth 0-10, 10-30 and 30-60 cm due to long-term effects of continuous cropping with and without P fertilizer application from a Black Vertosol at Colonsay, Darling Downs, Queensland. Data in parentheses are in per cent of total soil P decreases at 0-60 cm depth.

Treatment	depth	Change in P fractions and total P (kg P ha^{-1}) Resin-P + NaHCO$_3$-P	NaOH-Pi	NaOH-Po	HCl-P	Residual-P	Total-P
				1. Reference-2003^A			
P0	0-10 cm	-24.7 (8)	-16.6 (5)	-10.9 (4)	-56.6 (19)	-44.8 (15)	-144.3 (55)
	10-30 cm	-4.7 (2)	-8.4 (3)	-8.1 (3)	-52.6 (17)	-38.4 (13)	-94.0 (35)
	30-60 cm	-0.5 (0)	-0.3 (0)	-6.1 (2)	-11.0 (4)	-22.1 (7)	-26.0 (10)
P20	0-10 cm	+34.3	+7.1	-15.1	+11.8	-29.1	+40.0
	10-30 cm	-0.6	-3.7	-12.5	-48.9	-37.9	-84.0
	30-60 cm	-0.5	-0.6	-9.3	-9.8	-18.9	-33.6
				2. 1994-2003^B			
P0	0-10 cm	-2.6	-1.2	-1.4	-20.2	-25.6	-46.5 (53)
	10-30 cm	-1.5	-1.1	+1.9	-17.0	-19.1	-31.8 (36)
	30-60 cm	-3.1	+0.9	+1.3	-6.3	-9.5	-9.5 (11)
P20	0-10 cm	+19.0	+10.7	+0.7	+9.1	-10.1	+31.3
	10-30 cm	+0.7	+1.5	-1.1	-21.2	-16.9	-25.4
	30-60 cm	-2.2	+1.3	+1.9	-3.2	-6.3	-9.5

[A] Difference in P fractions between soil samples in 2003 and the reference soil (uncropped since start of trial (1985)

[B] Difference in P fractions between soil samples in 2003 and those in 1994.

8.3.4. P balance

Grain P removal by the crops during these 18 years was estimated as 210±11 and 280±10 kg ha^{-1} for unfertilized and fertilized soil, respectively. By comparison with the reference site, an estimated 260 kg P ha^{-1} had been removed from the top 60 cm of the unfertilized soil, which is slightly higher than the grain export in these 18 years (Figure 8.3). Where P fertilizer was applied at 20 kg P ha^{-1} crop^{-1}, it was expected that approximate 80 kg P ha^{-1} should have accumulated in the soil (difference between the P input and grain removal) (Figure 8.3). Although increases in labile and NaOH-P$_i$ fractions in the top 10 cm can explain 55% of this balance, concomitant decreases in the residual fraction and subsoil pools resulted in a decrease in total soil P level (Fig. 3). When compared with the soil sample in 1994, total P decrease in the unfertilized soil in 2003 only contributed to about 80% of crop uptake from 1994 to 2003, suggesting that crops had exploited additional P sources outside the surface 60 cm. In comparison, with the P input (20 kg ha^{-1} crop^{-1}), approximately 30 kg ha^{-1} P was missing from the top 60 cm profile over 10 years.

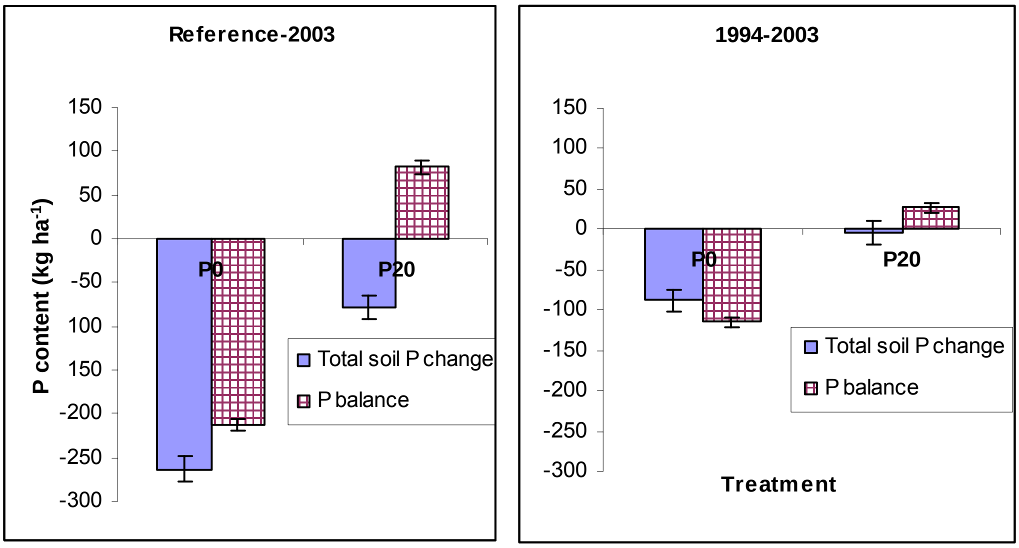


Figure 8. 3. Total soil P change and P balance from 1994 to 2003 and from 1985 (represented by the reference site) to 2003. Total P balance was calculated as the difference between P input from the fertilizer and outputs in the crop grain. Total soil P changed was the difference between the measured total soil P content (kg ha^{-1}) at depth 0-60 cm in 1985, 1994 and that in 2003. Error bars represent the standard error of means of 6 replicates.

8.4. Discussion

8.4.1. P balance

In the literature, unaccounted P balances have been frequently reported (Blake *et al.* 2003; Goel and Noguera 2006; Spears *et al.* 2003). The magnitude of discrepancies depends on many factors (e.g. rainfall, soil properties, root distribution, etc) that influenced the input and output balance of a certain soil system, or on the sampling method, estimation of P concentration in the grain and laboratory analysis, although the latter is relatively less significant (less than 30 kg P ha^{-1} in my study). Blake *et al.* (2003) suggested a loss of P from surface soil, when more than 150 kg P ha^{-1} was missing during 20 years of continuously cultivation with and without P input on a Chromic Luvisol and Eutric Gleysol. The P loss by surface runoff is less likely to occur at the site in this study since the whole experiment area is quite flat. Since larger errors in P balance were observed between the reference site and the 2003 data than that between the 1994 and 2003 data**,** we suggest that grass growing at the reference site could have redistributed P from between depths, which may subsequently raise the total P level from 0 to 60 cm depth at the reference site. Further reference sampling at the site and in the surrounding areas may allow better definition of the "native" soil P fractions.

This also raises issues regarding the on-going management of long-term agronomic experiments. The loss of soil samples collected at the commencement of this experiment inhibits the ability to elucidate changes in soil P pools since the start of the experiment. Communication to staff members within operating organizations regarding the value of archived samples and the potential opportunities that may exist in the future for investigation is key ingredients in successful sample retention (Martin and Grace 1998).

8.4.2. Accumulation of applied fertilizer P on the surface

Nearly all the applied P was retained within the surface 10 cm and minimal vertical movement was observed (Table 8.2). Under the no-till system, P was expected to accumulate in the soil surface due to minimal mixing of surface-applied fertilizer (banded with seed) with soil, and cycling of P from deep soil layers to surface layer by crop residue return (Eckert 1991; Robbins and Voss 1991). Similar results were obtained by Saleque *et al.* (2004) and Han *et al.* (2005) who reported that P addition did not influence the concentration of any fraction below the plow layer. In addition, my results revealed that this retained fertilizer residual P in the surface soil was preferentially accumulated in the labile inorganic P and moderately labile P pools. Strong *et al.* (1997) also found an increase in P availability with successive P application at any rate of 4, 8, 12 and 25 kg ha^{-1} in wheat-growing soils of the Western Downs, Queensland. Transformation of P into the acid pool was evident and most likely associated with the high Ca content in this soil. This is consistent with the findings of McKenzie *et al.* (1992b) and Song *et al.* (2007) who reported that acid P increased due to fertilizer P during long-term crop production. However, Ca-P originating from liming and fertilizer P could be among the least stable P compounds in specified soil types and can be readily re-mobilised back into the labile pools (O'Halloran *et al.* 1987).

8.4.3. Depletion of residua-P pools

It was evident that acid and residual-P pools were depleted due to grain P removal without P addition, hence they appear to act as the main buffering pools replenishing plant available P in the unfertilized soil. This is consistent with the dominance of these fractions in the soil. The dynamics of the residual pool is more complex than those of labile pools due to its unknown nature. This pool is routinely considered as the least plant available fraction. However, despite application of P, the residual pool depletion in this study implies either buffering of the labile fractions for plant uptake, or direct removal from this fraction by the crop.

In the literature, changes in residual-P under cropping system showed two contrasting patterns. Residual-P declined in slightly weathered soils (Agbenin and Goladi 1998; Hedley *et al.* 1982b; McKenzie *et al.* 1992a; McKenzie *et al.* 1992b), but seemed to build up gradually in highly weathered soils (Guo *et al.* 2000). These suggest that the residual pool separated by the same sequential fractionation method, was not of equal availability to plant in different soils. The nature of the residual pool could differ greatly among soil types. Tiessen *et al.* (1992) reported a high positive correlation between $NaHCO_3$-P_o and residual-P in Ultisols and suggested that the contribution of the residual fraction may come from its organic portion. However, Guo *et al.* (2000) demonstrated a relatively faster decline of residual-P in the Lualualei soil with higher calcite content and postulated that some plant available P in the residual fraction in that soil was Ca-P. Dalal and Mayer (1986a) reported that for Waco type soils (black earths) organic P is a property moderately affected by cultivation. Therefore, it is possible that the residual-P pool in the black Vertosol at Colonsay acted as one of the important sources of replenishment, and its contribution was mostly lying in its organic portion. Further research is needed to identify the nature of this ‘labile’ residual pool since the total organic P pool was not determined in this study.

An alternative hypothesis for the depletion of residual pools may be related to the P acquisition strategies of plants. Some crop species are capable of taking up P from stable pools, due to its specialized root morphology and physiology. A well-known example is white lupin which forms cluster roots and exudes large quantities of organic acids (Shen *et al.* 2003), and accesses up to six times more P from bound P fractions than a range of crop plants (Braum and Helmke 1995; Watt and Evans 2003). Sorghum may also possess some strategies to mobilize P from stable (acid and residual) pools down the whole soil profile. It has already been reported that sorghum could acidify the rhizosphere at low soil P status and caused an obvious P depletion zone (Ortas *et al.* 1996). Mycorrhizal infection could also be associated with the efficiency of P uptake in sorghum (Ortas *et al.* 1996; Osonubi 1994). Further opportunities in research exist to establish which hypotheses is the mechanism for the decline in the residual-P pool.

8.4.4. Depletion of subsoil (10 to 30 cm) P pool

In comparison to the reference soil, total P at 10-30 cm depth decreased greatly due to long-term cultivation with and without P input. Little published data are available on the quantitative measurement of subsoil P resource contribution. Subsoil P supply could substantially contribute to P uptake by sorghum-sudangrass in pot culture when surface P was low (Pothuluri *et al.* 1986). In a long-term field experiment, Richards *et al.* (1995) described an unaccounted P input in the 0-20 cm after 10 years of continuous cultivation without P application and implied the possibility of contribution from subsoil P pools. In addition, Kuhlmann and Baumgartel (1991) described a method which allowed the quantification of the potential uptake of P from the subsoil (>30 cm) by spring wheat. Their results showed that P uptake from the subsoil increased from 37-85% of total P uptake, with decreasing P supply in the topsoil.

These above studies suggest that low P in the surface soil results in subsoil exploitation. However, the data from this study implies that subsoil utilization occurred even when the surface soils remain relatively P fertile (Table 8.2). Part of the explanation for utilization of subsoil P is uptake of P from where moisture is also present. The Colonsay site is located in the northern cereal-belt in southern Queensland which has a summer dominant rainfall pattern. The self-mulching nature of Vertosols can create conditions where unsaturated hydraulic conductivity is negligible at the surface. Moreover, no-tillage operations and surface-banded fertilizer P tend to accumulate P in the top few centimetres of the soil profile. A relative accumulation of P near the soil surface may decrease nutrient availability to plants because of the increased likelihood of dry conditions in this zone. Therefore, the subsoil P acquisition in this experiment possibly resulted from the water stress-induced P deficiency in the surface soil. When the topsoil dries out during the growing season, P in this enriched zone would become unavailable and immobilized. Plants had to explore deeper for both water and nutrients. Crop root length density and distribution were considered to decrease exponentially with the soil depth (Gregory 2006). However, under water limiting conditions, this growth pattern could be reversed (Merrill and Rawlins 1979; Merrill *et al.* 2002). Moroke *et al.* (2005) reported that the root length density of tropical species, such as sorghum, tended to decrease at soil surface while increase at deep depths throughout the growing season. In addition, Pan and Hopkins (1991) showed a water stress-induced P deficiency in an eroded top-sequence and demonstrated an apparent P deficiency during grain filling of the crop even when P was band-applied at recommended rates during planting. Considering the decreased P availability and root distribution on the dry top soil, surface replenish of P will make the P acquisition by crops more difficult. This may help explain the lack of P response during some years.

8.4.5. Conclusion

From a resource sustainability viewpoint, Colwell P appears a poor indicator of overall P status in Vertosol. With no P input, acid and residual fractions declined steadily and acted as the main source pools at top 10 cm. In the subsoil (10-30 cm), these two pools also tended to be depleted regardless of P application. It is apparent that the alkaline reagent $NaHCO_3$ used in the Colwell P method did not result in the mobilization of some potentially plant available P present in the acid and residual P pools. The build-up of P in labile pools from fertiliser inputs accounts for the continued increase in Colwell P, despite a negative P balance. The extent to which the acid and residual pools can be depleted with regard to the long-term sustainability and productivity of grain production are questions for further research. Further subsoil sampling and fractionation of cropping soils across the northern grains region is recommended to provide further information for soil P status, and will allow further examination of appropriate extractants for estimation of subsoil P reserves.

Chapter 9: Preliminary comparisons of the effect of long-term production of cotton (*Gossypium hirsutum* L.) or wheat (*Triticum aestivum* L.) on phosphorus fractions in Vertosol profiles

9.1. Introduction

Efficient P fertility management of agricultural soils requires an understanding of the P sinks and sources in the soil. Soil P fractionation schemes have frequently been used to characterize soil P pools, and may provide a direct measure of the fate of applied P in the soil under long term agricultural use. Change in soil P pools over time has been reported on various soil types (Blake *et al.* 2003; Dalal 1997; Han *et al.* 2005), with input of different P sources (Saleque *et al.* 2004; Verma *et al.* 2005; Zhang *et al.* 2004), and under different tillage or rotation practices (Bünemann *et al.* 2006; Rossella *et al.* 2007; Vu *et al.* 2008).

Little information exists, however, on the P status of the soil following continuous cropping of different crop species. In Vertosols of northern NSW and southern QLD, cotton and wheat production are the two dominant farming systems. Cotton production requires regular P fertilizer inputs (~ 20 kg P ha^{-1}) in order to maintain soil P fertility and high lint yields, especially in the increasingly productive last 20 years (Dorahy *et al.* 2004). The amount of P removed by irrigated cotton ranges from 20 to 30 kg P ha^{-1}, and of this, approximately 15 to 26 kg P ha^{-1} is present in the seed while less than ~0.5 kg P ha^{-1} is in the lint (Dorahy *et al.* 2004; Rochester and Peoples 1998). Traditionally, leaves and stalk materials are returned to the soil and nutrients in these residues become potentially available to future crops. Thus, P removed by cotton occurs through seed removal and can constitute a significant P loss to soil reserves. In the northern grain region, recorded P removal by dryland wheat is ~10 kg P ha^{-1} (Dalal 1997; Holford and Doyle 1993; Norrish 2003). Lester *et al.* (2009) demonstrated a greater P removal rate of 16 kg P ha^{-1} with higher wheat yields at Colonsay and Tulloona, northern eastern Australia. Total P removed in the wheat straw is less than 4 kg P ha^{-1} (~20% of total P removal) (Prasad and Prasad 1983). Hence it is likely that cotton removes slightly more P on average per hectare than wheat systems. In addition, recent studies have demonstrated that wheat is superior in acquiring sparingly soluble $AlPO_4$ (Pearse *et al.* 2007; Pearse *et al.* 2006b; Chapters 4 and 6), whilst cotton is inefficient in utilizing less soluble P sources (Chapters 3, 4 and 6). The contrasting root morphology and P acquisition strategies between cotton and wheat might suggest differences in the pools of P that are used in these two cropping systems.

Traditionally, P nutrition studies have focused on the topsoil with little attention given to the subsoil P fractions. It is generally accepted that P in the surface 10 cm is the major source of plant-available P, especially considering that is the site of fertiliser P replenishment. Nevertheless, Kuhlmann and Baumgartel (1991) reported that P uptake by spring wheat from the subsoil (below 30 cm) increased from 37 to 85% of total P uptake, when P supply was decreased at the surface. Providing that sufficient P is available in the subsoil, Nayakekorala and Taylor (1990) found that root length density of cotton located at the 30 to 60 cm depth was comparable to that located at the 0 to 30 cm layer, and P uptake from deeper soil layers provided 30% of total P uptake at the square-forming stage, and 50% at maturity. In addition, the previous chapter revealed a depletion of subsoil P pools, including the HCl-P and residual-P pools, following long-term continuous cereal cropping with and without P addition (Chapter 8). Considering the potential importance of subsoil P for the P nutrition of some crops, knowledge on full profile P status is necessary for the sustainable management of soil P fertility.

The aim of the study documented in this chapter is to identify any differences in P stratification arising from the cotton and wheat cropping systems at soil depths down to 60 cm. The study will test the hypothesis that P accumulation and depletion patterns that occur in the whole soil profile, compared with an adjacent virgin soil profile, will differ between cotton and wheat production systems. The inclusion of virgin, fenceline, sites will delineate differences due to both the cropping system and continuous cropping effect.

9.2. Materials and methods

9.2.1. Site selection

Agronomists were consulted to identify properties with a history of cotton and wheat crops for more than 20 years. Sites were selected as continuous wheat or cotton where more than 75% of crops grown in that paddock were wheat or cotton respectively. Twenty years cropping history was chosen because yields and nutrient removal are likely to be higher than in the preceding 20-50 years, based on historical production trends. Cotton fields were generally irrigated but not laser leveled. Wheat production was predominantly dryland, as is typical of the northern grain region of Australia. Separate virgin (native vegetation or long-standing fencelines) sites were selected for each crop species if paddocks identified as wheat and cotton were more than 500 m apart. Otherwise common virgin sites were used for comparisons. These criteria limited the number of suitable sites considerably (Table 9.1). Only 5 sites with continuous wheat and 2 sites with cotton were identified. A third cotton/wheat site was eliminated from the study due to high P application rates immediately prior to sampling.

9.2.2. Soil sampling and analysis

Eight cores were collected at two locations in the paddock and divided into the 0-10, 10-20, 20-30, 30-45 and 45-60 cm soil layers. All samples from the same location in the paddock were bulked for each depth. Two locations should provide an indication of the soil variation within the paddock. Essentially replication in this sampling strategy is across the sites. However, when the defined systems contained only one site, the two locations were used as two replicates for the statistical analysis. All samples were air-dried and then ground to <1 mm. A modified version of the Hedley P fractionation scheme was used to sequentially fractionate soil P (Guppy *et al.* 2000). Residual P was analyzed using concentrated H_2SO_4:$HClO_4$ (20:1) acid digestion. Inorganic phosphate in the extracts was determined colorimetrically using the malachite green method (Motomizu *et al.* 1980). The pH was measured using a Thermo Orion 720 pH meter after extraction in 0.01 M $CaCl_2$ solution (1:5 w/v soil: solution ratio) by shaking for 17 h on an end-over-end shaker. Soil electrical conductivity (EC) was measured in a 1:5 soil:water suspension using a conductivity meter (CDM210). Soil organic carbon (OC) and total nitrogen (N) were analyzed using an Elemetar Vario EL III (Elementar Analysensysteme GmbH, Germany). Total organic P (P_o) was determined by the acid ignition method using 0.1 M H_2SO_4 (Walker and Adams 1958).

Table 9. 1. Location and management history of the sampling site, in northern NSW and southern Queensland.

Site	Location	Management and cultivation period	P fertilizer application
Moree (wheat)	Moree	Mostly wheat since 1960	10 kg P ha^{-1} year^{-1} for 10 years
Virgin	(North NSW)	Native grass	-
Dalby 1 (wheat)	Dalby	Mostly wheat since 1960	10 kg P ha^{-1} year^{-1} for 5 years
Virgin	(South east QLD)	Native grass	-
Goondiwindi 1 (cotton)	Goondiwindi	Mostly cotton since 1980	20 kg P ha^{-1} year^{-1} for 10 years
Virgin	(South QLD)	Native grass	-
Goondiwindi 1 (wheat)		Mostly wheat since 1980	40 kg P ha^{-1} year^{-1} for 20 years
Virgin		Native grass	-
Dalby 2 (wheat)	Dalby	Mostly wheat since 1936	10 kg P ha^{-1} year^{-1} for 10 years
Virgin	(South east QLD)	Native grass	-
Goondiwindi 2 (cotton)	Goondiwindi	Mostly cotton since 1980	10 kg P ha^{-1} year^{-1} for 2-3 years
Goondiwindi 2 (wheat)	(South QLD)	Mostly wheat since 1970	10 kg P ha^{-1} year^{-1} for 2-3 years
Virgin		Native grass	-

9.2.3. Calculation and statistical analysis

Sites were blocked according to whether there was a positive or negative P balance. Statistical analyses of data were performed by an unbalanced two-way ANOVA for the effect of long-term cropping (cultivated vs. virgin) and soil depth on each P fraction using Genstat (11th version). Changes in P pools following wheat and cotton cropping were calculated as the difference in P fractions between the cultivated sites and the corresponding virgin sites. All data were then pooled across the sites and analyzed by an unbalanced three-way ANOVA with cropping systems (wheat and cotton), P balance and soil depths as three factors. Changes in soil chemical properties were subjected to a two-way ANOVA without separating the sites with different P balances.

9.3. Results

9.3.1. Soil chemical characteristics

Table 9.2. Soil chemical properties at soil depth of 0-10, 10-20, 20-30, 30-45 and 45-60 cm in Vertosol soil collected from sites cultivated with continuous cotton (*Gossypium hirsutmum* L.) and wheat (*Triticum aestivum* L.) for more than 20 years, and from adjacent virgin sites in northern NSW and southern QLD.

Site	Depth (cm)	Organic C (g kg^{-1})		Total N (g kg^{-1})		pH		EC1:5 (μs cm^{-1})	
		Cultivated	Virgin	Cultivated	Virgin	Cultivated	Virgin	Cultivated	Virgin
Moree (wheat)	0-10	9.7	13.7	0.7	0.9	6.0	6.4	59	97
	10-20	5.8	7.5	0.3	0.3	6.7	6.9	90	110
	20-30	6.3	6.9	0.1	0.2	7.2	7.4	79	118
	30-45	5.9	5.9	0.1	0.3	7.3	7.4	80	147
	45-60	4.3	4.4	0.3	0.3	7.5	7.5	110	185
Dalby 1 (wheat)	0-10	9.8	15.0	0.7	1.1	7.4	7.3	169	131
	10-20	8.6	10.8	0.6	0.6	7.4	7.5	161	162
	20-30	6.5	6.7	0.6	0.5	7.5	7.7	189	201
	30-45	8.2	6.2	0.5	0.5	7.5	7.5	237	306
	45-60	6.6	5.3	0.4	0.5	7.6	7.5	308	533
Dalby 2 (wheat)	0-10	12.4	15.4	0.8	0.6	6.4	6.2	153	214
	10-20	12.1	12.5	0.6	0.6	6.9	7.4	122	197
	20-30	12.3	11.8	0.6	0.6	7.2	7.5	153	194
	30-45	12.0	12.7	0.6	0.7	7.2	7.3	162	194
	45-60	12.9	12.9	0.6	0.7	7.4	7.3	185	196
Goondiwindi 1 (wheat)	0-10	12.9	16.8	1.1	1.1	5.7	5.8	103	79
	10-20	11.5	12.4	0.8	0.7	6.0	6.3	66	65
	20-30	10.6	11.0	0.7	0.6	6.7	6.8	78	96
	30-45	10.2	10.5	0.7	0.6	6.9	7.3	99	216
	45-60	8.8	8.6	0.5	0.6	7.4	7.6	156	404
(cotton)	0-10	5.9	8.3	0.7	0.6	7.1	7.2	198	181
	10-20	4.9	5.9	0.3	0.3	6.4	7.4	64	139
	20-30	4.2	5.4	0.2	0.2	7.1	7.6	89	187
	30-45	4.3	5.7	0.3	0.3	7.7	7.8	310	318
	45-60	4.6	4.9	0.2	0.3	7.8	7.8	235	354
Goondiwindi 2 (cotton)	0-10	11.1	16.7	0.7	1.1	6.6	6.6	137	174
	10-20	7.8	8.8	0.5	0.6	6.7	7.0	93	126
	20-30	7.9	9.2	0.6	0.6	7.2	7.4	178	213
	30-45	8.4	8.9	0.6	0.6	7.2	7.0	266	451
	45-60	8.4	8.9	0.5	0.5	7.1	7.0	517	860
(wheat)	0-10	9.4	16.7	0.6	1.1	6.6	6.6	141	174
	10-20	7.3	8.8	0.5	0.6	6.6	7.0	161	126
	20-30	7.6	9.2	0.5	0.6	7.1	7.3	277	213
	30-45	7.5	8.9	0.5	0.5	7.0	7.0	341	451
	45-60	7.4	8.9	0.5	0.5	7.2	7.2	641	860
Analysis of Variance (F probability)									
Site (block)		<0.001		<0.001		<0.001		<0.001	
Depth		<0.001		<0.001		<0.001		<0.001	
Cropping status (cultivated vs. virgin)		<0.001		0.078		<0.001		0.002	
Depth × Cropping status		0.001		0.134		0.041		0.028	

At all sites, soil pH and EC increased with increasing soil depth, in contrast to the decreased soil organic C (OC) and total N with soil depth (Table 9.2). Total N was less affected by the long-term cropping (Table 9.2, Table 9.3). However, long term cropping with either wheat or cotton resulted in a noticeable pH decrease at the soil depth of 10 to 30 cm (Table 9.2, Table 9.3). Soil OC in the surface 10 cm was invariably higher in the virgin site (13.7~16.8 g kg^{-1}) than the corresponding cultivated sites (11.1~12.9 g kg^{-1}) (Table 9.2). There was no significant difference between long term wheat and cotton farming in either pH, EC, total N or soil OC (Table 9.3).

9.3.2. Distribution of soil P fractions

Generally, the soil P fractions were more concentrated in the surface 20 cm than below 20 cm (Table 9.4). Regardless of the site and the soil depth, residual-P was the dominant P pool in the soil and comprised about 60% to 80% of $\sum P_f$ (sum of all P fractions), followed by the HCl-P pool (~20%) (Table 9.4). Less than 10% of $\sum P_f$ was found in labile NaHCO$_3$-P$_i$ and NaOH-P$_i$ pools. Total organic P (P$_o$) in the top 10 cm ranged from 31 to 128 mg kg^{-1} (Table 9.4). It decreased with soil depth in a similar manner to soil OC.

Table 9. 3. Changes in soil chemical properties with soil depth due to long-term (>20 years) continuous cropping with cotton (*Gossypium hirsutmum* L.) and wheat (*Triticum aestivum* L.) on Vertosols in northern NSW and southern Queensland.

Depth (cm)	pH (0.01M $CaCl_2$)	EC 1:5 (µs cm^{-1})	Organic C (g kg^{-1})	Total N (g kg^{-1})
		Wheat-virgin (5 sites)		
0-10	-0.04	-14.17	-4.68	-0.18
10-20	-0.30	-12.08	-1.34	0.00
20-30	-0.26	-9.26	-0.46	0.00
30-45	-0.12	-79.86	-0.08	-0.04
45-60	+0.10	-155.26	-0.02	-0.06
		Cotton-virgin (2 sites)		
0-10	-0.10	-10.56	-9.0	-0.15
10-20	-0.65	-54.45	-1.0	-0.05
20-30	-0.35	-66.95	-1.25	0.00
30-45	+0.05	-96.95	-0.95	0.00
45-60	+0.05	-213.35	-0.40	-0.05
		Analysis of Variance (F probability)		
Depth (D)	0.002	0.03	<0.001	0.08
Systems (S)	0.18	0.22	0.34	0.94
D × S	0.21	0.30	0.67	0.89

9.3.3. Effect of cropping on soil P pools

In comparison with the virgin site, all soil P fractions underwent change following continuous cropping with either wheat or cotton (Table 9.4). For paddocks in positive P balance, the NaHCO$_3$-P$_i$, NaOH-P$_i$ and HCl-P fractions increased by about 10 mg kg^{-1} in the surface 20 cm,

Table 9. 4. Distribution of different soil P fractions (mg kg^{-1}) at soil depths of 0-10, 10-20, 20-30, 30-45 and 45-60 cm in Vertosol soils collected from sites cropped with continuous cotton (*Gossypium hirsutmum* L.) and wheat (*Triticum aestivum* L.) for more than 20 years, and from adjacent virgin sites in northern NSW and southern QLD.

Site	Depth (cm)	NaHCO$_3$-P$_i$ (mg kg^{-1})		NaOH-P$_{i\ i}$(mg kg^{-1})		HCl-P (mg kg^{-1})		Residual-P (mg kg^{-1})		Sum of all P fractions (mg kg^{-1})		Total organic P (mg kg^{-1})	
		Cultivated	Virgin	Cultivated	Virgin	Cultivated	Virgin	Cultivated	Virgin	Cultivated	Virgin	Cultivated	Virgin
Moree (wheat)	0-10	22.3	8.5	20.4	8.6	9.2	7.8	143.1	159.2	195.0	184.1	90.4	128.9
	10-20	10.2	5.5	8.7	3.3	10.3	5.8	121.5	124.0	150.7	138.6	33.6	56.5
	20-30	3.9	3.9	2.4	2.7	18.2	15.6	111.4	120.2	135.9	142.4	4.2	19.8
	30-45	5.3	5.8	3.7	3.9	1.9	1.1	105.5	106.6	116.4	117.4	32.5	35.9
	45-60	4.5	5.0	3.7	3.8	8.5	11.1	117.8	123.2	134.5	143.1	22.8	19.8
Dalby 1 (wheat)	0-10	36.8	32.1	43.8	33.6	82.1	64.9	182.4	210.4	345.1	341.0	60.5	96.1
	10-20	15.4	8.4	17.5	7.6	53.8	22.0	172.4	181.4	259.1	219.4	49.2	57.3
	20-30	7.5	4.7	4.8	2.1	27.5	17.4	136.4	138.3	176.2	162.5	29.7	56.0
	30-45	4.4	2.8	2.7	1.2	17.9	11.9	123.5	128.6	148.5	144.5	63.8	52.0
	45-60	3.0	2.4	2.6	1.7	14.7	11.5	123.8	121.9	144.1	137.5	49.4	46.9
Dalby 2 (wheat)	0-10	26.1	3.3	35.0	5.2	31.1	13.6	208.4	224.6	300.6	246.7	74.3	88.4
	10-20	15.5	2.2	9.0	5.2	15.0	12.1	198.1	201.1	237.6	220.6	52.5	56.3
	20-30	4.4	3.3	6.9	4.2	15.5	15.7	183.2	197.1	210.0	220.3	51.3	57.2
	30-45	2.4	3.7	2.3	3.8	9.3	11.9	179.8	193.4	193.8	212.8	71.2	79.9
	45-60	2.6	3.7	3.5	5.6	11.4	14.6	192.5	197.8	210.0	221.7	49.8	53.5
Goondiwindi 1 (wheat)	0-10	34.1	31.2	59.0	53.0	144.2	134.1	282.9	301.1	520.2	519.4	49.0	64.5
	10-20	27.3	15.5	41.5	26.4	118.2	100.4	295.5	305.0	482.5	447.3	56.3	63.2
	20-30	13.8	13.1	21.3	17.3	83.7	86.1	265.6	272.1	384.4	388.6	71.7	78.7
	30-45	11.4	8.6	15.6	8.7	89.4	83.0	210.2	220.2	326.6	320.5	69.1	84.8
	45-60	9.9	10.0	10.5	9.1	92.8	109.5	203.5	194.8	316.7	323.4	52.2	59.8
(cotton)	0-10	22.5	13.3	26.4	15.9	33.9	21.6	186.6	210.8	269.4	261.6	67.5	92.3
	10-20	19.0	5.8	18.2	3.5	27.1	18.9	175.5	187.9	239.8	216.1	24.3	37.8
	20-30	7.6	5.5	8.7	3.7	14.3	11.1	171.4	181.9	202.0	202.2	36.7	52.6
	30-45	4.6	4.2	3.5	3.3	11.6	15.4	141.2	148.2	160.9	171.1	35.7	42.7
	45-60	4.7	5.3	4.8	4.7	14.5	23.3	130.4	134.7	154.4	168.0	14.4	14.3
Goondiwindi 2 (wheat)	0-10	12.4	22.9	15.2	29.4	34.3	51.2	221.7	245.5	283.6	349.0	35.5	46.7
	10-20	7.0	8.6	9.9	13.2	31.9	41.1	218.3	235.0	267.1	297.9	39.8	53.6
	20-30	3.9	6.9	3.2	6.5	9.5	27.9	173.7	185.9	190.3	227.2	21.3	38.0
	30-45	5.0	7.7	3.0	7.8	25.9	39.3	170.6	181.4	204.5	236.2	42.5	66.4
	45-60	6.1	8.6	4.7	9.9	28.8	39.8	157.1	160.9	196.7	219.2	42.9	42.1
(cotton)	0-10	11.8	22.9	14.5	29.4	34.3	51.2	225.0	245.5	285.6	349.0	31.5	42.0
	10-20	10.7	8.6	13.0	13.2	25.6	41.1	208.1	235.0	257.4	297.9	34.5	53.6
	20-30	4.9	6.9	2.6	6.5	11.8	27.9	169.9	185.9	189.2	227.2	30.0	48.0
	30-45	5.2	7.7	3.2	7.8	25.9	39.3	168.8	181.4	203.1	236.2	29.5	46.4
	45-60	6.9	8.6	5.2	9.9	25.6	39.8	157.1	160.9	194.8	219.2	38.5	35.0
Analysis of Variance (F probability)													
Site (block)		<0.001		<0.001		<0.001		<0.001		<0.001		0.001	
Depth		<0.001		<0.001		0.004		<0.001		<0.001		<0.001	
Cropping status		0.004		<0.001		<0.001		<0.001		<0.001		0.001	
Depth × Cropping status		0.003		0.020		0.043		0.031		0.032		0.010	

Table 9. 5. Changes in different P fractions (mg kg^{-1}) at soil depths of 0-10, 10-20, 20-30, 30-45 and 45-60 cm following long-term continuous cropping (>20 years) with cotton (*Gossypium hirsutmum* L.) and wheat (*Triticum aestivum* L.) on a Vertosols in northern NSW and southern QLD.

Cropping system		Change in					
		mg P kg^{-1}					
P balance	Depth (cm)	$NaHCO_3$-P_i	NaOH-P_i	HCl-P	Residual-P	Sum of all P fraction	Total organic P
Wheat-virgin							
Positive P balance	0-10	+11.06	+14.50	+15.88	-20.92	+20.58	-25.93
(4 sites)	10-20	+9.23	+8.56	+14.40	-6.02	+26.89	-10.43
	20-30	+1.24	+2.18	+2.51	-7.78	-1.33	-13.70
	30-45	+0.66	+1.70	+2.68	-7.45	-2.41	-4.00
	45-60	-0.26	+0.06	-4.87	-0.03	-5.05	-1.45
Negative P balance	0-10	-10.45	-14.20	-16.90	-23.85	-65.40	-11.20
(1 site)	10-20	-1.55	-3.25	-9.25	-16.65	-30.70	-13.80
	20-30	-2.80	-3.30	-18.40	-12.25	-36.75	-16.70
	30-45	-2.60	-4.90	-13.40	-10.80	-31.70	-23.9
	45-60	-2.45	-5.10	-10.50	-3.80	-21.50	+0.8
Cotton-virgin							
Positive P balance	0-10	+9.20	+10.40	+12.30	-24.25	+7.65	-24.8
(1 site)	10-20	+13.15	+14.75	+8.25	-12.00	+24.15	-13.5
	20-30	+2.10	+5.00	+3.15	-10.45	-0.20	-15.9
	30-45	+0.45	+0.10	-3.70	-7.35	-10.30	-7.0
	45-60	-0.50	+0.05	-8.85	-4.30	-13.55	+0.1
Negative P balance	0-10	-11.10	-14.85	-16.80	-20.50	-62.40	-10.5
(1 site)	10-20	-2.40	-0.20	-15.60	-26.90	-44.60	-19.1
	20-30	-1.80	-3.90	-16.15	-16.60	-38.85	-18.0
	30-45	-2.50	-4.60	-13.45	-12.00	-32.15	-16.9
	45-60	-1.70	-4.65	-14.20	-3.8	-24.30	+3.5
LSD (P=0.05)		3.61	4.53	7.54	10.14	11.97	14.78
	Depths	***	**	***	**	**	***
	P balance	***	***	***	*	***	n.s.
	Systems	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Depths × P balance	***	***	**	*.	***	n.s.
	Depths × Systems	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	P balance × Systems	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
	Depths × P balance × Systems	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s., $P>0.05$; *, $0.01>P>0.05$; **, $0.001>P>0.01$; ***, $0.001>P$

Table 9. 6. Significant correlation coefficients (*P*<0.05) between soil chemical properties and each P fractions.

Variable	Cotton					Wheat					Virgin				
	pH	EC	Organic C	Total N	Total P_o	pH	EC	Organic C	Total N	Total Po	pH	EC	Organic C	Total N	Total P_o
$NaHCO_3$-Pi	-	-	0.45	-	0.40	-	-	-	-	-	-0.55	-0.31	0.43	0.53	0.29
NaOH-Pi	-	-0.36	0.40	-	0.43	-0.48	-0.27	-	0.53	-	-0.63	-0.28	0.50	0.57	0.28
HCl-P	-	-	0.37	-	-	-	-	-	-	-	-0.44	-	0.33	0.35	-
Residual-P	-	-	0.39	-	0.37	-	-	-	-	-	-0.47	-0.27	0.41	0.40	0.27
Total P_o	-	-	0.37	0.38	-	-0.36	-	0.46	0.60	-	-0.48	-0.30	0.56	0.67	-

- Not significant at *P* = 0.05

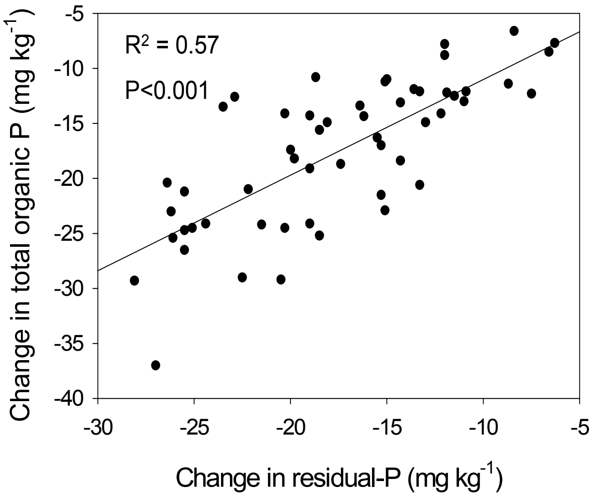


Figure 9. 1. Relationship between change in total organic P (mg kg^{-1}) and that in soil residual-P (mg kg^{-1}) occurring at each depth, at each site.

relative to the virgin site (Table 9.5). However, the surface residual pools of all cropped paddocks were approximately 20 mg kg^{-1} lower than that of the virgin site. For paddocks in negative P balance, all surface P pools were significantly depleted by continuous wheat and cotton cropping (Table 9.5). Below 10 cm, $NaHCO_3$-P_i and NaOH-P_i underwent minimal changes while HCl-P and residual-P for paddocks in negative P balance declined by more than 10 mg kg^{-1}, compared with the virgin site. In addition, long-term cropping reduced total organic P in the surface 30 cm regardless of P balance (Table 9.5). Changes in total organic P were significantly correlated to changes in soil residual-P (R^2 =0.57, *P*<0.01, Figure 9.1). There was no significant effect of cropping system on the change in each soil P pool, nor were there any interaction between the cropping system and other factors (Table 9.5).

Soil organic C was positively correlated with all P pools under cotton and virgin system, but only with residual-P under wheat system (Table 9.6). Similarly, the total organic P was positively related to the $NaHCO_3$-P_i, NaOH-P_i and residual-P for the cotton and virgin systems. As pH and

EC increased with soil depth, soil P pools significantly decreased, especially in virgin sites.

9.4. Discussion

9.4.1. Effect of cropping system on soil P fractions

Phosphorus depletion from individual P pools was not related to the ability of a crop species to use sparingly soluble P sources. For example, wheat is now documented for its high efficiency in utilizing P from $AlPO_4$ (Pearse *et al.* 2007; Pearse *et al.* 2006b; Chapters 4 and 6). However, P depletion from NaOH-P_i (P mainly bond with Al and Fe) did not vary between cotton and wheat systems. Moreover, although cotton and wheat were inefficient in accessing P precipitated with Ca, such as hydroxyapatite (Chapters 3, 4 and 6), the HCl-P pool was depleted in the soil profile under both systems when total P balance was negative. Possibly, the NaOH-P_i and HCl-P depleted from these Vertosols mainly represents the P adsorbed to the surface of calcite and amorphorus Al, which is more plant-available than sparingly soluble forms. A possible mechanism is the depletion-driven desorption of weakly held P from the $NaOH_i$ and HCl-P pools.

Subsoil P was involved in the nutrition of both wheat and cotton systems. However, the depth from which these plants derived their P did not differ between cotton and wheat despite their contrasting root morphology. The depletion of subsoil P by plant uptake is well documented (Garz *et al.* 2000; Gransee and Merbach 2000). Without subsoil constraints, wheat and cotton roots are able to penetrate deeply into the soil, and have noticeable amount of roots present at depth 0 to 60 cm (Bloodworth *et al.* 1958; Carmi *et al.* 1993; Incerti and O'Leary 1990; Zhang *et al.* 2004). Kuhlmann and Baumgartel (1991) found that if subsoil contained about 1/4 to 1/3 of total root length, it could supply 20-30% of the total P uptake of the crop. Nevertheless, the contribution of subsoil P sources to plant P uptake would also depend on many other factors, including the moisture level of the topsoil, soil texture and the presence of P sources in the subsoil (Kuhlmann and Baumgartel 1991; Chapters 7 and 8).

9.4.2. Effect of long-term cropping on soil P fractions

In both systems, $NaHCO_3$-P_i, NaOH-P_i and HCl-P pools in the soil acted as the major P sinks when P applied exceeded crop removal. Previous studies have also shown a preferential accumulation of P in the labile and acid-extractable pools in Vertosols (Chapter 8; Vu *et al.* 2008). Phosphorus fixation in Vertosols was mainly attributed to P adsorbed to the surface of calcite or amorphorus Al and Fe-oxides (Assimakopoulos *et al.* 1998; Bertrand *et al.* 2003). Under negative P balances, not only was the labile $NaHCO_3$-P_i depleted, but less labile pools such as NaOH-P_i and HCl-P were also reduced, suggesting moderate availability of these two pools in Vertosols. This agrees with previous findings that P adsorbed to the surface of fine soil carbonates and amorphous Al- and Fe-oxides was a weakly held form of P and was relatively plant available in less weathered soils such as Vertosols (Dorahy *et al.* 2007; Guo and Yost 1998; Holford and Mattingly 1975; Soils and Torrent 1989).

Regardless of level of P fertilizer addition, residual P decreased substantially following continuous cropping with either wheat or cotton. Residual-P in the sequential fractionation scheme is normally considered as precipitated inorganic P, plus humified organic P, both of which are considered to be of low availability to plants (Hedley *et al.* 1982b). However, numerous studies suggested that residual P in some soils, such as Vertosols and Alfosols, is partly plant-available (Agbenin and Goladi 1998; Guo and Yost 1998; Vu *et al.* 2008; Chapter 8). Zhang and Mackenzie (1997a) demonstrated that nearly 98% of the variation in the residual P pool caused by long-term cropping, was due to the presence of organic P components. In this study, the decline in the residual pool occurred in parallel with a decrease in the total organic P (Table 9.5), and the regression analysis showed that 57% of decrease in total organic P would be explained by the variation in the residual P pool (Figure 9.1). This might indicate that a routine fractionation scheme used for separating soil P pools according to its relative availability is not entirely accurate for the organic P fractions.

Loss of organic P was in line with the loss of organic C, confirming the general observation that soil organic matter is easily lost with continuous cropping and cultivation (Agbenin and Goladi 1998; Dalal and Mayer 1986b; Solomon *et al.* 2002). The important role of soil organic matter in P nutrition of cotton is indicated by the significant correlation between organic C and the size of each soil P fraction (Table 9.6). This would be explained by the fact that cotton systems could have considerable amounts of P returned as organic P to the soil during defoliation. However, routine crop residue return, although partly replenishing organic C and P pools in the topsoil, would not be able to maintain soil organic P in the subsoil.

9.4.3. Subsoil (10-30 cm) acidification by both wheat and cotton root

The subsoil (10-30 cm) layer underwent acidification following continuous cropping with either cotton or wheat. Addition of N fertilizer as urea or monoammonium phosphate (MAP) and export of alkaline products from the soil-plant system by harvesting crops could produce acidity at this depth. Kohn *et al.* (1986) also found soil acidification to 30 cm after 18 years of a wheat-pasture rotation. Nitrification followed by nitrate leaching had been well recognized as the major cause of soil acidification in surface soil layers (Tang *et al.* 2000; Tang *et al.* 1999). When compared with virgin sites, several studies revealed that the rate of acidification resulting from different agriculture systems ranged from near zero to 3-5 kmoles H^+ ha^{-1} yr^{-1}, depends on the soil texture and rainfall conditions (Chartres *et al.* 1990; Helyar *et al.* 1990; Ridley *et al.* 1990). The lack of acidification in the surface soil in this study possibly suggests that acidification generated from either NO_3^- leaching or crop removal was counterbalanced by the alkalinity released from crop residue, since mineralisation of organic matter is an alkalising process in the topsoil. The assimilation of leached nitrate by the crop could lead to an alkalization in the subsoil (Black 1992; Tang *et al*. 2000). Poss *et al*. (1995) attributed the higher rate of alkalization in the 25-90 cm layer from wheat cropping to root development and nitrate uptake from this layer. However, the relatively low permeability and the high pH buffering capacity of the Vertosol possibly mitigate against the occurrence of subsoil alkalization in this study.

9.5. Conclusion

Soil P accumulation and depletion in the profile did not differ between the cotton and wheat systems. The cropping system effect expected from species variation in root morphological and physiological characteristics was undetectable. Possibly, other factors in the system, such as tillage, irrigation or P fertilizer application, masked species differences. Alternatively, variation in changes in P pools, relative to the virgin site, originated from the undefined cropping history in the proceeding 20 years, was significant enough to counterbalance the system effect in the recent 20 years. Nevertheless, it can be concluded that long term cropping could lead to a depletion of residual, organic and subsoil P pools which are not measured by the common soil tests. For long-term sustainability, the extent to which these pools are depleted are questions for further research.

Chapter 10: General discussion and conclusion

This thesis examined the P acquisition characteristics of cotton from P pools that varied in availability and with soil depth. The key findings are summarized as follows: 1) the low responsiveness of cotton to P fertilizers is less likely to be attributed to the acquisition of P from sparingly soluble inorganic P pools at the soil surface; 2) hydraulic lift does not aid cotton in P acquisition from dry top soil; 3) long-term cultivation with cotton led to a significant depletion of organic and residual P pools from top and subsoil layers, regardless of P fertilizer application history; and 4) cotton plants are considerably less able to utilize the sparingly soluble Al-P than wheat plant.

Except for excretion of phosphatase, the cotton plants lack the ability to manipulate its rhizosphere chemistry, and to mobilize non-labile inorganic P sources. Significant exudation of carboxylates was not detected in the rhizosphere of cotton in response to P deficiency (Chapter 3). In addition, cotton was not superior in using sparingly soluble P sources such as Al, Fe and Ca phosphates, when compared with both wheat and white lupin (Chapters 4 and 6). While Dorahy *et al.* (2004) found a strong correlation between the P uptake by cotton and the Al- and Fe- soil P fractions in a field experiment, these pools in Vertosols possibly represent P weakly adsorbed to Al and Fe oxides (Holford and Mattingly 1975; Soils and Torrent 1989). Nevertheless, the enhanced acid phosphatase activity associated with P deficiency of cotton could promote the utilization of P from soil organic pools, as demonstrated by the concurrent depletion of NaOH-P_o in the rhizosphere soil (Chapter 3). In the literature, I am unaware of any published studies examining P acquisition characteristics of cotton from sparingly soluble P sources.

This study also highlights the fact that subsoil P pools act as additional P sources for either cotton or cereal P uptake. This is evidenced by a significant depletion of subsoil P pools, e.g. HCl-P, residual and organic P, following long-term cotton and cereal cropping (Chapters 8 and 9). Norrish *et al.* (2003) also reported a greater contribution of subsoil P sources (10-60 cm) to P uptake of wheat in a field experiment in the north-eastern grain zone of Australia. However, the contribution of subsoil P sources to plant P uptake depends on many factors, including moisture level of the topsoil, soil texture and the presence of roots and labile P sources in the subsoil (Chapters 7, 8 and 9). Consistent with many studies (Jarvis and Bolland 1991; Simpson and Pinkerton 1989; Singh *et al.* 2005), surface dryness reduced root growth in top soil layers and increased root proliferation in the subsoil (Chapter 7). Phosphorus uptake from the Vertosol was more strictly regulated by soil water content than from the sandy soil, and hydraulic lift detected for cotton in the Vertosol did not aid P uptake from the drying topsoil (Chapter 7). Thus, exploration of subsoil P by cotton roots would act as an important root morphological adaptation to the unpredictable availability of P in the topsoil. The depletion of subsoil P following long-term continuous cotton cropping could probably attributed to the transient or ongoing dryness of topsoils frequently encountered in cotton production area.

In contrast to cotton, wheat demonstrated an outstanding ability in using sparingly soluble Al-P (Chapters 4 and 6). This is in line with the recent discoveries by Pearse *et al.* (2007; 2006b). Mobilization of Al-P by wheat has frequently been attributed to the Al toxicity-induced root malate release (Lambers *et al.* 2002; Pearse *et al.* 2006b). However, Pearse *et al.* (2007; 2006b) demonstrated that wheat was capable of efficiently utilizing Al-P despite releasing less carboxylates. The rhizosphere pH detected in all these studies was actually too high (>6) to induce Al toxicity. Thus, the role of carboxylates in mobilizing Al-P by wheat still remains unclarified and warrants further research. On the other hand, my study demonstrated that sparingly soluble Al-P showed an increased availability with prolonged incubation (Chapter 5). In this case, wheat with higher root length density than cotton (2.15 cm cm^{-3} vs. 0.56 cm cm^{-3}) (Chapter 4) could possibly be better equipped to acquire this progressively available P source. The higher accumulation of Al in the root of wheat (~2.2 mg g^{-1}) than cotton (~0.8 mg g^{-1}) (Chapter 4) might further drive the reaction towards the dissolution of Al-P. Elucidating how wheat utilized Al-P would be an exciting area amenable to further research, considering that wheat is widely grown in soils containing significant amount of Al-P.

The depletion of NaOH-P, HCl-P and residual-P pools following long-term cultivation of both cotton and wheat with a negative P balance (Chapters 8 and 9) would indicate that all these P pools in Vertosols are potentially labile and available for plant uptake. In the literature, ample evidence exists to support this contention. For example, NaOH-P pools in a Vertosol represent P weakly adsorbed on Al and Fe oxides and are relatively labile (Holford and Mattingly 1975; Soils and Torrent 1989). Also, under intensive cropping, both HCl and residual P act as the main buffering pool for the plant-available P in a Vertosol (Guo and Yost 1998; Guo *et al.* 2000; McKenzie *et al.* 1992b). The decline in residual-P pools following long-term cropping in this study was accompanied with a concurrent depletion of total organic P from all soil depths, regardless of P fertilizer history (Chapter 9). Correlation analysis further showed that 57% of the decrease in total organic P was from the residual P pool (Chapter 9). Thus, future P fertilizer management for a sustainable cotton and wheat production on Vertosols should emphasize the replacement of organic P pools through addition of organic sources (crop residue, manure, compost) to the soil, in addition to the inorganic P fertilizer.

While the Hedley's fractionation scheme has been widely used for many soils, this study questions its reliability to establish the relative availability of each P fraction in Vertosols. This applies particularly to the residual-P, which is normally considered least available to plants in the sequential fractionation scheme (Hedley *et al.* 1982b). However, this study revealed that residual-

P fraction in the Vertosol contained noticeable amounts of the exchangeable P (Chapter 5) and was subjected to a gradual depletion following long-term continuous cropping with and without P fertilization (Chapters 8 and 9). The availability of the residual P fraction had been shown to be attributed to its organic portion, which is in line with studies from Zhang and McKenzie (1997b) and Tiessen *et al.* (1992). However, Guo *et al.* (2000) postulated that it was the P bonded with Ca that contributed to the labile nature of the residual pool. Research is still required on isolating and elucidating these available portions of residual P fractions in the Vertosol.

Phosphorus acquisition by cotton plants from Vertosol soils could be depicted as a root morphological exploration of organic P and relatively labile inorganic P sources from both topsoil and subsoil layers. The high soluble nature of organic P in neutral to alkaline soil (Pinto 1974; Samadi and Gilkes 1998), and the root excretion of phosphatase (Chapters 3 and 4) facilitate the acquisition of organic P by cotton from Vertosols. On the other hand, root morphological traits, such as increased root to shoot ratios (Chapter 3) or AM associations (Duggan *et al.* 2008; McGee *et al.* 1997; Rich and Bird 1974) could result in a high root P-absorption surface area. Considering that Vertosols had relatively low P-fixing capacities (Chapters 5, 8 and 9), it is desirable to place P fertilizer in the way that favors a higher probability of root-P contact. In contrast to the low responsiveness of cotton to the P fertilizer applied in the topsoil (Bronson *et al.* 2001; Dorahy *et al.* 2004), a significant yield response was detected for cotton to the deep P placement (Singh *et al.* 2005). Possibly, the effectiveness of deep P placement in increasing cotton yield under field conditions is due to an enhanced contact between root and fertilizer during the later stages of growth, and also a sustained P availability under periodic surface drought conditions.

It can be concluded that routine soil P tests, using alkaline bicarbonate extraction solutions with soil samples collected from the topsoil layers (above 10 cm), do not adequately estimate P responsiveness of cotton to P fertilizers for several reasons. The first is because P pools, such as HCl-P, residual and organic P, in the Vertosols which have been proved to be potentially plant-available, are not measured by the standard bicarbonate extraction procedure (Chapters 8 and 9). The second reason is that the cotton plant takes up significant quantities of P from deeper soil layers below 10 cm. Low responsiveness of cotton to P fertilizers applied in the surface soil with low soil test values, indicates that cotton plants are able to meet their P requirement from these P pools, without the need for P fertilizers. Since P fertilizers are costly and non-renewable, the efficient use of soil P resources would be an important strategy to minimize P inputs as fertilizers. This would also result in a lower risk of building up soil P status and surface P runoff. On the other hand, since organic and subsoil P pools can not be replenished by routine inorganic P fertilizer input, the extent to which these pools can be depleted with regard to the long-term sustainability and productivity of crop production are questions for further research. Efforts on finding a soil testing procedure that can adequately predict the bioavailability of soil P in

Vertosols will be a real challenge in the future. The role that mycorrhizae play with cotton plants in Vertosol soils is also worthy of future investigation.

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