1. General Introduction

The Australian cotton industry has a farm gate value of $3 billion per year. Australia produces some of the world's highest yielding cotton, and set a new national production record of five million cotton bales in the 2011/2012 growing season. Queensland (Qld) and New South Wales (NSW) produce 44 and 56 % of the annual cotton crop respectively in Australia. The major cotton growing areas are located in one of Australia's most important agricultural regions, known as the northern grains region (NGR) of eastern Australia. The NGR covers an area of approximately four million hectares, which extends from central NSW (~ 32.25 ° S) to central Qld (~ 22.82 ° S) (Figure S1, Supporting Information).

1.1. Climate

The NGR has a low and variable rainfall distribution, and a median average rainfall between 600 to 800 mm per year. Rainfall becomes increasingly more variable and summer dominant from northern NSW to central Qld. The NGR can experience frequent surface droughts due to high evapotranspiration rates of between 1600 to 2000 mm per year. Average maximum temperatures in the summer and winter range from 27 – 33 °C and 12 – 20 °C respectively, and 12 – 20 °C and 0 – 9 °C for the average minima summer and winter temperatures respectively.

1.2. Soil

Vertosols are the dominant soil type used for cotton (and grain) production in the NGR (Dorahy 2002; Norrish 2003). In general, Vertosol soils contain more than 35 % clay, which has a uniform distribution down the soil profile to at least one metre below the soil surface, exhibit 'self-mulching' or shrink/swell characteristics due to the presence of smectite clay minerals, and often contain lenticular peds and calcite nodules (Hubble 1984). Vertosols are also likely to contain a neutral to alkaline pH, a high cation exchange capacity dominated by calcium, and are considered to have a moderate to high fertility compared to other Australian soils (e.g., Chromosols) (Hubble 1984). The main subsoil constraints of Vertosols include sodicity and compaction.

1.3. Agronomy

Cotton is a warm season crop usually grown from late Spring when soil temperatures are at least 16 °C in the NGR. Cotton is a deciduous and a perennial crop species with an indeterminate growth habit. This requires cotton to be 'defoliated' at optimum boll maturation prior to harvesting in Autumn. The main factors affecting cotton yield are ambient temperature (expressed as day

degrees) and soil moisture (COTT300 2006). The latter is often overcome with a 6 to 18 month fallow prior to sowing cotton, and is the biggest factor affecting cotton yield in dryland systems. The use of single and/or double 'skip' rows is also used to maximise soil moisture for cotton. However, 80 % of Australian cotton growers use irrigation to ensure adequate water throughout the growing season.

Cotton is usually grown in rotation with cereals and/or pulse crops every three to five years, although some continuous cotton operations do exist. The main cool season crop species are barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), chickpea (*Cicer arietinum* L.), and faba bean (*Vicia faba* L.), whereas the main warm season crop species are mungbean (*Vigna radiata* L.), sorghum (*Sorghum bicolor* L.), and soybean (*Glycine max* L.). If adequate soil moisture is available 'double cropping' can occur with a cool season crop sowed directly after the cotton harvest.

1.4. Phosphorus in cotton

1.4.1. Cotton physiology and response to phosphorus

Phosphorus (P) is an essential macronutrient for plants. Phosphorus is required for the metabolism of carbohydrates, the molecules that carry genetic code (i.e., DNA and RNA), and the storage and derivation of energy by photosynthesis (Vance *et al.* 2003). Plant roots absorb orthophosphate ions in the soil solution. If P is non-limiting to cotton the root cell hydraulic conductivity increases, and results in leaf expansion and an increase in transpiration rate (Radin and Eidenbock 1984; Skinner and Radin 1994). Whereas under P limiting conditions the rate of atmospheric carbon assimilation and carbohydrate metabolism decrease, and protein and nucleic acid synthesis is reduced due to an inability of protein amino group assimilation (Grant *et al.* 2001; Longstreth and Nobel 1980; Radin and Eidenbock 1986). These physiological processes are expressed phenotypically, which enables a visual diagnosis of P deficiency in plants (Grundon *et al.* 1997).

The expression of P deficiency in cotton may not be as visually striking in comparison to other crop species (e.g., wheat) (COTT300 2006). Typically, reductions in root and shoot dry weight occur giving a stunted appearance (Ahmad *et al.* 2001). Crop establishment and seedling vigour are inhibited, and a general darkening of the canopy eventuates (Hodges 1992; Howard *et al.* 2001). Under sever P deficiency, a purple discolouration can occur on the foliage between the veins on the leaves (Dorahy 2002). In cotton, P deficiency induces delayed boll set, reduced boll size and frequency, which negatively affects lint yield (Duggan *et al.* 2008). Some studies have found fibre quality to be adversely affected by P deficiency (Duggan *et al.* 2008), but not in all

cases (Girma *et al.* 2007). Nelson (1949) found fibre quality was unlikely to be affected by soil P content post boll filling.

1.4.2. Phosphorus translocation in cotton

During cotyledon development plant P demand is supplied by seed P reserves. Thereafter, soil phosphorus reserves are the main source of cotton P uptake (Dorahy *et al.* 2007; Eaton and Ergle 1957). Cotton P demand rapidly increases at boll formation, and then peaks during boll filling (Halevy *et al.* 1987). At maturity, no more than 80 % of total plant P uptake is contained in the boll and bud (Eaton and Ergle 1957). Rochester (2007) found cotton P uptake was on average 27 kg P/ha, which 18 kg P/ha was removed by the seed.

1.4.3. Critical phosphorus tissue concentrations

Plant tissue testing occurs on the youngest leaf blade or the petiole of the youngest lead bladet early square or flower (Reuter *et al.* 1997b). Phosphorous concentrations in plant tissue decrease with age because tissue P concentration is a function of dry weight, where the amount of P in the plant becomes more 'diluted' as plant biomass increases. A consistent sampling technique (e.g., plant growth stage) is needed to minimise the effect of climate and systematic variability (COTT300 2006). In general, cotton P deficiency occurs when tissue P concentrations are below 0.2 %, whereas tissue P concentrations are considered high above 0.5 % (Reuter *et al.* 1997a).

The ability to diagnose cotton P deficiency by analysing cotton tissue is difficult (Dorahy 2002). There is limited data and agreement in the literature on the critical tissue P concentration in cotton (Reuter *et al.* 1997a). If a P deficiency is detected it is likely P fertilisation will have little impact on yield for the current crop (Dorahy *et al.* 2004; Joham 1951).

1.5. Soil phosphorus

Vertosol soils contain variable concentrations of total P, which usually range between 150 to 1200 mg total P/kg (McLaren *et al.* 2012b). The quantity of total soil P is related to parent material, environmental factors and other soil formation process (Cross and Schlesinger 1995; Walker and Syers 1976). In recent times (i.e., on a geological scale), agricultural practices have influenced the accumulation or depletion of total soil P. The primary mechanisms that cause the accumulation of total soil P are P fertilisation and the redistribution of soil P from subsurface layers to the topsoil by plants (biocycling) (Negassa and Leinweber 2009). Whereas the primary mechanisms that cause the depletion of total soil P are crop removal, tillage and soil loss (e.g., wind erosion) (Dalal 1997; Negassa and Leinweber 2009). Soil phosphates exist in a variety of forms, and their chemical

552 structure is often related to their thermodynamic stability in soil (Hedley *et al.* 1982; Lindsay
553 1979).

554 The cycling of P in the soil ecosystem is complex. Phosphorus is highly reactive in soil
555 and its solubility decreases over time (Mengel and Kirkby 2001). However, quasi equilibrium
556 processes regulate the distribution of soil phosphates between the mineral and solution phase
557 (Mengel and Kirkby 2001). The three main pathways responsible for this are; 1)
558 dissolution/precipitation, 2) adsorption/desorption, and 3) mineralisation/immobilisation (Figure
559 1.1).

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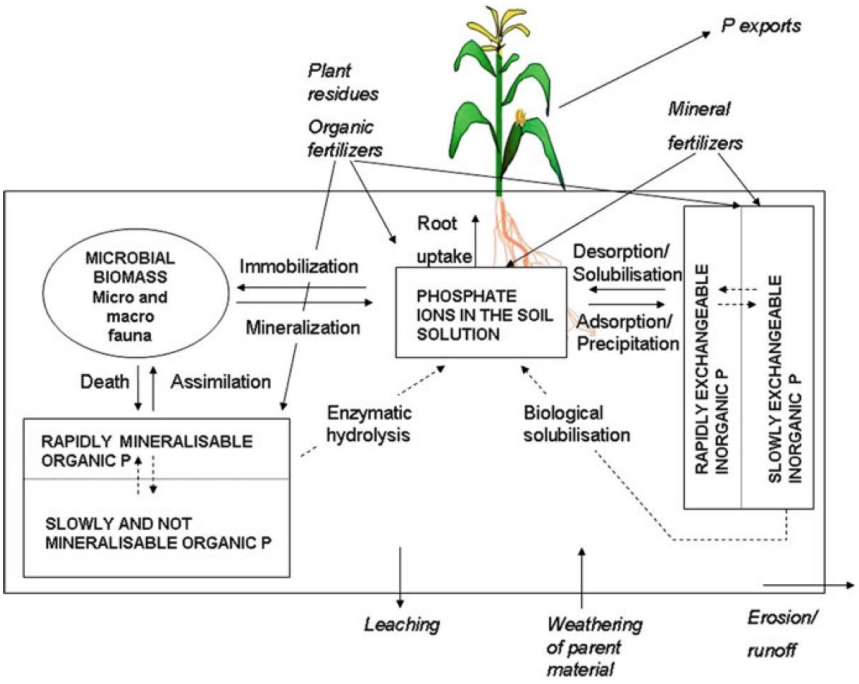

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562 **Figure 1.1: The soil phosphorus cycle (taken from Frossard *et al.* (2011)).**

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564 ### 1.5.1. Mineral phosphorus

565 A large proportion of total P exists in mineral form associated with aluminium (Al),
566 calcium (Ca), iron (Fe) and manganese (Mn) (Lindsay 1979). Calcium phosphates (e.g., apatites)
567 are the most abundant form of soil phosphates in Vertosols, and their abundance is often related to
568 the extent of chemical weathering during soil development (Cross and Schlesinger 1995). The
569 chemical weathering of apatites result in the formation of secondary P minerals such as Al and Fe
570 phosphates, and precipitated forms of Ca phosphates (Cross and Schlesinger 1995; Sanyal and De
571 Datta 1991). In general, Al and Fe phosphates are a minor fraction of total soil P in Vertosols, due

to minimal soil weathering and their low thermodynamic stability in alkaline soils (Cross and Schlesinger 1995; Lindsay 1979; Wang *et al.* 2007).

The main forms of Ca phosphates include apatites ($Ca_{10}(X)(PO_4)_6$, where X can represent Cl^-, F^-, CO_3^{2-} and OH^- ions), and its derivatives (dicalcium phosphate dihydrate (DCPD), octo-calcium phosphate (OCP), tri-calcium phosphate (TCP), etc.) (Condron *et al.* 2005; Lindsay *et al.* 1989). Calcium phosphates that are thermodynamically stable tend to be present in highly weathered soils (Guo *et al.* 2000; Williams *et al.* 1967). These include TCP and hydroxyapatite (HA), which are slowly available over time because of their very low solubilities in alkaline soils (Sposito 2008). Readily available forms of Ca phosphate (i.e., with higher solubility products) tend to be present in less weathered soils, such as DCPD and OCP (Lehr and Brown 1958).

In alkaline soils, DCPD or OCP are the most likely compounds formed after applying P fertilizer to soil (Lindsay *et al.* 1962; Lombi *et al.* 2006). These compounds can persist in the soil environment for weeks or months, and are considered relatively plant available (Fixen *et al.* 1983; Havlin and Westfall 1984). Invariably, Ca phosphates of low thermodynamic stability transform into more thermodynamically stable Ca phosphates such as TCP and HA (Lindsay *et al.* 1989). The rate at which this occurs is dependent on several factors such as soil P and cation concentrations, pH, temperature and time (Bell and Black 1970; Lindsay *et al.* 1962; Lombi *et al.* 2006).

1.5.2. Sorbed phosphorus

The quantity of P in the soil solution is low, and is typically less than 1 % of total soil P (Pierzynski *et al.* 2005). Orthophosphate ions are the dominant form of P in the soil solution, which can be directly absorbed by plant roots (Frossard *et al.* 2000; Mengel and Kirkby 2001). It is therefore vital that sufficient quantitates of P are maintained in the soil solution for plant uptake. The primary sources of P that maintain soil solution P are those sorbed to Al and Fe oxy-hydroxides, clay minerals, and the surfaces of calcite (Thomas and Peaslee 1973).

Sorbed P is a relatively small fraction of total P in Vertosols (Cross and Schlesinger 1995). However, much research has been undertaken to understand the quantity and supply of sorbed P in soil (Beegle 2005; Thomas and Peaslee 1973). This is because of the strong relationships found between the concentrations of sorbed P in soil and the quantity of P accessible to plants (Thomas and Peaslee 1973).

1.5.3. Organic phosphorus

Vertosols contain low quantities of soil organic P, and are estimated to be less than 20 % of total P (Dalal 1997; Steward and Oades 1972; Williams and Anderson 1968). The dominant form of soil organic P in Vertosols are humic compounds of high molecular weight (Doolette *et al.* 2011b). These compounds are relatively stable in soil and are unlikely to be an important source of plant available P during a single crop (Bünemann *et al.* 2008; Doolette *et al.* 2010). However, the turnover of microbial P can be an important source of P for plant uptake (McLaughlin *et al.* 1988).

1.6. Soil phosphorus measurements

1.6.1. Soil phosphates

The chemical structure of soil phosphates is often related to its thermodynamic stability in soil (Lindsay 1979). The Hedley et al. (1982) P sequential fractionation (SF) method and similar modifications (Condron *et al.* 1990; Guppy *et al.* 2000), are the most commonly used methods used to separate and identify soil phosphates and their bio-availability (Condron and Newman 2011). The two most commonly used reagents in various sequential fractionation procedures are the 0.1 M NaOH and 1 M HCl extractants (Pierzynski *et al.* 2005). The 0.1 M NaOH reagent is designed to estimate Al and Fe phosphates, sorbed P and the more stable organic P pools that would not be removed by a preceding bicarbonate reagent (Hedley *et al.* 1982; Thomas and Peaslee 1973). The 1 M HCl extractant is designed to estimate the quantity of Ca phosphates, and the difference between this measurement and total P is referred to as "residual-P" (Hedley *et al.* 1982). However, there has been no direct validation of the soil phosphates removed by the 0.1 M NaOH and 1 M HCl extractant in soils, and it is unknown to what extent these chemical reagents may dramatically alter the soil chemical environment for accurate soil P speciation (Kar *et al.* 2011; Kruse and Leinweber 2008).

Advances in synchrotron X-ray techniques have been successfully applied to soil P studies using X-ray absorption near-edge structure (XANES) (synonymous with near-edge X-ray absorption fine structure (NEXAFS)) on the P K-edge (Ajiboye *et al.* 2007a; Beauchemin *et al.* 2003; Kruse *et al.* 2009; Lombi *et al.* 2006; Schefe *et al.* 2011). X-ray absorption near edge structure spectroscopy has the ability to characterise both amorphous and crystalline P minerals (Ingall *et al.* 2011; Shober *et al.* 2006). Phosphorus K-edge XANES is a direct analysis of soil phosphates, however, accurate determination of soil phosphates can be hindered due to a lack of spectral features of some P compounds, high method detection limits, and the heterogeneity of soil (Ajiboye *et al.* 2008). Nevertheless, P K-edge XANES is the best available technique available for measuring inorganic soil phosphates (Doolette and Smernik 2011).

1.6.2. Plant available P

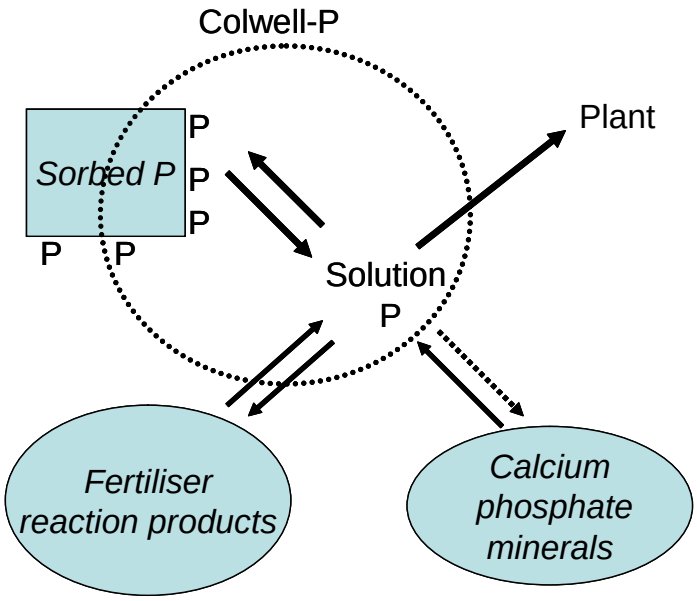
The supply of P to the soil solution can be described using two phases: 1) a rapid release of labile P (readily plant available) and, 2) a slow release of non-labile P (slowly plant available) (Lookman *et al.* 1995; McLaughlin *et al.* 1999). In the rapid release phase, soil solution P is supplied from P desorbed from Al and iron Fe oxy-hydroxides, and soil carbonates (Figure 1.2) (Holford 1997). In general, the Colwell (1963) method is correlated with these P pools that supply phosphate to plants.

The Colwell (1963) bicarbonate method is a common soil P test used to predict plant available P in the NGR (Dorahy *et al.* 2004). However, the Colwell extractant can underestimate the contribution of slowly available P to the soil solution (Kirkby and Johnston 2008; Syers *et al.* 2008). This is because the Colwell extractant is a single, short term extraction (16 h) that cannot take the long term replenishment of soil solution P into account (Colwell 1963; Wang *et al.* 2007). Recent studies have suggested the Colwell soil P is being replenished from soil phosphates not measured by the Colwell extractant P in Vertosols, particularly Ca phosphates (Wang *et al.* 2007).

Due to the importance of Ca phosphates in Vertosols, a dilute acid extractant (a modified Truog (1930) technique known as the BSES extractant (Kerr and von Stieglitz 1938)) was investigated to estimate the quantity of Ca phosphates in Vertosols, which would have normally been estimated using sequential fractionation procedures (Wang *et al.* 2007). The main pathway that Ca phosphates supply the soil solution is by dissolution processes (Lindsay 1979). The BSES extractant was investigated as an alternative to the sequential fractionation technique because the later is not commercially available to primary producers of the NGR.

Calcium phosphates are often considered soluble in dilute acids (e.g., 0.005 M H_2SO_4) (Thomas and Peaslee 1973; Truog 1930). Acidic reagents primarily act as solvents, dissolving soil P minerals in descending order; Ca phosphates > Al phosphates > Fe phosphates (Thomas and Peaslee 1973). Although, the acidic anion can also competitively desorb phosphate associated with Al and Fe oxy-hydroxides (Figure 1.3) (Rayment 1993; Truog 1930).

The P sorption capacity of a soil is a measure of the soil's ability to resist changes in soil solution P, and is typically measured using a single point index (e.g., PBI) (Burkitt *et al.* 2008; Moody 2007). Vertosols have a relatively narrow, and low to moderate capacity to sorb P, and the use of PBI in the NGR has been limited (Bertrand *et al.* 2003; Norrish 2003). Nevertheless, in some soils PBI is combined with Colwell-P to improve P fertiliser response predictions (Burkitt *et al.* 2008).



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673 **Figure 1.2: A conceptual diagram of the soil phosphates removed by the Colwell extractant**
674 **(taken from Moody *et al.* (2012)).**

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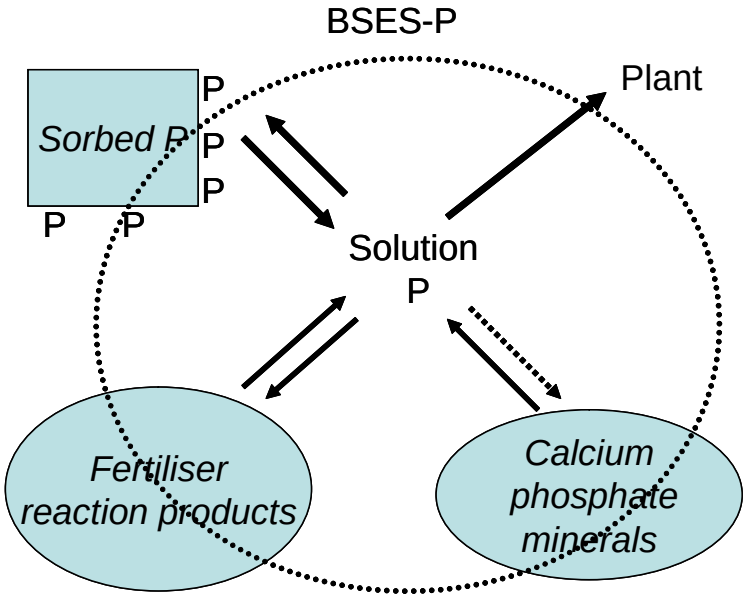

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677 **Figure 1.3: A conceptual diagram of the soil phosphates removed by the BSES extractant**
678 **(taken from Moody *et al.* (2012)).**

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1.7. Justification of the project

Over the past 30 years, soil P fertility has declined due to the removal of ~ 20 kg of lint P/ha/yr (Rochester 2007). Consequently, over the past 25 years the amount of P fertiliser used by the cotton industry has increased six-fold, with some growers applying P fertiliser based on estimated crop removal without taking into account current plant available P (Dorahy *et al.* 2004). However, the response of cotton to P fertiliser has been variable and frequently low compared to other crops (e.g., wheat), and few studies have investigated why this may be the case (Dorahy *et al.* 2004; Hibberd *et al.* 1990).

Recent studies by Dorahy *et al.* (2007; 2004; 2008) and Wang *et al*. (2011; 2007; 2008; 2010) have improved our understanding of soil P dynamics in the cotton systems of the NGR. Dorahy *et al.* (2004) found the response of cotton to applied P was likely to occur when Colwell-P concentrations were below 6 mg P/kg, although P fertiliser recovery was highly variable (0 – 67 %) across all 17 field sites. Dorahy *et al.* (2007) also found that ~ 95 % of P accumulated by the cotton plant originated beyond the fertiliser band, demonstrating the importance of dispersed P (native soil P pools and residual P fertilisers) for cotton P uptake. In addition, Wang *et al*. (2011; 2010) found that sparingly soluble Al, Fe and Ca phosphates were not easily accessible to cotton roots, and that arbuscular mycorrihizae fungi (AMF) associations may be critical for cotton P acquisition in low P environments. Wang *et al.* (2007) also found substantial decreases in the 1 M HCl-P and 'residual P' fractions of up to 50 and 20 % respectively after 18 crop cycles, suggesting that slowly available P can contribute to plant available P. Wang *et al.* (2007) reported that crop P removal was greater in the surface 0 – 10 cm (55 % of total P removal) layer compared to the 10 – 30 cm (35%) and 30 – 60cm (10%) layers in unfertilised plots. However, when P fertilisers were applied annually, labile soil P pools (resin and $NaHCO_3$) increased in the topsoil, whereas 1 M HCl and residual P pools were depleted in the topsoil and subsoil irrespective of P fertiliser input.

Lester *et al.* (2003) found Colwell-P concentrations were not altered after grain containing 200 kg P/ha was removed in crops grown in a Vertosol soil of the NGR. This finding suggests that Colwell-P can be replenised by slowly available inorganic P pools (Johnston and Poulton 1992; Wang *et al.* 2007). This is unsurprising given that the Colwell (1963) method can underestimate the contribution of slowly available P to the soil solution, because it is a single short term extraction (16 h) that cannot take the long-term replenishment of soil solution P into account (Holford 1997; Lester *et al.* 2003). It is not known to what extent the soil solution is supplied from the readily and slowly available P pools, nor is the rate of P release from these pools known (Kirkby and Johnston 2008). These difficulties are pronounced in Vertosols as they contain highly variable amounts of what may be considered slowly available P (Vu *et al.* 2010; Wang *et al.* 2007). Research is needed to understand the form and release dynamics of slowly available P to the soil solution in Vertosol soils of the NGR (Kirkby and Johnston 2008; Wang *et al.* 2007).

This thesis has been arranged in journal style format, so that relevant literature is reviewed at the start of each experimental chapter. The author recommends several excellent literature reviews on the current understanding of soil/plant P dynamics, including:

- Dorahy (2002), Norrish (2003) and Wang (2009) for a comprehensive review on soil P dynamics in the Vertosol soils of the NGR.
- Pierzynski *et al.* (2005) and Sims and Pierzynski (2005) for current understanding on the form and cycling of P in soil.
- Beegle (2005), Holford (1997) and Rayment (1993) for understanding common soil P tests used in cropping systems and the soil phosphates they are likely to remove.
- Condron and Newman (2011) for a comprehensive review on the benefits and limitations of sequential fractionation procedures for estimating soil phosphates.
- Lombi and Susini (2009) and Doolette and Smernik (2011) for an understanding of the use of spectroscopic techniques for soil P speciation.
- Richardson *et al.* (2009) and Richardson *et al.* (2011) for a comprehensive review on various strategies on improving P use efficiency using plant and biological parameters.
- Kirkby and Johnston (2008) and on current understanding of P fertiliser reactions and availability in soil.

The implications and significance of the thesis will be collated in the General Discussion section (Chapter 5). In general, there is limited research on the slowly available P pool in Vertosols of the NGR (Syers *et al.* 2008; Vu *et al.* 2010; Wang *et al.* 2007). This thesis aims to understand the contribution of Ca phosphates to supply or replenish the readily available P pool in Vertosols of the NGR. This thesis will:

1. Investigate the response of faba bean and cotton to readily and slowly available P in the subsoil measured by the Colwell and BSES extractants respectively (Chapter 2). This chapter will also investigate the crop response of different P fertiliser placement strategies in the subsoil.
2. Investigate the supply of slowly available P to the soil solution in Vertosols (Chapter 3). This work will demonstrate the usefulness of the BSES extractant to estimate the supply of slowly available P to the soil solution in Vertosols.
3. Identify and quantify the soil phosphates removed by the 0.1 M NaOH and 1 M HCl extractants in Vertosols, and how this compares to those removed by the BSES extractant

(Chapter 4). This work will identify the benefits and limitations of the 0.1 M NaOH, 1 M HCl and BSES extractants to measure soil phosphates in Vertosols.

Appendices – Establish a new method for total element determination in soil and plant tissue using PXRF. This work will demonstrate the applicability of PXRF for agricultural purposes, and provide a rapid and cost-effective technique for total element determination that can be used throughout this thesis (and elsewhere).