CHAPTER ONE

General introduction

General introduction

1.1 Project overview

Organic farming is an alternative to conventional agriculture for sustainable food and fibre production with high consumer demand. Organic growers commonly report that weeds, pests and diseases are their greatest challenges; however managing soil fertility also remains a major limitation. In Australia, farmers face particular challenges including infertile soils, high climatic variability and large distances between farms and organic input sources (Malcolm et al. 1996).

Fertility management on Australian organic farms may not be adequately addressed with European methods. Of specific concern are the findings of Penfold (2000) and Kirchmann and Ryan (2004) which indicate that plant available phosphorus (P) is a limiting factor in broad-acre organic farming due to the low natural abundance and slow rate of release from organic-permitted fertilisers. The type and quantity of inputs, seed materials permitted in organic farming and some derogations from organic regulations at times to avoid economic loss for organic farmers (Groot and Lammerts van Bueren 2003) is regulated world-wide by the International Federation of Organic Agriculture Movement (IFOAM) and in Australia by several organisations such as Australian Certified Organics (ACO) and the National Association for Sustainable Agriculture Australia (NASAA) who are accredited by the Australian Quarantine and Inspection Service (AQIS 2002). Rock phosphate and composted manures are two common permitted P fertilisers used in organic agriculture, while other permitted P sources such as blood and bone are less often used (IFOAM 2005). Organic standards require that seeds or plant propagation material be derived from crops grown organically (Neeson and Howell 2003). However, a derogation rule allows the use of non organic seed material if suitable organically certified material is not available in sufficient quality and quantity (Groot and Lammerts van Bueren 2003).

Knowledge about the P fertility status of organic vegetable farms in Australia is limited. One investigation in New South Wales has shown that organic vegetable production

methods can maintain adequate soil fertility which draws support from a case study (Cornish and Stewart 2002) although it remains unclear whether it is generally true that organic production is inherently nutrient-limited in Australia. Gross differences in soil type, climate and enterprise structure between geographic locations may produce greater differences in relative soil fertility levels than management system alone. This emphasises the need for documenting and researching the fertility status, especially for P, in Australian organic vegetable farms and for further exploring strategies that may enhance P cycling in organic vegetable production.

This thesis reports on a field investigation and several glasshouse experiments. Adjacent organic and conventional vegetable farms were investigated in the commercial vegetable growing regions of Gatton, Stanthorpe and Dorrigo with differing soil types (Vertosol, Tenosol, Ferrosol respectively), climate and proximity of key input sources and output destinations. Farm management details were obtained for the five years prior to sampling.

Several glasshouse experiments were conducted to investigate short term P utilisation strategies in sweet corn cultivars and also to examine the potential of legumes to provide increased soil P availability for subsequent crops. Early experiments investigated sweet corn cultivars currently used in commercial organic vegetable production. Later studies involved measuring the temporal changes in P accumulation by faba beans and field peas, evaluating the potential of legumes to mobilise P in contrasting soil types and an isotopic tracer study to quantify the P contribution of winter legume green manure to a subsequent corn crop.

1.2 Thesis format

This thesis is presented in Journal Article format (UNE Handbook 2007). The review of literature (Chapter 2) discusses soil fertility in organic and conventional farming systems around the world and in Australia; P management in agriculture; genotype by fertiliser interaction; and the P benefits of legumes for subsequent crops. Short introductions highlighting specific research relevant to each chapter are presented in each chapter.

Chapter 3 reports on a field investigation carried out at three locations in 2005 and 2006. This work was submitted to *Agriculture, Ecosystem and Environment* as "Comparison of soil chemical and microbial properties in organic and conventional vegetable farms in eastern Australia" and is currently being revised. Chapter 4 describes the glasshouse experiment on P management strategies in sweet corn varieties in a paper titled "Short term phosphorus fertiliser source utilisation by sweet corn (*Zea mays* L.) in organic production". This paper will be submitted to the *Agronomy Journal.*

Chapter 5 reports on a series of glasshouse experiments on the "Evaluation of the potential of two legumes to enhance P nutrition in a following crop under organic production in Australia". This includes two glasshouse experiments which determine the temporal changes in plant P accumulation and P acquisition by legumes and its potential to enhance soil nutrients in two different soil types when supplied with different P sources. This work will be submitted to the *Agronomy Journal.* Chapter 6 describes a glasshouse isotopic dual-labelling experiment on the "Isotopic tracing of phosphorus from ^{33}P labelled legume residues and ^{32}P fertilisers to subsequent corn in legume-corn rotation using dual labelling technique". This work has been submitted to the journal *Plant and Soil.*

Chapter 7 presents a general conclusion that describes the key findings from each chapter, discusses the limitations of the work and highlights some future research needs arising out of this study.

All the units of measurement within each chapter are in a consistent format, but the unit format differs slightly between chapters (e.g. kg/ha or kg ha^{-1}). Appendices include a range of preliminary experiments and additional data from glasshouse experiments that have less importance to the focus of this thesis, but are provided as additional information. A paper published in *Journal of Microbiological Methods* and short papers published in conference proceedings are also included in the Appendix.

1.3 Objectives

The objectives of the research described in this thesis were:

1. to assess the nutrient status of organic and conventional commercial vegetable farms in a range of contrasting soil types and climatic conditions.
2. to study the genotype x environment interaction, where P source is the environmental variable and identify plant traits beneficial to P uptake, and
3. to evaluate the potential of winter legumes (faba beans and field peas) to enhance soil P availability to a subsequent crop.

CHAPTER TWO

Literature review

Literature review

This chapter reviews differences in soil chemical and biological properties that occur between organic and conventional farming systems around world, and focuses on such farming systems in Australia. An overview of phosphorus (P) fertilisers will be presented along with a discussion on the effects of cover crops or green manures on a subsequent crop with special focus on winter legumes and corn.

2.1 Overview of organic farming around the world and Australia

Agricultural intensification and increased productivity from agro-ecosystems are designed to meet the increasing demand to supply food and fibre to a burgeoning world population. To feed the projected global population of 9.3 billion by 2050, it has been predicted that global food output must rise by 110 % (FAO 2002). However, this agricultural intensification has the potential to cause large scale environmental problems, especially degradation of soil and water resources (Drinkwater and Snapp 2007; Millennium Ecosystem Assessment 2005).

Organic and low input farming systems are an alternative to conventional agriculture and have potential to enhance agricultural sustainability and food quality (Macilwain 2004). The international food standard, Codex Alimentarius, defines organic agriculture as a "holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity. It emphasises the use of management practices in preference to the use of off-farm inputs, taking into account that regional conditions require locally adapted systems. This is accomplished by using, where possible, agronomic, biological, and mechanical methods, as opposed to using synthetic materials, to fulfil any specific function within the system" (FAO 1999). In contrast to conventional farming systems which tend to be reactive to problems which arise in regard to production or the environment, organic farming aims at preventing those problems (Ramesh et al. 2005; Watson et al. 2002a). Although organic principles and practices originated in

northern Europe, they have been adopted with varying success around the world in regions with vastly different soils and climates under different environmental conditions and production systems (Alexandra and May 2004).

According to recent statistics, 31 million ha of cultivated land is managed organically on a global scale with the largest percentage in Australia (12.1 million ha) followed by China (3.5 million ha), and Argentina (2.4 million ha) (Willer and Yussefi 2006). In terms of cereal production Italy, USA and Germany are the top three respectively, but there are no clear statistics available for organic vegetable production (Willer and Yussefi 2007). In Australia, growth in organic production is estimated at 15-25% annually and is expected to continue because of strong domestic demand for organic products such as fruits, vegetables meat and cotton and also because of Australia's ability to supply expanding markets overseas, especially in Asia (Alexandra and May 2004). These organic production systems include small-scale market gardens, orchards and vineyards, broad-acre cereal and grain legume cropping as well as intensive and extensive animal production systems.

World wide, organic producers commonly report that weeds, pests and diseases are their greatest challenge followed by managing soil fertility (Walz 1999). While the economic prospects can be promising for Australian organic production, many growers face particular challenges due to infertile soils, high climatic variability and large distances between farms and input sources (Malcolm et al. 1996) and the use of stockless systems is also common amongst organic growers (Hudson 1996) reducing the opportunities for on-farm nutrient cycling (Stockdale et al. 2000).

Killham (2002) stated that although organic farming presents an ecological, rather than a chemical approach to food production, it too has its own set of environmental and economic challenges to address. Some of these are finding alternative sources of plant nutrients to replace those exported off-farm, the use of crop rotations to counteract pests, diseases and

weeds within the context of integrating whole-farm operations to increase soil organic matter and maximise the retention of nutrients on-farm.

There are ongoing debates about whether organic and low input farming systems are a viable alternative to conventional farming systems with the potential to enhance agricultural sustainability and food quality (Reganold et al. 2001). Some critics of organic agriculture argue that it will not be productive enough to feed the increasingly large world population (Trewavas 2004). Organic agriculture may, in the long term, result in less land degradation, e.g. increased macro-aggregation (Cong and Merckx 2005). In a 21 year study of various organic and conventional farming systems in Europe, Mäder *et al* (2002) found that although crop yields were 20% lower in organic farms, the inputs of energy and fertilisers were reduced by between 34 and 53% and pesticides by 97%. In addition, soil fertility and soil faunal biodiversity were enhanced over the conventional systems. Methods using this ecological farming approach are still being developed and refined with the aim of increasing production in conjunction with sustaining general soil health.

Fertility management in some organic farming systems in Australia may not be adequately addressed with the traditional European methods of organic agriculture. Soil fertility management in organic farming aims at long term sustainability by on-farm nutrient cycling with some off-farm inputs also permitted under organic standards (IFOAM 2005). Most of the organically approved inputs may be poorly soluble in the soil solution and less available to crops immediately after application (Davis and Abbott 2006). Of specific concern are the findings of Penfold (2000) and Kirchmann and Ryan (2004) which indicate that plant available P is a limiting factor in organic farming in Australia due to the low natural abundance and slow rate of release from organic-permitted fertilisers, usually rock phosphate (RP).

In Australia the total area under vegetable production (70,760 ha) is less than one per cent of total vegetable production area in the world (51 million ha). Australia produces around 2

million tonnes of vegetables compared to 875 million tonnes in the world (FAO 2004). Most of the vegetable production systems around the world, whether organic or conventional, are intensive, with higher levels of external inputs such as fertilisers and crop protection products; high levels of irrigation; shorter, more frequent cropping sequences; high tillage use and so on. Positive soil nutrient budgets are common in vegetable production farms with the possibility of high levels of accumulated nutrients causing off-farm environmental effects (Chan et al. 2007; Watson et al. 2002b; Wells et al. 2002). However, there is limited information available on soil fertility in commercial organic vegetable farms in Australia (Cornish and Stewart 2002).

2.2 Soil properties in organic and conventional farms

2.2.1 Soil biological properties

Soil health partly depends on the micro-organisms in soil that aid plant growth directly (symbiosis) and indirectly by enhancing chemical fertility through mineralisation and solubilisation of nutrients (Davis and Abbott 2006). Organic farmers are particularly concerned about the soil biological health as they recognise the central role of soil biota and microbes in promoting soil chemical (Tu et al. 2006) and physical fertility (Lundquist et al. 1999). Organically approved fertilisers such as RP are sparingly soluble or insoluble in soil and the processes that enhance the release of nutrients, directly or indirectly depend on the mediation of soil organisms (Stockdale et al. 2002). Decomposer soil biota and micro-organisms in particular, supply the plants with nutrients from their form of stored sources in the soil e.g. crop residues or manure (Penfold et al. 1995).

The level of activity and size of the soil microbial biomass may differ according to which management practices have been used (Bulluck et al. 2002; Toyota and Kuninaga 2006). Soil biological activity is generally found to be higher under organic systems than conventional systems (Drinkwater et al. 1995; Dumaresq and Greene 2001; Fließach and Mäder 2000; Glover et al. 2000; Gunapala and Scow 1998; Marinari et al. 2006; Riffaldi et al. 2003; van Diepeningen et al. 2006). This has been attributed to the use of diverse crop

rotations, which may include legumes, and to the absence of pesticide use that may have undesirable effect on microbes (Bünemann et al. 2006b). Organic matter building in organic systems will encourage decomposer microbial activity, speeding up nutrient release from organic residues and suppressing the pathogenic effect of some soil borne diseases (Weller et al. 2002). In addition, the benefit to plant growth may be derived indirectly by facilitation of growth through improved soil biological conditions rather than the direct effect of soil biota on chemical and physical fertility (Knudsen et al. 1999). In some studies total C and microbial biomass C show no consistently significant differences in soils under organic and conventional management (Watson et al. 2002b). A similar observation in soil organic carbon and microbial biomass was reported by Burkitt *et al.* (2007) between biodynamic and conventional dairy farms in Australia and Penfold *et al.* (1995) found no differences in microbial biomass in organic, biodynamic and conventional broad-acre farming systems in South Australia after 6 years. The lack of differences between farming systems can be attributed to the length of time the farms has been under organic management, e.g. compost application and the specific farming practices (Monokrousos et al. 2006; Zaller and Kopke 2004), together with the nutrient content and quality of organic matter residues added (Marinari et al. 2006; Shepherd et al. 2002; Stockdale et al. 2002) as well as its quantity (Watson et al. 2002a).

Compost application to soil contributes to chemical and biological soil health in many regions of the world including South Asia (Manna et al. 2003), Australia (Wells et al. 2000) and the United States (Drinkwater et al. 1995). Compost can increase beneficial soil microbial populations compared to the application of chemical fertilisers (Bulluck et al. 2002). This has been attributed to enhanced physical and chemical fertility. Other organic materials such as composted cotton gin trash and straw mulching effectively increased soil microbial biomass in an organic tomato farming system in the USA (Tu et al. 2006). However, Toyota and Kuninaga (2006) found farmyard manure (FYM) application along with chemical fertiliser

had less influence on increasing the total number of soil bacteria compared to chemical fertiliser alone, though it helped to increase certain specific groups of bacteria. This was in contrast to the findings of Widmer *et al* (2006) that FYM had a positive effect on soil microbial biomass. The quality (or nutrient content) of organic amendments will depend on the quality of the original residues and their age (Berry et al. 2002).

The above review on soil microbial activity suggests that the indigenous soil microbial population can readily adapt and multiply with respect to changes in the soil environment such as additional of organic or inorganic amendments.

2.2.2 Soil organic carbon, cation exchange and pH

Organic management under South Australian broad-acre farming conditions, including green manuring, could only increase the soil organic carbon (SOC) by 1% in 20 years (Penfold et al. 1995) and further slow built up of SOC is reported by Marinari *et al.* (2006) who observed no consistent increase in SOC over seven years of organic management of field crops. The relationship between SOC and CEC of soil is tend to be linear (Ata Rezaei and Gilkes 2005) and an increase in CEC could be expected if there is an increase in SOC over years. Bulluck *et al.* (2002) observed organic amendments enhancing cation exchange capacity (CEC) in the second year after addition and Wells *et al.* (2000) observed an increase in CEC after 3.5 years of intensive vegetable production, indicating the long term benefits to soil fertility of organic inputs.

Manures and compost are known for their liming effect (increase in soil pH) (Eghball 1999), although Van Diepeningen *et al.* (2006) did not observe any significant difference in soil pH between organic and conventional farms due to overlap of organic inputs between farming systems. However several other studies reported an increase in soil pH over time (Clark et al. 1998; Dumaresq and Greene 2001; Mäder et al. 2002; Riffaldi et al. 2003; Zaller and Kopke 2004) which could be due to possible liming effect of organic inputs. It is important that the system described here has high compost inputs.

2.2.3 Nitrogen in organic and conventional farms

Several strategies are available to supply nitrogen (N) in organic farming systems for example, using crop rotation with legumes, green manuring and applying manure or compost (Ryan et al. 2004). The availability of N can limit yield in some organic production because of low levels of immediately available mineral N from organic sources (Watson et al. 2002a). Nitrogen needs to be mineralised from these sources, a biological process which is temperature and moisture dependant, and the timing of N release should coincide with crop growth requirements (Berry et al. 2002). The yields of organic arable systems can be limited by N by 50% to 95% of those in conventional agriculture, the shortfall being linked to difficulties faced by farmers in managing soil N fertility to meet the crop demand (Watson et al. 2002a).

On the other hand, the slow mineralisation of N from organic sources in organic systems may reduce N leaching and consequent soil acidification and contamination of ground water supplies (Kramer et al. 2006; Wells et al. 2002). Drinkwater *et al.* (1995) observed that though soil inorganic N in organic farms was 25% lower than conventional vegetable farms in California, potentially mineralisable N was three times higher in organic farms, suggesting that the soil N would be available in future. Marinari *et al.* (2006), observed that the total N content of organic soil was always higher than conventional soil but the ratio of organic N varied in different seasons due to difference in mineralisation.

Soil N fertility status depends on soil type (van Diepeningen et al. 2006) and seasonal rainfall, which can result in high microbial activity favouring N fertility such as high population nitrifying bacteria may occur under moist conditions (Chao et al. 1996; Marinari et al. 2006) and also the number of years of specific management (Monokrousos et al. 2006). Under manure and compost application, the crop response will generally be related to N, and there is possibility of excessive P accumulation. This is due to the ratio of P to N being higher in manure than plant requirements (Eghball and Power 1999). Thus, the biggest issue being

reducing the manure and compost inputs and to manage N and subsequently P. One option being employed successfully was using leguminous cover crops to manage N (Stopes et al. 1996), however the requirement of P while using leguminous cover crops need further study.

2.2.4 K and secondary nutrients in organic farms

The concentration of potassium (K) and secondary nutrients such as sulphur (S), calcium (Ca) and magnesium (Mg) in soil is influenced by its parent material, weathering and leaching of soil minerals, clay mineral, texture, organic mater content and fertilisation history (Peverill et al. 1999). Most of the organic amendments used in organic cropping are based on N and P requirement (Eghball 2002; Eghball and Power 1999) and the content of K, S and secondary nutrients may not be accounted for, ultimately affecting soil fertility and nutrient status in the long term (Clark et al. 1998). Organic farmers rely on manures, compost and minerals such as feldspar as source of K (IFOAM 2002). Usually surplus K budgets were reported in range of enterprises such as vegetable, dairy and arable in organic farming systems (Watson et al. 2002b). However, excess K as a result of large external inputs in intensive vegetable production (Wells et al. 2000) may tend to reduce the uptake of other cations by displacing them from available sites (Lampkin 1990).

Calcium is largely used to modify the pH of soil, as most plants will grow successfully grow in a limited pH (5-8) (Naramabuye et al. 2007). Liming is standard practice in organic farming although certain Ca sources such as slaked lime and quick lime are not permitted (IFOAM 2005).

2.3 Phosphorus in agriculture

2.3.1 Phosphorus in soil and plants

Phosphorus (P) is an essential macronutrient for plants and P deficiency is found to limit crop yield (Fletcher et al. 2006). Phosphorus is the second major limiting nutrient in Australian broad-acre agriculture, partly due to the inherently low P status of Australian soils (Penfold 2000). The poor P fertility of Australian soils is due to their coarse-textured parent materials (Peverill et al. 1999). Phosphorus occurs in most plants in concentration between

0.1 and 0.5%, with critical P concentrations for each crop varying at different stages of their crop growth (Reuter and Robinson 1997). Phosphorus is a constituent of nucleic acids, phytins, phospholipids and numerous substances involved in biochemical reaction such as photosynthesis, respiration, etc. (Hazelton and Murphy 2007). Plants absorb P as either $H_2PO_4^-$ or HPO_4^{2-}. Phosphorus plays a vital role in stimulating early root development and growth (Marschner 2002), and acts as an energy currency within plants.

2.3.2 Phosphorus in organic and conventional agriculture

In conventional agriculture in Australia, there is increasing wide-scale use of readily soluble P sources such as single superphosphate (FIFA 2006). However, management of P in organic agriculture has been of particular concern in Australia, with large areas of ancient depleted soils which are not being able to replenish sufficiently to meet the crop demand by the organically approved fertilisers such as rock RP (Kirchmann and Ryan 2004; Penfold 2000). Penfold *et al.* (1995) observed between 9 and 12% decline in extractable soil P in organic and biodynamic farms from the baseline as compared with 6 and 19% increase in integrated and conventional treatments after six years due to a low and less soluble P source in the organic and biodynamic treatments. Organic farming is criticised as it could be said that it depletes or "mines" soil P which has built up during earlier conventional management with inorganic sources of P (Gosling and Shepherd 2005). A recent study on 10 paired conventional and biodynamic dairy farms in Australia found negative P balances in biodynamic farms compared to conventional, but found no difference in total P in the subsurface layers of soil (>10cm). It was suggested that more P imports were required to ensure the future sustainability of the biodynamic farms tested (Burkitt et al. 2007). Soil type may have a greater influence on soil P than management type, for example, in arable soils in the Netherlands, van Diepeningen *et al.* (2006) observed a decline in soil P status in organic clayey soil and an increase under sandy soil and this could be attributed to the use of different

management practices like plough depth, crop or cover crop type or to the management history of the soil.

Watson *et al.* (2002b) compiled data from 47 nutrient budgets for N, P and K in European mixed dairy farms. They concluded that farm management, such as nutrient inputs, crop rotation and tillage practices, had more influence than farming system *per se* (i.e. organic, conventional, biodynamic, etc.) on nutrient budgets, with horticultural systems showing the highest average P surplus. In general, Australian vegetable farms are reported to have high soil P fertility (Chan et al. 2007). Recent research on intensive vegetable production in Australia showed that although the available P was lower in organic systems compared to conventional, the available P increased during the 6 year trial in the organic plots and the P level was more than adequate for vegetable growing (Wells et al. 2000). Excessive P accumulation due to use of compost and manures to meet N requirement and off-farm environmental effects are reported in few studies into vegetable production in Australia (Cornish and Stewart 2002; Wells et al. 2002).

Further research is necessary on two aspects of P management in Australia on

1. enhancing the P supply to crops in broad-acre organic farming given the slower release of P from the certified organic P sources such as RP (Evans et al. 2003; Evans et al. 2006).
2. reducing the excessive P accumulation in intensive vegetable production.

2.3.3 Effect of different inputs on soil phosphorus

Rock phosphate is often used as a source of P in Australian broad-acre organic farming, but the time for conversion to plant-available P can be long, from more than a year for RP, compared with immediately after application for SP (Watson et al. 2002a). Most of the Australian studies investigating on RP have reported a lack of dissolution of even reactive RP over the long term due to insufficient hydrogen ions and lack of rain during growing seasons (Bolland et al. 1997). Rock phosphate is less commonly used in vegetable growing, where

manures, compost and blood & bone can be more affordable alternative sources of P (McCoy and Parlevliet 2001). Regardless of the farming system, the efficient use of P fertilisers is hindered by sorption (Ayaga et al. 2006) and immobilisation in the soil, and methods for improving P availability are sought after (Richardson 2001), especially in alkaline soils.

The response of a crop to RP application mainly depends on the solubilisation rate which depends on conducive soil environment such as low P, low calcium and sufficient moisture (Bolan et al. 1990; Rajan 1996). Though direct application of RP is not recommended for non-acidic soils, its utilisation can be improved incorporating legume crop rotation (Vanlauwe et al. 2000). Certain legume species are known for altering the phosphorus chemistry in the rhizosphere and solubilising or mobilising the partly soluble P (Horst et al. 2001; Kamh et al. 1999; McLenaghen et al. 2004; Pypers et al. 2006). The above review suggests that legumes may convert RP into a more available source.

Annual P-based manure application has been found to build up soil fertility and achieve higher phosphorus use efficiency (Eghball and Power 1999). Sikora and Enkiri (2005) observed similar performance of crops under inorganic and organic P sources, while Ramirez and Lizaso (2006) reported the poor performance organic P sources compared with inorganic soluble sources in maize under acidic soil conditions. Earlier research on poultry manure versus other organic amendments in acid soil revealed that poultry manure was more efficient than other amendments in producing higher maize yield which was associated with increases in pH, Ca, Mg and P, and a decrease in Al (The et al. 2006). Research comparing the effect of poultry litter compost and triple super phosphate on P uptake by fescue (*Festuca* sp.) found similar responses between both treatments (Sikora and Enkiri 2005). Dao *et al.* (2001) has reported that water and Mehlich-3 soluble P content was similar in poultry manure before and after composting.

Soil P pools such as solution P, labile P and non labile P are differentially available and the efficiency of added mineral fertiliser to increase the soil test P is less than 20% (Mattingly

1975). The availability of manure P and its subsequent effect on soil P is quite different from P in mineral fertiliser (Griffin et al. 2003). Earlier studies comparing poultry manure with mineral fertiliser showed that 3-4.5 kg P ha^{-1} from poultry manure was required to raise the Mehlich-3-P by 1 mg kg^{-1} compared with 16.5 kg P ha^{-1} from mineral fertiliser (Lucero et al. 1995). Other research supported this finding with 5.6 kg P ha^{-1} and 17.9 kg P ha^{-1} from poultry manure and mineral fertiliser required to increase the Olsen P by 1 mg kg^{-1} in soybean wheat rotation (Damodar Reddy et al. 1998). Griffin *et al.* (2003) reported the superiority of poultry manure over beef, dairy and swine manures in P supply to soil. An increase in the organic acid concentration as a result of manure decomposition was responsible for the reduction in P sorption, and the concentration increased more with manure than inorganic P over the long term (Laboski and Lamb 2003).

Compost and manures can supply phosphorus in soils where soluble mineral P is not applied (Eghball and Power 1999; Mkhabela and Warman 2005; Mohanty et al. 2006). With P concentrations in manures and composts high enough to increase the plant available P when applied to soils (Goodwin et al. 1998; Nguluve et al. 2004), P mineralisation from applied manure depends on its C:P ratio. The use of low C:P ratio manure is important for favouring P supply and poultry manure has a low C:P ratio and is suitable for phosphorus management (Bahl and Toor 2002). This suggests that manures with a low C:P ratio could be a possible option for P management in low external input systems.

2.3.4 Phosphorus and microbial activity

Methods to enhance the biological cycling of phosphorus (P) are needed in P limiting soils (Ayaga et al. 2006) and bio-fertilisers (soil inoculants) have been suggested as a means of increasing soil fertility and crop production in sustainable farming (Sivapalan et al. 1993). Some naturally occurring fungal organisms that enhance P uptake in plants, e.g. vesicular-arbuscular mycorrhiza (AMF), are maintained under organic farming but may be severely depressed under conventional farming (Penfold 2000) as, when soil P levels increase, AMF

infection rates on roots were reduced. King and Williamson (1992) found a decline in AMF presence in white clover roots with increasing levels of superphosphate application to pasture soil over 43 years. Though AMF can potentially increase P uptake by acting as an extension of the plant root system, it was found to have negligible effect in P mobilisation under intensive organic or conventional mixed cropping systems in southern Australia (Ryan and Ash 1999; Ryan et al. 2000). Biodynamic and conventional soils have been found not to develop significantly different processes to enhance plant nutrient uptake (Ryan and Ash 1999) and the high colonisation of AMF in an organic system did not overcome the serious P deficiency experienced in that system (Kirchmann and Ryan 2004). Thus, AMF's biological activity is not sufficient to mobilise or solubilise the phosphorus nutrition requirement of crops in the current season, though they could release them slowly in the long term. Although the potential exist for developing of phosphorus solubilising, microbial inoculants such as *Penicillium* sp.(Wakelin et al. 2004), *Bacillus polymyxa* and *Pseudomonas striata* (Manna et al. 2003), their widespread application is limited due to various reasons which includes inconsistent performance over a range of environments due to a poor understanding of their ecology and population dynamics in soil (Richardson 2001).

2.3.5 Phosphorus management using cover crops and cultivars

There may be scope to increase P cycling and availability in organic systems through the use of P-accumulating plants. In a study in south-eastern Australia with mixed plantings of *Acacia mearnsii* and *Eucalyptus globulus*, the *Acacia* species not only fixed significant quantities of N, but also enhanced P cycling through litter fall (Forrester et al. 2005b). The value of tropical P accumulating plants such as *Tithonia* has also been reported in other low-input farming systems (Kwabiah et al. 2003a) and temperate and sub-tropical winter legumes such as fababeans and field peas in Australia (Nuruzzaman et al. 2005a), highlighting the potential use of perennial and annual crops in P cycling. A detailed review of P management using cover crops particularly legumes, is presented in Section 2.5.

Another option for P management is using the cultivars that have higher P efficiency (Clark 1983; Fageria and Baligar 1997; Fageria and Baliger 1999). A discussion of the literature related to crop cultivar effects on P nutrition is presented in Section 2.4

2.3.6 Methods of measuring soil P

Similar nutrient cycling processes occur in organic and conventional soils, but their magnitude and rates may differ and during the absence of a readily available nutrient source, nutrient reserves in less available pools will be of greater significance (Stockdale et al. 2002). Low inorganic N or P reported earlier in several organic soils cannot be considered to indicate infertile soils, because of the high demand; the released nutrients were taken up without changing soil chemistry significantly as nutrients released are rapidly taken by plants or microbes (Davis and Abbott 2006). Standard soil testing methods may not be sufficient to predict the P fertility due to difference in mobility of P ions in a different farming system (Oberson et al. 1993). Therefore, studies on different methods of P estimation in soil are reviewed.

Soil testing is a useful tool for assessing the plant available P (Mallarino and Atia 2005) and for making agronomic recommendations (Ketterings and Flock 2005). Several methods have been used to measure the soil available P. Each method has its own advantages and disadvantages. The Bray-1 method by Bray and Kurtz (1945) is used to test acidic to neutral soils with little or no calcium carbonate. The sodium bicarbonate (pH 8.5) extraction method was originally reported by Olsen *et al.* (1954). A modification to the Olsen method was described by Colwell (1963) where the extraction time was increased from 30 minutes (in Olsen method) to 16 hours (in Colwell method). At present, many laboratories follow Mehlich-3 test as the standard soil test (Mehlich 1984).

An alternative method for chemical extraction using anion exchange membranes (AEM) which act as simulators of plant roots in absorbing nutrients from solution (Yang et al. 1991) has been shown to be a good indicator of soil nutrient availability measured at soil pH

compared with chemical extraction (Mallarino and Atia 2005; Qian et al. 1992; Schoenau and Wang. 1991; Zheng et al. 2003; Ziadi et al. 1999). Saggar *et al.* (1999) concluded "The Resin test is more suitable than the current Olsen test for assessing the plant available P status of soils previously fertilised with fertilisers of varying solubility". Saggar *et al.* (1990) developed a simplified extraction technique using strips of resin membrane which eliminated the procedural limitations of resin beads usage in a bag (Sibbesen 1977). Sata and Comerford (2006) who compared a multiple AEM method with a sequential AEM concluded that these methods should be tested on a range of soil types to determine their suitability in developing P desorption isotherms. Resin P is usually measured in diluted soil solution and is a good indicator of available P.

Based on the review above about methods of soil P measurement, Resin P represents the exchangeable P in soil. It gives the available P status at the existing soil pH and thus differs from other methods such as Olsen, and Colwell, where the test is performed at higher pH (8.5) than soil and there is a possibility to overestimate as Fe and Al-P in acid soils may unbound at high pH and some available P in alkaline soils might precipitate with Ca under high pH. The Resin P method could be the best method for comparison of available P for field soils with pH ranging from acidic to alkaline.

2.3.7 Use of radioisotopes in phosphorus uptake studies

There are several studies involving radio isotopes in agriculture and in particular using P isotopes to study soil and plant nutrition. The techniques used to measure the transfer of orthophosphate from the solid phase of soil to solution involves measuring isotopically exchangeable P by adding ^{32}P or ^{33}P orthophosphate to soil suspension and measuring the decay of isotopes over time (Fardeau 1996). Earlier studies have used other methods using bioassays and measuring the specific activity in the plant dry matter grown in ^{32}P or ^{33}P labelled soil (Larsen 1952; Russell et al. 1957).

^{33}P has a higher half-life (25.4 days) to ^{32}P (14.3 days) and is superior as a radiotracer for P in most biological applications (Robinson 1969), however, the availability of ^{32}P and ^{33}P makes it possible to use dual labelling research in plant nutrition (IAEA 2001), a technique that has been successfully employed in several older studies (McLaughlin and Alston 1986; McLaughlin et al. 1988a; b). Recently, the dual labelling technique was used to determine the short-term fate of inorganic P fertilisers in clay soils used for cotton production in Australia (Dorahy et al. 2007). The ^{33}P and ^{32}P dual labelling technique is also a valuable tool to quantify the proportion of P uptake from green manures and inorganic fertilisers (Bah et al. 2006).

2.4 Genotype x fertiliser interaction in corn

2.4.1 Overview of corn production

Corn or maize (*Zea mays* L.) can be categorised as a cereal and also as a vegetable based on variety, harvesting time and final usage. Although few statistical distinctions are available for corn production as cereals or vegetables, the Food and Agricultural Organisation (FAO) has summarised that total corn production in the world in 2005 was 701,666,160 tonnes from 147,576,740 ha and that of Australia is 312,000 tonnes from 75,000 ha (FAO 2004). The United States was the largest corn producer (43% of world production) followed by Asia (25%), Latin America and the Caribbean (13%) and Africa (7%) (IITA 2006). Sweet corn is grown in most Australian states with NSW alone producing half of the national production of 80,467 tonnes (ABS 2006).

2.4.2 Need to explore plant traits in organic production

The lack of detailed knowledge about cultivar performance under organic farming conditions limits the development of specific varieties that are known to perform better under organic conditions and constrains the choice of cultivars and quality of seed available to farmers (Lammerts van Bueren et al. 2003).

Gene expression of the phenotype is dependant on the environment (Kang 1998). Variation in genotypic responses to environmental factors such as temperature, soil moisture,

and soil fertility are the function of interactions between genotype x environment (GxE). Where genotypes perform differently in different environments, this is termed as GxE interaction (Baker 1988). Therefore, cultivars developed for one management regime need to be investigated before adoption in other management systems (Cooper et al. 2001).

Although organic standards require that crops be grown from organically produced seed or plant propagation materials, if suitable organic material is not available in sufficient quality and quantity, a derogation rule allows the use of non-organic material (Zeijden 2002). Currently there is a lack of readily available, high quality organic-specific cultivars globally, and for varieties suited to Australian conditions in particular. There is a growing number of commercial and non-profit community groups that breed, select and grow out a diverse array of organic vegetable lines, although issues about quality and regularity of supply have been raised (Neeson and Howell 2003).

Corn uses large amounts of P from the soil, to the extent that it is used in P reduction studies in USA (Eghball et al. 2003). Past studies on the estimates of overall efficiency of applied fertiliser in corn have been reported to be about (or lower than) 50% for N, 40% for K and less than 10% for P. Increased nutrient use efficiency in plants is vital to enhance yield and quality, and to reduce input costs and to improve soil quality. Intra-specific (cultivar) variations in plant growth and mineral nutrient use efficiency are modified by interactions with environmental variables. Identification of traits such as nutrient absorption, transport, utilisation, and mobilisation in plant cultivars could greatly enhance fertiliser use efficiency (Baligar et al. 2001).

2.4.3 Plant adaptation to phosphorus deficiency

Root growth and function is important at an early stage for crops to extract soil P as P is immobile in many soils. Root architecture, the spatial configuration of a root system in the soil, can vary between and among species and plays an important role in belowground resource acquisition (Lynch 1995; Lynch and Beebe 1995). Greater nutrient acquisition has

been associated with increased soil exploration by roots in surface layers, especially in the case of immobile nutrients such as P (Ho et al. 2004; Silberbush and Barber 1983). Plants adapt to P deficiency by altering their morphological and physiological responses (Schachtman et al. 1998) such as increasing root:shoot ratio, increased root hair proliferation, root hair elongation, accumulation of anthocyanin pigments, proteoid root formation (Anghinoni and Barber 1980; Rosolem et al. 1994; Schenk and Barber 1979; Walk et al. 2006; Zhu and Lynch 2004), increased association with mycorrhizal fungi (Hinsinger 2001; Marschner et al. 2007; Raghothama 1999) and exudation of various compounds including organic acids in the rhizosphere (Jones 1998). These modifications help plants increase P availability in the rhizosphere and thus enhance crop P uptake (Hinsinger 2001). These differences in root morphology and functions, and their subsequent rhizosphere effect, have been explored between cultivars to select suitable varieties for P deficient conditions (Liu et al. 2004; Wang et al. 2004). High P use efficiency can involve adaptive traits that allow plants to produce more dry matter for each unit of applied P, and to take up P at lower concentrations in the soil solution and from less available sources (Marschner 2002).

2.4.4 Cultivar x phosphorus interaction in corn

Differences in the P uptake between cultivars has been found in a wide range of crops such as legumes (Caradus et al. 1998; Hill and Jung 1975; Vesterager et al. 2006), pasture species (Bélanger et al. 2002; Caradus et al. 1998; Hill and Jung 1975; Missaoui et al. 2005; Sleper et al. 1977; Zobel et al. 2006), cereals (Egle et al. 1999; Saito et al. 2006) and corn (Ciarelli et al. 1998; Liu et al. 2004; Machado and Furlani 2004; Nielsen and Barber 1978). By testing corn cultivars using different organic P sources it is possible to identify cultivars (and traits) that perform better under P limiting conditions (Fageria and Baliger 1999). It has been suggested that P efficiency is a multi-gene controlled quantitative trait (Chaubey et al. 1994; da Silva et al. 1992), so identification of P-efficient genotypes and understanding of

underlying physiological mechanisms of efficiency are important for parent selection in a traditional breeding program.

Variation in adaptive traits for P uptake within corn cultivars under different management conditions has been reported by many authors. Nielsen and Barber (1978) observed differences in P uptake among corn cultivars and concluded that root parameters for acquiring P, especially from low soluble P sources, were important. The most favourable characteristics for P uptake and use efficiency of parental genotypes were also observed in the derived hybrids, indicating that P-efficiency characters are heritable and under genetic control (Ciarelli et al. 1998). Machado and Furlani (2004) identified genotypic resistance to P stress. Low P-tolerant corn genotypes adopt different mechanisms such as increasing roots exudates or increasing the root growth to overcome the P stress (Liu et al. 2004). Ramirez and Lizaso (2006) reported cultivar differences for RP application, concluding that cultivars that combine high uptake efficiency (P uptake/unit root length) and high conversion efficiency (biomass production/unit P uptake) would improve RP utilisation by corn.

The above section has focussed on factors affecting corn varieties in low P soils, such as those fertilised with RP, and how cultivars differ in their P use efficiency in similar environmental conditions. The following discussion reviews the literature on soil changes induced by cover crops and effect of cover crops, particularly winter legumes, in enhancing the soil fertility and possible benefits for subsequent crops, particularly P nutrition.

2.5 Legumes and phosphorus benefits

Cover crops are an important component of crop rotation, forming one of the key factors in organic crop production (IFOAM 2002). The fact that winter legumes can provide significant nitrogen inputs is well established (Groffman et al. 1987; Stopes et al. 1996) and the effect of winter legumes on soil N dynamics and crop N uptake has been fairly well studied (Müller et al. 2006). However, there has been considerably less research done on the effect of winter legumes on other crop nutrients. In countries such as Australia, where the natural P abundance is low, P accumulating cover crops could form an important component

in organic crop rotations. Information about the P benefits of legume cover crops could provide a further option for P fertility management in organic farming. The use of a dual purpose grain legume is recommended for crop rotation, rather than adopting a herbaceous legume to get higher economic benefits (Pypers et al. 2007).

2.5.1 Influence of cover crops on soil pH

Cover crops can influence the soil pH and nutrient availability (Groffman et al. 1987). Phosphorus solubilisation in legume crops were related to exudation of organic acids (Jemo et al. 2006) such as citrates, malates and carboxylates and its large roots morphology (Nuruzzaman et al. 2005a). The growth of cover crops usually results in a decrease in soil pH due to the release of protons, but, soil pH can also increase initially after the application of legume residues due to the return of accumulated organic anions back to the soil (Yan et al. 1996). The fate of fertiliser applied to legume cover crops soil also depends on plant species that modify the conditions in the rhizosphere through water and mineral uptake, changes in pH and redox potential and through rhizodeposition that stimulates microbial growth (Nguyen 2003).

2.5.2 Changes in soil after plant residue addition and P availability

When a green manure or legume cover crop is incorporated into the soil, several processes are likely to occur that enhance the P availability to subsequent crop (McLenaghen et al. 2004). After incorporation, P is released from residues by several processes which includes autolysis of residues by desiccation (Bromfield and Jones 1972) and microbial decomposition which indirectly depends on soil temperature (Till and Blair 1978), soil water (Martin and Cunningham 1973) and the presence of living plant roots (Blair and Boland 1978). After incorporation, cover crops initially retain the nutrients within plant tissues and reduce sorption in soil (Harrison 1985), Later, phosphorus released from residues preferentially moves to the soil solution is mineralised and made available to plants (Nziguheba et al. 2000). Minimising the interaction of the released P with soil to reduce

immobilisation may be achieved by the presence of active roots of a subsequent crop that acquire the released P before it is subject to adsorption or fixation (Friesen et al. 1997). Higher infection rates of mycorrhiza in legume roots indirectly benefits the subsequent crop in soil P availability (McLenaghen et al. 2004). Legume organic matter may enhance the soil physical conditions, thereby indirectly aiding the microbial activity, plant foraging and nutrient uptake (Kabir and Koide 2002).

The release of P from organic residues was reportedly limited by the total P content of residues (Kwabiah et al. 2001) under sub humid tropical conditions. Increase in soil pH (Yan et al. 1996) after green manure (*Tithonia* sp.) residue addition increased the solubilisation of P fixed by Al in acidic soils (Cong and Merckx 2005). In contrast, acidification of soil by release of H^+ ions can contribute to the release of P from calcium phosphates in alkaline soils (Gahoonia et al. 1992).

Slow N mineralisation in oven-dried residues compared with fresh residues was explained as the reason for the difference in soil pH (Yan et al. 1996). The increase in soil pH after residue addition was identified as a liming effect of the applied residues which is related to macromolecules, mainly pectinates in the residues (Yan and Schubert 2000).

2.5.3 Partitioning of nutrients by root and shoot in legumes

Understanding nutrient partitioning and mobilisation within plants helps to interpret results obtained in plant analyses. Under low P conditions, much of the total plant P is partitioned to the shoots. Roots accumulate more P when there is surplus of P in the soil (Reuter and Robinson 1997). Nuruzzaman *et al.* (2005b) reported that the shoot P concentration of faba beans and field peas at 50% flowering stage varied from 1 to 2 mg g^{-1} of dry matter and root P concentrations ranged from 1-1.5 mg g^{-1}. Under soil nutrient deficient conditions, an increase in root production improves nutrient acquisition. However, the effect depends on the successful movement of newly developed roots to nutrient-undepleted zones in the soil (Lynch 1995; Steingrobe 2005). Franchini *et al* (2004) showed that the legume

species *Vicia sativa* accumulated 60% of total plant P in their roots, suggesting potential as an efficient cover crop for P cycling.

2.5.4 Organic P mineralisation rate of applied green manures

Organic P mineralisation from applied green manure is a slow process (Birch 1961) and the long term benefits of residue incorporation could be studied using experiments that can detect the temporal changes after several crop rotations (Nuruzzaman et al. 2005b). The rate of organic P mineralisation in silt loam soil after the addition of green manure (*Lupin* sp.) was 0.27 mg P kg^{-1} day^{-1} compared with the unamended soil with 0.06 mg P kg^{-1} day^{-1} up to 35 days (Randhawa et al. 2005). A long term organic cropping trial in Switzerland revealed that organic P mineralisation rate was as high as 1.7 mg P kg^{-1} day^{-1} after 3 and 10 days of incubation, but declined to 0.2 mg P kg^{-1} day^{-1} after 56 days after incubation (Oehl et al. 2001). Organic P mineralisation in sterilised soils is reported to be between 0.6 and 3.8 mg P kg^{-1} day^{-1} compared with an immobilisation rate of 0.0-4.3 mg P kg^{-1} day^{-1} (Zou et al. 1992). The quality of the applied manure and especially the C:P ratio, influence the mineralisation of organic P from residues and its availability (Zaharah and Bah 1997), and the dynamics of organic P is closely related to dynamics of carbon in the soil in long term (Bünemann et al. 2006a). The difference in mineralisation rate of various residues depends on plant species, chemical composition and soil type and, to some extent, the native P fertility of the soil. Though organic P mineralisation is a slow process, the slower mineralisation rate is important in the context of low input organic system, especially in the early phase of crop growth after organic residue addition.

2.5.5 Effect of cover crops on P benefits in subsequent crop

As indicated earlier, research on the role of cover crops or green manures in supplying nutrients to subsequent crops has commonly concentrated on N. Few studies in Australia have accounted for the P benefits while the crop is grown for N benefits (Forrester et al. 2005a; Nuruzzaman et al. 2005a). In tropical countries research on P accumulating crops such as

Tithonia sp. and their role in P cycling and availability has been reported (Kwabiah et al. 2003b). In general, cereal crops depend on their root morphological adaptation to acquire P in soil (Gahoonia and Nielsen 2004), compared with legumes which depend on soil processes induced by secretion of organic acids and root exudates (Hinsinger 2001; Jones 1998). Studies conducted on evaluating the potential benefits of legumes on subsequent crop indicate that legumes such as lupin (*Lupinus* sp.) (Kamh et al. 1999), chick pea (*Cicer arietinum*) (Hens and Hocking 2004), faba beans, field peas (Nuruzzaman et al. 2005b) and velvet bean (*Mucuna* spp.) (Pypers et al. 2007) mobilise more P than that needed for their own requirement and could be useful for subsequent crops. Legumes are known to acquire more P under P stress condition than cereals and the reverse under high P conditions (Bolland 1999; Nuruzzaman et al. 2005a). Legumes have varying potential to mobilise P for subsequent crops depending on soil type, cover crops species and the level of readily accessible P pools (Cavigelli and Thien 2003; Nuruzzaman et al. 2005b).

2.5.6 Contribution of residues and fertilisers to crop growth and P uptake

Incorporation of cover crops can reduce external P inputs and improve recycling of the existing P from plant residues (Kamh et al. 2002). The combination of legumes green manures with RP can improve the utilisation of RP by a subsequent crop (Carsky et al. 2001). For example, Bah *et al.* (2006) reported a four-fold increase in the effectiveness of reactive RP when applied with legume green manure compared to RP application alone in crops grown in acid tropical soil. Although the supply of P from green manure was less than 5% of the total P uptake, P uptake from soil P was two to five times greater than fertiliser alone (reactive RP or triple super phosphate). The improved utilisation might be achieved by mineralisation of applied residues coupled with solubilisation of residual P (McLenaghen et al. 2004).

The effect of previous cover crops on subsequent cash crops is due to several factors including crop-induced changes in soil such as increasing soil N and modifying microbial

ecology. Growth chamber studies on the effect of incorporation of medic (*Medicago* sp.) residues on subsequent wheat have shown that the residues supplied 25% of soil P from that of fertiliser and 20% of total P in the wheat plant (McLaughlin and Alston 1986). In other studies, 5.4% of P from medic residues was recovered in wheat and 22-28% recovered in microbial biomass, with the rest in soil in either organic or inorganic forms (McLaughlin et al. 1988a; b; c).

In conclusion, this review of legume cover crops and phosphorus benefits suggests that legumes have the potential to provide phosphorus benefits to subsequent crops and that possible mechanisms influencing the benefits are generally understood. However, there is lack of information about the quantity of P contributed by the roots and shoots of legumes, separately and in combination.

2.6 Conclusions and research gaps

The review of soil properties in organic and conventional production systems indicates that soil properties varied with the type, quantity and quality of inputs supplied rather than farming system per se. There are overlaps in management practices, with conventional farms often receiving organic inputs. The various methods for enhancing the P nutrition cannot meet crop demand in broad-acre organic farming systems in Australia. However, limited research on vegetable production in Australia has indicated that methods for enhancing the P nutrition are able to meet crop demand, with an increase in P and other nutrients over time. This needs further investigation to determine whether those findings are generalisable to commercial organic and conventional vegetable farms across a range of soil and climatic conditions.

Also, the manure and compost applications to meet the N requirement generally lead to P accumulation in soil as the P to N ratio is higher in the inputs than for crop requirements. Hence alternatives sources of N such as legumes could be explored for P management in organic production. There is evidence for P benefits from winter legumes to a subsequent cereal crop, but further information is needed on the amount and form of P derived both from

root residues and whole plant. Differences in P efficiency between cultivars suggests the need to compare the performance of plant cultivars under the P deficient conditions that commonly occur in Australian organic production systems in order to maximise nutrient use efficiency.

The above mentioned knowledge gaps (section 2.6) have been used to develop the hypotheses for the empirical work in this thesis. The following chaper (chapter 3) involves a field investigation exploring soil fertility status and nutrient management history of organic and conventional vegetable farms at three localities in eastern Australia.

CHAPTER THREE

Comparison of soil chemical and microbial properties in organic and conventional vegetable farms in eastern Australia

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Comparison of soil chemical and microbial properties in organic and conventional vegetable farms in eastern Australia

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Abstract

Organic agriculture is considered as one of the alternatives to conventional agriculture. While there are reports that soil fertility management, especially phosphorus (P) can limit production in organic broad-acre farming systems in Australia, there is also some evidence to show that vegetable farms have similar soil properties to their adjacent conventional farms. The soil properties (0-10 cm) of two farming systems, organic (OF) and conventional (CF) vegetable farms at three locations (Gatton, Stanthorpe and Dorrigo) were tested in 2005 and 2006. Examination of farm management records revealed substantial overlap between organic inputs at all localities with CF also using organic inputs, e.g. green manure and composts. The soil properties do not vary across the two years. Soil chemical properties (pH, electrical conductivity, total nitrogen (N), resin P, KCl-40 sulfur, nitrate-N and ammonium-N) and microbial biomass carbon were similar between organic and conventional vegetable farms, in contrast with earlier Australian studies into broad-acre organic farming systems. Extractable soil P pools were medium to high across farm types (average Colwell P >100 mg kg^{-1}). Total carbon and effective cations exchange capacity (ECEC) was higher in organic vegetable farms at Dorrigo (Ferrosol). The application of manures and compost produced a liming effect (higher soil pH) and increased the exchangeable calcium irrespective of farming system at two locations, i.e. Gatton (Vertosol) and Dorrigo (Ferrosol). It is clear that soil biological and chemical properties were strongly influenced by crop management practices and soil type, rather than the type of farming system as a whole. While the results did not support the hypothesis that organic vegetable farms are lower in soil fertility than the adjacent conventional vegetable farms, the medium to high status of measured chemical parameters

indicate all are high input systems. High extractable P levels were the result of manure and compost applications to meet the N requirement of vegetables. Crop management in vegetable production in eastern Australia (whether organic or conventional) should consider the baseline soil nutrient status to avoid over fertilisation and off-farm environmental effects.

Key words: soil, organic, conventional, vegetable farms, Australia

3.1 Introduction

Agricultural intensification to feed the burgeoning global population (FAO 2002), has lead to large scale environmental problems especially degradation of soil and water resources (Drinkwater and Snapp 2007; Millennium Ecosystem Assessment 2005). Organic farming is considered an alternative to conventional farming, providing sustainable crops with high export demand and lower environmental impact (Badgley and Perfecto 2007; Wood et al. 2006). Although organic principles and practices originated in northern Europe, they have been adopted around the world in regions with vastly different soils and climates under different environmental conditions and production systems (Kristiansen and Merfield 2006). Australian organic growers are likely to face particular challenges due to relatively weathered infertile soils, high climatic variability and large distances between farms and input sources (Malcolm et al. 1996). Nevertheless, in Australia, growth in organic production is estimated at 15-25% annually, and this is expected to continue because of strong domestic demand and expanding markets overseas, especially in Asia (Alexandra and May 2004).

Soil biological activity varies across farming systems with research findings indicating either that it is commonly higher under organic systems than conventional systems (Drinkwater et al. 1995; Dumaresq and Greene 2001; Fließach and Mäder 2000; Glover et al. 2000; Gunapala and Scow 1998; Marinari et al. 2006; Riffaldi et al. 2003; van Diepeningen et al. 2006) or that activity is not different between the two farming systems (Burkitt et al. 2007; Watson et al. 2002b). Similarly, many soil chemical properties such as available nitrogen (N), extractable potassium (K), sulfur (S) and exchangeable cations show no consistent trend between organic and conventional farming systems (Burkitt et al. 2007; Clark et al. 1999;

Drinkwater et al. 1995; Gosling and Shepherd 2005; Marinari et al. 2006; van Diepeningen et al. 2006). The inconsistency of findings across farming systems is due to the differences in the type of enterprise being evaluated (eg. stockless systems, vegetable production systems, pasture etc.), specific management practices and environmental factors such as aridity. Organic farming is defined well with list of permitted inputs (IFOAM 2002), whereas the conventional farming lacks clear distinction from organic farming as some conventional growers also tend to use organic inputs and some may not (van Diepeningen et al. 2006). Soil fertility level of farms depends on length of time a farm has been under a particular management regime (Monokrousos et al. 2006; Zaller and Kopke 2004), the nutrient content and quality of inputs (Marinari et al. 2006; Shepherd et al. 2002; Stockdale et al. 2002) as well as their quantity (Watson et al. 2002a) and soil type (van Diepeningen et al. 2006).

Organic production in broad-acre farming relies on nutrient reserves, especially P built up during conventional management (Penfold et al. 1995) due to the low natural availability of P especially in Australian soils and slow rate of release from organic-certified fertilisers such as rock phosphate. However, research on organic vegetable production reports excessive P due to the higher level of inputs per unit area (Chan et al. 2007; Watson et al. 2002b; Wells et al. 2002). Whether they are organic or conventional, vegetable production systems are known to present possible environmental concerns such as leaching of accumulated nutrients (Chan et al. 2007; Wells et al. 2002). A single case study has reported (Cornish and Stewart 2002) that soil fertility of organic vegetable gardens were not sustainable with C and N declining and P accumulating due to excess manure and compost. Although Wells *et al.* (2000) have shown that organic vegetable production methods can maintain adequate soil fertility in Australia, their findings are based on a single site and the trials were conducted on a research station for five years, not on working organic farms with longer histories of organic management. Therefore, it is unclear whether it is generally true that organic vegetable production methods are not inherently nutrient limited under typical conditions for growing

commercial vegetables in Australia. Do gross differences in soil type, climate and enterprise structure affect the ability of organic vegetable growers to maintain adequate soil fertility?

Soil properties should be discussed in conjunction with environmental and management aspects. A comparative study of the fertility status of organic and conventional vegetable farms in eastern Australia was conducted in 2005 and 2006. The research also sought to compare different inputs used by vegetable farmers to manage soil fertility. It was hypothesised that soil chemical and biological fertility in organic vegetable farms would be lower than adjacent conventional vegetable farms, and that specific management practices, not farming system *per se,* is a better indicator of soil fertility levels.

3.2 Materials and methods

3.2.1 Initial soil sampling and characteristics

Soil samples (0-10 cm) were collected from paired organic farms (OF) and neighbouring conventional farms (CF) on similar soils at three localities in eastern Australia where vegetables are commonly grown commercially (Figure 3.1). The Gatton region (27.5° S, 152° E, 94 m elevation) has a warm sub-tropical climate and a wide range of summer and winter vegetables are grown. The soil type is predominantly Vertosols (Isbell 1996), uniform medium to heavy cracking clays. The Stanthorpe region (28.6° S, 152° E, 872 m elevation) has a cold sub-tropical climate with large areas of Tenosols (Isbell 1996), deep sandy soils derived from granite. Cooler climate vegetables are grown throughout the year. The Dorrigo area (30.34° S, 152.7° E, 734 m elevation) has cool sub-tropical weather and fertile Ferrosols (Isbell 1996), which are acidic red clay soils derived from basalt and are suitable for growing potatoes, garlic and leafy herb and vegetable crops.

Drip irrigated, conventional tillage systems are the most wide-spread production methods used in Gatton and Stanthorpe with black plastic mulching and fertigation common. In Dorrigo, overhead irrigation is more common, plastic mulches are not generally used and regular tillage is not a standard practice. Details of individual farms are presented in Tables 3.1. All the farmers completed a questionnaire about farm management history over the last

five years regarding the type of green manure or cover crops (GM), bulky manures such as cow manure, sheep manures, poultry manures and their composts (CTBM) and the use of synthetic fertilisers not permitted in organic systems (CF). Since, the type of inputs used was very diverse and the quality of some questionnaire responses was variable, the data were classified as "Yes" or "No" indicating if the management practice was used or not.

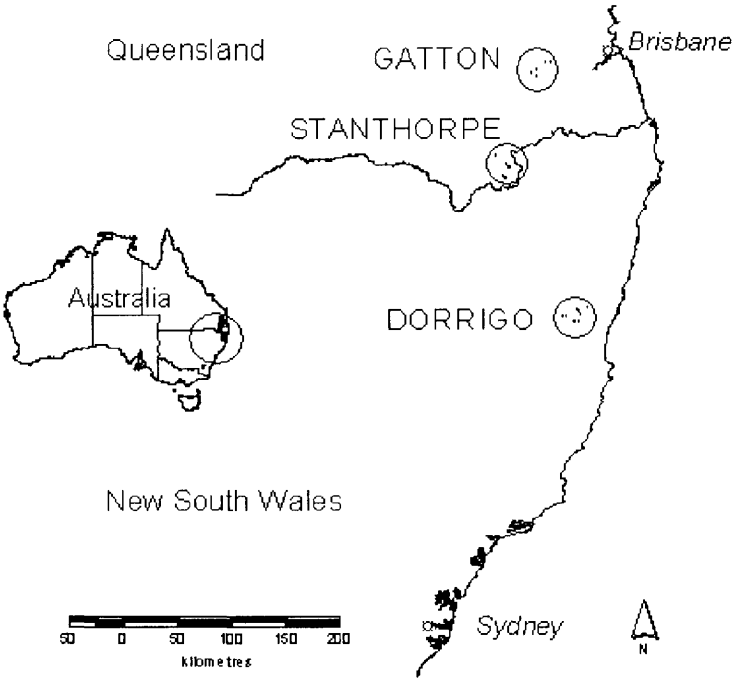

Figure 3.1. Map of Australia showing the location of vegetable farms investigated in this study

Soil samples were taken over a span of four days during the last week of February in 2005 and 2006. Each pair of organic and conventional farms was sampled on the same day. Samples were collected using a 10 cm deep soil core sampler at 20 sites placed randomly across the farm, bulked and transported in cool containers to the laboratory. The samples were sieved using 5 mm sieve, and a sub-sample taken for microbial analysis and stored at 4°C until measurement. The rest of the samples were air dried at 40°C for 48 hours and stored at room temperature for textural and chemical analysis. Two sub-samples were taken for chemical analysis and passed through 2.0 mm and 0.5 mm sieves respectively.

Table 3.1. Details of farms using particular fertilisation practices of organic and conventional vegetable farms in Eastern Australia

Farm	Farming system	Years[1]	Texture	GM[2]	CT/BM[3]
		Gatton			
Farm 1	Organic	5	Clay Loam	Yes	Yes
Farm 2	Conventional	50	Clay Loam	Yes	No
Farm 3	Organic	18	Clay	Yes	Yes
Farm 4	Organic	5	Silty Clay	Yes	Yes
Farm 5	Conventional	50	Clay Loam	No	Yes
Farm 6	Conventional	50	Clay Loam	No	Yes
Farm 7	Organic	14	Silty Clay	Yes	Yes
Farm 8	Organic	10	Clay	Yes	Yes
		Stanthorpe			
Farm 9	Conventional	30	Loamy Sand	No	No
Farm 10	Organic	7	Sand	Yes	Yes
Farm 11	Organic	12	Loamy Sand	No	Yes
Farm 12	Organic	10	Loamy Sand	Yes	Yes
Farm 13	Conventional	15	Loamy Sand	Yes	Yes
Farm 14	Conventional	50	Loamy Sand	No	Yes
Farm 15	Organic	14	Loamy Sand	Yes	No
Farm 16	Conventional	50	Loamy Sand	Yes	Yes
		Dorrigo			
Farm 17	Organic	5	Sandy Clay Loam	No	Yes
Farm 18	Organic	5	Clay Loam	Yes	Yes
Farm 19	Organic	5	Sandy Clay Loam	Yes	Yes
Farm 20	Organic	5	Sandy Loam	Yes	Yes
Farm 21	Organic	5	Sandy Clay Loam	Yes	Yes
Farm 22	Conventional	3	Sandy Clay Loam	No	No
Farm 23	Conventional	25	Clay Loam	Yes	Yes
Farm 24	Organic	10	Sandy Clay Loam	Yes	Yes

[1]Years: years under current management system; [2]GM: Green manure or cover crops produced on-farm; [3]CT/BM: Compost or bulky manure imported to the farm.

3.2.2 Soil microbial biomass carbon

Soil microbial biomass carbon (MBC) was determined using the Substrate-Induced Respiration (SIR) method (Anderson and Domsch 1978). Soil was broken into small clumps and stones, large invertebrate animals, stones and roots were removed before the soil moisture was adjusted to approximately 75% field capacity where microbial respiration is optimal. The moistened soil was incubated at 25°C for two days and then CO_2 evolution rate was measured in an electronic respirometry system (Respicond III, Nordgren Innovations AB, Terrangvagen 3A S-903 38 Umea, Sweden). Average basal respiration rate was measured over 48 hours (mg CO_2/hour/100 g dry matter soil). The increase in respiration rate after the addition of a glucose substrate is used to calculate the amount of MBC in soil (mg Microbial C/100 g dry matter soil), using the following formula:

MBC (mg microbial C/100g dry matter soil) = (40.4 * SIR) + 0.37

3.2.3 Chemical and textural analysis

The dried soils were analysed for pH, electrical conductivity (EC), nitrate-N, ammonium-N, resin P, KCl-40 sulfur, and exchangeable cations such as K, Ca, Mg, Na, Al and Mn in 2005 and 2006. The details of methods used are listed in Table 3.2. Total carbon (C) and N was measured using a Carlo Erba NA1500 solid sample analyser in 2005 and 2006. Soil texture was measured using the hydrometer method (Gee and Bauder 1986) in 2005. Colwell P (Colwell 1963) and Olsen P (Olsen et al. 1954) were estimated for soils collected in 2005 and results were correlated with those of resin P.

Effective cation exchange capacity (ECEC) and exchangeable Na percentage (ESP) were calculated as follows

ECEC= Exchangeable (Ca+ Mg+Na+K+Al)

ESP = Exchangeable Na/ECEC

Table 3.2. Methods followed for chemical analysis of soil

S.No	Parameters measured	Extractant used	Reference
1	Soil pH (1:5)	Water suspension	
2	Electrical conductivity (1:5)	Water suspension	
3	Nitrate-N	2 M KCl	Page et al., (1982)
4	Ammonium-N	2 M KCl	Page et al., (1982)
5	Resin P	Resin strips, 0.7 M NaCl	Guppy et al., (2000)
6	KCl-40 sulfur	0.25 M KCl	Blair et al., (1991)
7	Exchangeable cations	0.1 M $NH_4Cl/BaCl_2$	Gillman and Sumpter (1986)

3.2.4 Statistical analysis

Data were analysed with two-way analysis of variance (ANOVA) using the statistical program R (R Development Core Team 2006). Farming system and location were the two factors tested. Differences between sampling dates (i.e. 2005 vs 2006) were not significant for almost all of the measured variables ($P > 0.05$), as well as interactions with farming system and locations ($P > 0.05$); hence the data was pooled across years in the analyses. The farms were also classified based on management variables such as green manuring, compost or manure application and chemical fertiliser application, and the data were analysed as two-way

ANOVAs with location and each management variable as the other factor. Diagnostic plots were used to check the normality of the data and homogeneity of variance for each response variable and necessary transformations were estimated using the boxcox procedure. The correlation matrix (r value) was calculated for response variables and significant correlations are reported at the 5% level.

Plate 3.1. Vegetable farm used in soil sampling survey, Dorrigo, New South Wales (Ferrosol)

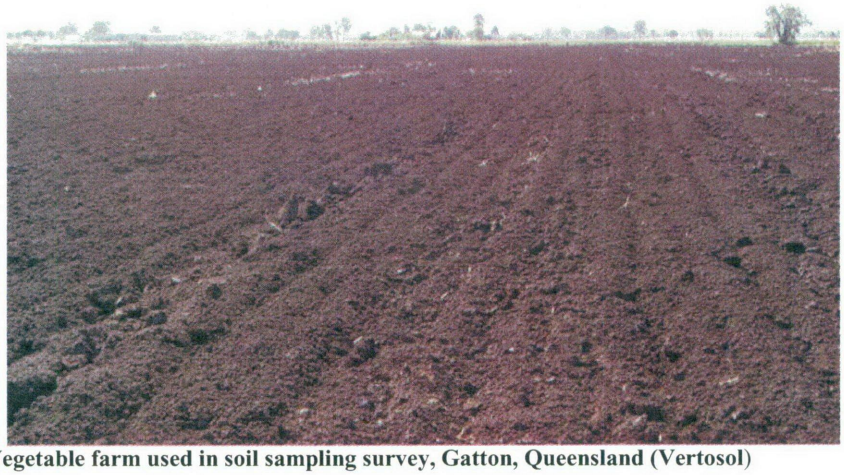

Plate 3.2. Vegetable farm used in soil sampling survey, Gatton, Queensland (Vertosol)

Plate 3.3. Vegetable farm used in soil sampling survey, Stanthorpe, Queensland (Tenosol)

3.3 Results

3.3.1 Management variables

All the conventional farms were supplied with chemical fertilisers in the previous 2-4 years irrespective of location. As expected soil texture did not vary significantly between the farms in each location. The number of organic and convetional farms at Gatton, Stanthorpe and Dorrigo were 5 and 3, 4 and 4 and 6 and 2 respectively (Table 3.1). All farms received compost and/or bulky manures such as poultry manure imported from other farms, except three conventional farms each at Gatton, Stanthorpe and Dorrigo and one organic farm at Stanthorpe (Table 3.1). More than three-quarters of the organic farms received green manures produced on farm, irrespective of the locations.

3.3.2 Soil pH and EC

The average EC of the soils was similar across locations and farming systems with most soils in the average range of 0.13 to 0.30 dS m^{-1} (Table 3.3 and 3.4), indicating there were no potential limitations due to salinity (Hazelton and Murphy 2007), There was a difference in pH across locations (Table 3.3 and 3.4) with pH ranging from 7.7 to 8.7 at Gatton, 5.5 to 7.6 at Stanthorpe and 5.3 to 7.3 at Dorrigo. However soil pH did not differ between farming systems. Farms receiving bulky manure and/or compost had higher pH values (Figure 3.2) with those not receiving such inputs.

3.3.3 Microbial biomass and total carbon

Microbial biomass carbon (MBC) and total C differed across locations (Table 3.3 and 3.4), while total C also differed across farming systems ($P < 0.05$). Organic farms at Dorrigo had higher total C compared to conventional farms (Table 3.4). Total C ranged from 1.6 to 2.3% with a mean of 1.7% in Gatton, 0.84 - 4.5% with a mean of 2% in Stanthorpe and 1.9 - 8.9% with mean of 4.5% for conventional farms and 6.4% for organic farms at Dorrigo. Microbial biomass carbon was highly correlated with total C ($r = 0.61$), total N ($r = 0.66$), ammonium-N ($r = 0.47$) and nitrate-N ($r = 0.53$) irrespective of farming system and location.

Table 3.3. Level of significance for farming system (FS), location (Loc), green manures (GM), bulky manures and compost (CTBM)

Characteristics	FS	Loc	FS*Loc	GM	Loc	GM*Loc	CTBM	Loc	CTBM*Loc
pH	NS	***	NS	NS	***	NS	*	***	NS
EC[1]	NS	NS	NS	NS	NS	NS	NS	NS	NS
Microbial biomass	NS	***	NS	NS	***	NS	NS	***	NS
Carbon	*	***	NS	NS	***	NS	NS	***	NS
Nitrogen	NS	***	NS	NS	***	NS	NS	***	NS
C:N ratio	NS	NS	NS	NS	NS	NS	NS	NS	NS
Resin P	NS	*	NS	NS	*	NS	NS	**	NS
KCl-40 Sulfur	NS	NS	NS	NS	NS	NS	NS	NS	NS
Ex. Al	NS	**	NS	NS	**	NS	NS	**	NS
Ex. Ca	***	***	**	NS	***	NS	***	***	*
Ex. Mg	***	***	NS	***	***	NS	***	***	NS
Ex. K	NS	**	NS	NS	***	**	NS	***	***
Ex. Na	*	***	NS	*	***	NS	**	***	NS
Ex. Mn	NS	*	NS	NS	*	**	NS	*	NS
Nitrate	NS	NS	NS	NS	NS	NS	NS	NS	NS
Ammonium	NS	***	NS	NS	***	NS	NS	***	NS
ECEC[2]	***	***	**	NS	***	NS	***	***	*
ESP[3]	NS	***	NS	NS	**	NS	NS	**	NS

*: significant at 5% level, **: significant at 1% level, ***: significant at 0.1% level, NS: non significant, [1]EC-Electrical conductivity, [2]ECEC: Effective cation exchange capacity, [3]ESP: Exchangeable sodium percentage

Table 3.4. Soil characteristics of organic and conventional vegetable farms in eastern Australia.

	Farming system		SE	Farming system		SE
	Conventional	Organic		Conventional	Organic	
Location	Soil pH			Electrical conductivity (dS m^{-1})		
Dorrigo	6.3	5.8	**0.18**	0.30	0.19	**0.05**
Gatton	8.2	8.1	**0.08**	0.14	0.19	**0.02**
Stanthorpe	6.5	6.9	**0.21**	0.15	0.13	**0.02**
SE	**0.26**	**0.23**		**0.04**	**0.02**	
	Microbial biomass carbon[1]			Total C (%)		
Dorrigo	47	48	**4.6**	4.5	6.4	**0.48**
Gatton	28	23	**2.8**	1.7	1.7	**0.11**
Stanthorpe	19	30	**2.8**	2.0	2.0	**0.30**
SE	**3.9**	**3.4**		**0.36**	**0.52**	
	Total N (%)			C:N ratio		
Dorrigo	0.50	0.62	**0.04**	9.1	10.5	**0.44**
Gatton	0.17	0.18	**0.01**	10.2	10.0	**0.23**
Stanthorpe	0.24	0.21	**0.04**	8.9	10.0	**0.55**
SE	**0.04**	**0.05**		**0.30**	**0.34**	
	Nitrate-N (mg kg^{-1})			Ammonium-N (mg kg^{-1})		
Dorrigo	37	64	**12**	16	10	**2.6**
Gatton	33	24	**5**	3	5	**1.5**
Stanthorpe	38	21	**7**	5	3	**1.4**
SE	**6**	**7**		**2.2**	**1.3**	
	Resin P (mg kg^{-1})			KCl-40 sulfur (mg kg^{-1})		
Dorrigo	44	64	**15**	23	18	**2.9**
Gatton	134	107	**9**	8	10	**1.3**
Stanthorpe	96	99	**20**	34	23	**9.2**
SE	**15**	**12**		**6.8**	**3.5**	

[1]mg microbial Carbon/100g DM soil

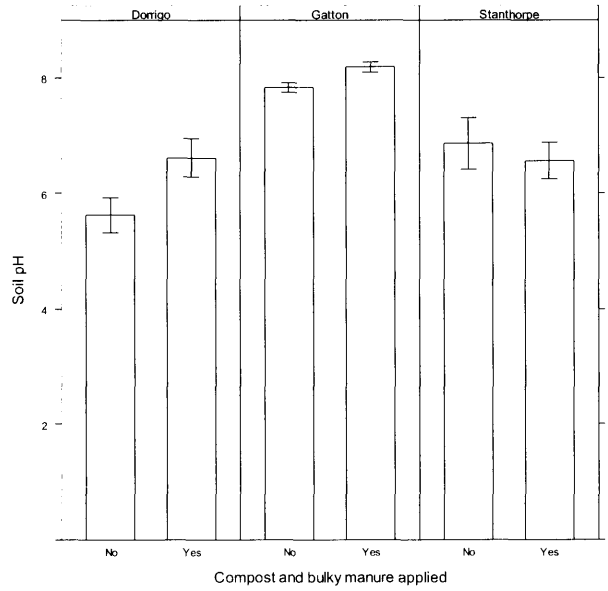

Figure 3.2 Soil pH (1:5 water) of vegetable farms using bulky manures and composts at three locations

3.3.4 Total N, C:N ratio, ammonium-N, nitrate-N

Total N and ammonium-N differed across locations ($P < 0.001$). C: N ratio and nitrate-N were similar across farming system and locations (Table 3.3). Total N ranged from 0.1 to 0.2% at Gatton, 0.1 to 0.4% at Stanthorpe and 0.4 to 0.9% at Dorrigo. Ammonium-N ranged from 2 to 24 mg kg^{-1} at Gatton, 1 to 20 mg kg^{-1} at Stanthorpe and 3 to 34 mg kg^{-1} at Dorrigo, while nitrate-N ranged from 1 to 132 mg kg^{-1} across the three locations. Soil nitrate was high (>30 mg kg^{-1}) in the soils from all conventional farms and the organic farms from Dorrigo. Soil nitrate levels in organic farms at Stanthorpe and Gatton was optimum (8-30 mg kg^{-1}) (Hazelton and Murphy 2007). C: N ratio ranged from 7.1 to 13.5 across three locations. Total N content was moderate (0.15-0.25%) in Gatton and Stanthorpe and very high in Dorrigo (>0.5%). Nitrate-N, ammonium-N, total N were significantly correlated with each other, with the following r values: nitrate-N ~ total N = 0.53, ammonium-N ~ total N = 0.47, nitrate-N ~ ammonium-N = 0.42.

3.3.5 Resin P and KCl-40 sulfur

Resin P differed across locations ($P < 0.001$), but not farming systems (Table 3.3). Analysing the data based on management variables also indicated a significant difference between locations but not between management practices. Resin P ranged from 64 to 173 mg kg^{-1} at Gatton, 13 to 236 mg kg^{-1} at Stanthorpe, 1 to 132 mg kg^{-1} at Dorrigo. In 2005, soil available P was analysed using the methods of Olsen *et al.* (1954) and Colwell (1963). The Colwell P results (Appendix 5, (Nachimuthu et al. 2007)) revealed that soil P levels were medium to high (average Colwell P >100 mg kg^{-1}) for vegetable production at all locations, irrespective of farming system (Peverill et al. 1999). The three methods were well correlated among each other with r values of 0.84, 0.91 and 0.92 for Colwell ~ Resin, Colwell ~ Olsen and Olsen ~ Resin respectively. KCl-40 sulfur was similar across farming system and locations with means ranging from 8 to 34 mg kg^{-1} (Table 3.4).

3.3.6 Exchangeable cations

All the exchangeable cations, ECEC and ESP varied between locations ($P < 0.05$). Exchangeable Ca, Mg, Na and ECEC varied between farming systems ($P < 0.05$) and between compost and manure applications (Table 3.3 and Figure 3.3). There was a significant interaction between FS and locations for exchangeable Ca and ECEC ($P < 0.01$). The mean exchangeable Ca and Mg (Table 3.5) were moderate to very high and exchangeable K was moderate to high across farming system and locations. Exchangeable Na content was very low to low at all locations (Hazelton and Murphy 2007). Exchangeable Al% of ECEC was less than 5% at all sampling units and were within the desired range for vegetable growth (Abbott 1989). Analysing the data using management variables showed that there was also a significant interaction between green manure use and farm locations for K and Mn ($P < 0.01$), and between farm locations and compost and manure applications for K, Ca and ECEC. Green manure-applied farms differed in exchangeable Na and Mg (Table 3.3) from those not supplied with green manure.

Table 3.5: Exchangeable cations of organic and conventional vegetable farms in eastern Australia

	Farming system		SE	Farming system		SE
	Conventional	Organic		Conventional	Organic	
Location	Al (cmol(+) kg^{-1})			Ca (cmol(+) kg^{-1})		
Dorrigo	0.10	0.18	**0.06**	7	24	**4.7**
Gatton	0.00	0.00	**0.00**	29	32	**2.2**
Stanthorpe	0.03	0.02	**0.02**	6	7	**0.6**
SE	**0.02**	**0.03**		**3.0**	**3.1**	
	Mg (cmol(+) kg^{-1}))			K (cmol(+) kg^{-1})		
Dorrigo	2	4	**0.46**	1.0	1.3	**0.23**
Gatton	22	23	**0.67**	1.0	0.8	**0.05**
Stanthorpe	1	1	**0.16**	0.5	0.4	**0.07**
SE	**2.4**	**2.2**		**0.14**	**0.11**	
	Mn (cmol(+) kg^{-1})			Na (cmol(+) kg^{-1})		
Dorrigo	0.02	0.04	**0.01**	0.08	0.13	**0.02**
Gatton	0.00	0.00	**0.00**	1.23	1.81	**0.18**
Stanthorpe	0.02	0.04	**0.01**	0.17	0.06	**0.04**
SE	**0.01**	**0.01**		**0.14**	**0.21**	
	ECEC (cmol(+) kg^{-1})			ESP (%)		
Dorrigo	10	30	**5.05**	0.9	0.6	**0.14**
Gatton	53	58	**2.60**	2.4	3.3	**0.40**
Stanthorpe	8	9	**0.73**	2.00	0.8	**0.44**
SE	**5.5**	**5.0**		**0.36**	**0.47**	

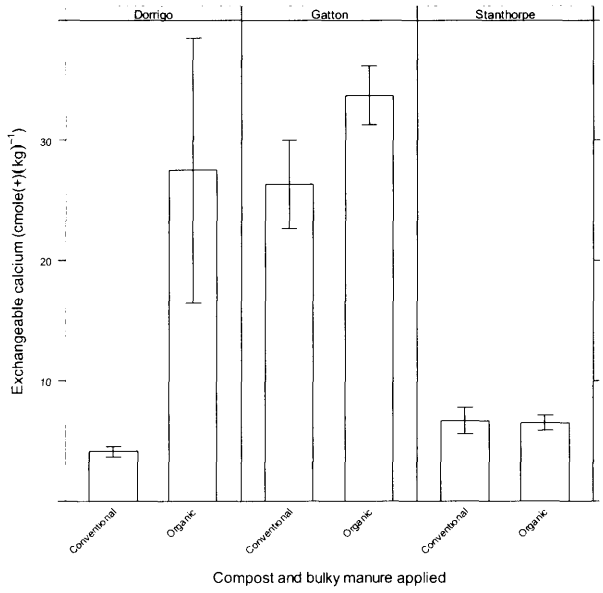

Figure 3.3. Exchangeable calcium of vegetable farms receiving bulky manures and composts at three locations

3.4 Discussion

3.4.1 Nutrient status and management history of vegetable farms

The similar soil nutrient status (pH, EC, total N, Resin P, KCl-40 sulfur, nitrate-N and ammonium-N) and MBC between the organic and conventional farms in three locations with contrasting soil types and climatic conditions indicated that organic vegetable farmers were not at a nutritional disadvantage compared to their conventional counterparts in the present study. The findings of this study challenges the generalisation that organic farming systems rely on nutrient reserves accumulated during prior conventional management as reported in arable mixed farms with lower input levels in UK (Gosling and Shepherd 2005). Classifying the farms based on the type of inputs received indicated that many conventional growers included substantial inputs of organic fertilisers (Table 3.1), so that management differences between organic and conventional systems were less distinct than might be expected, a finding earlier observed in Europe (van Diepeningen et al. 2006). For example, more than 50% of conventional farmers used imported bulky manures or compost in their farm management in all three locations and 50% of the conventional farmers used on farm produced green manures in two locations. While on farm green manures support closed nutrient cycling, imported manures and compost may lead to accumulation of nutrients in vegetable farms and may be of environmental concern (Cornish and Stewart 2002; Wells et al. 2002).

3.4.2 Soil pH

While some reports have indicated higher soil pH in organic farms compared to conventional farms (Lotter 2003), this research did not find any differences in soil pH between farming systems, although, there was a difference in soil pH between farms that used compost/manure and farms not using those inputs, irrespective of farming system at Dorrigo and Gatton (Figure 3.2). The increase in soil pH was likely to be due to use of manures and compost that contained substantial amount of calcareous material (Eghball 1999).

3.4.3 Soil microbial biomass

Organic farmers are particularly concerned about soil biological health as they recognise the central role of soil biota and microbes in promoting soil chemical (Tu et al. 2006) and physical fertility (Lundquist et al. 1999). The chemical fertility of soil indicated by total C, total N, nitrate-N and ammonium-N, was highly correlated (Section 3.3.3) with microbial biomass. The lack of difference in microbial biomass between farming systems might be attributed to the length of time the farms have been under current management (Monokrousos et al. 2006; Zaller and Kopke 2004) together with nutrient content and quality of organic matter residues added (Marinari et al. 2006; Shepherd et al. 2002; Stockdale et al. 2002) as well as its quantity (Watson et al. 2002a). Inorganic fertilisers appear to have a limited direct effect on soil microbes, but little work has been done on this immediate effect of these chemicals in Australia (Bünemann et al. 2006b). However they may indirectly facilitate the role of soil microbes in the longer term by increasing overall system productivity and its nutrient status, crop residue return and soil organic matter levels. Manures and organic amendments have additional benefits in that they provide direct sources of C for microbes, maintaining soil health and biological activity by providing an energy source, along with nutrients (Bünemann et al. 2006b).

3.4.4 C:N ratio and Total C

The average C:N ratio across three locations ranged from 9 to 10.5 indicating that all soil types had a suitable C:N ratio for crop growth (Hazelton and Murphy 2007). Most of the soils were sampled after the harvest of the crop or at least four months after the application of manures, composts or green manures. This is sufficient time for the decomposition of organic inputs and to lower the C: N ratio to the desired level of <12:1.

The organic farms at Dorrigo had higher total C than conventional farms which might be due to differences in the quality (Marinari et al. 2006; Shepherd et al. 2002; Stockdale et al. 2002) and quantity (Watson et al. 2002a) of organic matter incorporated into each farm due to

application of different inputs such as green manures, bulky manures and composts. This is in consistent with the findings of Wells *et al.* (2000).

3.4.5 Soil nitrogen

This study indicated that soil N fertility (total N, nitrate-N, and ammonium-N) did not vary between farming systems (Table 3.3 and 3.4), but total N and ammonium-N differed across locations. There was a good correlation between MBC and N fertility of soil which may reflect activity of nitrifying bacteria or an increase in the general microbial population as N fertility increased. Legume rotations may also increased the soil N (Beckwith et al. 1998).

3.4.6 Soil extractable P

The resin P levels were similar for conventional and organic farms at all locations and there was a good correlation between the three extraction methods (Olsen, Colwell and resin). The similar extractable P levels between organic and conventional farms measured by the three extraction methods in 2005 indicated that soil extractable P pools in organically managed farms were equivalent to those in conventionally managed farms with medium to high P levels in most farms (Peverill et al. 1999). Manure and compost applications to meet the N requirement of the vegetables may lead to P accumulation in the surveyed farms as the P to N ratio of the inputs were usually higher than the ratio required by the crop (Eghball 2002). This suggests that all farms (organic and conventional) were relatively high input systems with potential for adverse environmental effects, such as nutrient runoff (Wells et al. 2002; Zhang and MacKenzie 1997b) especially during high rainfall season as the topography of the farms at Stanthorpe were inclined enough to enhance such phenomenon. van Diepeningen *et al.* (2006) identified similar levels of P in organic and conventional farms in the Netherlands. Positive P balances have also been observed in different organic farming systems including vegetables in New South Wales, Australia (yellow earth - Luvic Ferrasol) (Wells et al. 2000) and cereal legume crop rotations (sandy clay loam) in Italy (Marinari et al. 2006).

Organic vegetable farms reported in this study have been under current management practices from 5-18, 7-14 and 5-10 years at Gatton, Stanthorpe and Dorrigo respectively. These timeframes would be sufficient to demonstrate a decrease in soil nutrient status if organic farming systems mined the nutrient reserves built up during past conventional management (Burkitt et al. 2007; Gosling and Shepherd 2005; Penfold et al. 1995). This indicates that the high soil fertility of organic farms was due to the relatively high quantity of inputs used and was not related to a lack of time to bring about detectable change in soil nutrient levels after conversion to organic management. The low correlation of resin P and years under organic management (r = 0.4) also confirms that soil extractable P is not in a systemic decline. Positive nutrients budgets over several years of organic horticultural production have been reported (Watson et al. 2002b). The results of our study confirm the work of Wells *et al.* (2000) on organic vegetable production in Australia and generalises those findings to a range of contrasting soil types and climate conditions. These results provide evidence from several agro-ecological zones that organic vegetable farming methods are not necessarily nutrient limited and that soil fertility is largely similar to conventional farms in the same region. These findings suggests that generalisations about organic farming being sustainable (Kirchmann and Ryan 2004; Trewavas 2004) based on changes in nutrient status, are unwarranted unless the fertiliser regime is specified The results also demonstrate that the soil P status of organic vegetable farms is quite different from broad-acre farms, probably because vegetable production systems are intensive, high input enterprises featuring the application of large amount of organic amendments such as green manure, manures and composts. Vegetable farms in Australia are reported to accumulate excess nutrients compared to other farms due to excessive cultivation and high rates of fertiliser and manure applications (Chan et al. 2007).

3.4.7 Exchangeable cations

Differences in exchangeable Ca, Mg and Na and ECEC between farming systems and compost and manure applications (Section 3.6) and the interaction between farming system and location for exchangeable Ca and ECEC could be due to the difference in both type and amount of clay content and amount of organic matter present in soil (Stockdale et al. 2002). High exchangeable Ca (Figure 3.3), Mg and Na (Table 3.3) with manure and compost-applied farms may be related to elevated levels of cations in manures such as poultry manure or compost added to the soil (Cooperband et al. 2002). The lack of difference in exchangeable K between farming systems suggests both the farming systems received similar amount of K inputs (Stockdale et al. 2002). The decrease in exchangeable K over the years irrespective of farming system reported in mixed cropping systems (Andrist-Rangel et al. 2007) is contrasted in this study and suggests that vegetable farms are high inputs systems in eastern Australia.

The results confirm that organic vegetable farms are not necessarily nutritionally disadvantaged compared with adjacent conventional farms. While most of the measured chemical parameters were above the required levels for crop growth (Hazelton and Murphy 2007) irrespective of farming system, options such as using either lower quantity of inputs or inputs with low nutrient contents can be used in vegetable production to better match the timing of crop growth.

3.5 Conclusion

Vegetable production systems in eastern Australia are generally high input systems. There was substantial overlap in farm management inputs between farming systems at all locations, with conventional farms routinely using organic inputs such as green manures, manures and composts. Consequently, soil microbial and chemical properties such as soil pH, EC, Total N, Resin P, KCl-40 sulfur, nitrate-N and ammonium-N were similar between farming systems and the results did not support the hypothesis that organic vegetable farms are lower in soil fertility than nearby conventional farms. Soil microbial and chemical properties were strongly influenced by crop management practices and soil type, rather than

the farming system as a whole. The application of manures and compost produced a liming effect and increased exchangeable Ca irrespective of farming system at Gatton and Dorrigo. Soil extractable P pools were medium to high (average Colwell P >100 mg kg^{-1}) across farm types due to the application of large quantities of manure and compost to meet the N requirement of vegetables. To be a sustainable system, crop management in vegetable production in eastern Australia (whether organic or conventional) should consider the baseline soil nutrient status to avoid over fertilisation and off-farm environmental effects.

The organic systems described in this chapter have history of high inputs. However, given that Australian soils are inherently low in soil P, changes in nutrient status during conversion to organic vegetable production on these soils would be uncertain and would depend, to a large extent, on management strategies employed and the type of organic fertilisers used. One such strategy yet to be explored in organic vegetable production in Australia is genotype x environment interaction (section 2.4). As phosphorus nutrition is the focus of this thesis, a minor investigation on impact of P source on locally grown corn cultivars was investigated in next chapter (chapter 4).

Statement of Originality:

All the work contained within this paper is the original research of the PhD candidate, Gunasekhar Nachimuthu.

Candidate:

Principal Supervisor:

Statement of Contribution by Others:

This paper has been prepared by the PhD candidate, Gunasekhar Nachimuthu. All coauthors are either PhD supervisors or provided technical assistance and have only contributed to this paper to the extent that would normally be expected of such roles. All coauthors have given their consent for having their contributions to this paper included in the thesis and accept the student's contribution as indicated in the Statement of Originality.

Candidate:

Principal Supervisor:

CHAPTER FOUR

Short term phosphorus fertiliser source utilisation by sweet corn (*Zea mays* L.) under organic production

Manuscript to be submitted to

Agronomy Journal

Short term phosphorus fertiliser source utilisation by sweet corn (*Zea mays* L.) cultivars

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Abstract

Organic agriculture is gaining momentum due to increased producer and consumer interest in clean and green agriculture. Appropriate phosphorus (P) nutrition is critical to successful organic agricultural production. A glasshouse experiment was conducted to evaluate the case for selecting corn (*Zea mays* L.) genotypes for specific adaptation to low P soil conditions and organic-approved P fertilisers Four traditional corn cultivars (Balinese, Golden Bantam, Hawaiian and Jolly Roger) and one conventional cultivar (Hybrid 424) were fertilised with either no P, (control), or P from rock phosphate (RP), poultry manure (PM) or single superphosphate (SP). Shoot and root dry matter and nutrient uptake were recorded at harvest. Soil solution phosphate, nitrate and ammonium concentration were recorded weekly over 28 days. Phosphorus use efficiencies of applied SP were six to eight times and one to two times higher than when the P source was RP and PM respectively. Organic cultivar responses relative to the Hybrid were similar in P acquisition under deficient and sufficient P levels. While this experiment did not support the hypothesis that organic cultivars were adapted for better P acquisition under low P status further research should focus on exploring the root morphology and exudates of cultivars e.g. Golden Bantam for high translocation efficiency to verify if it contains any useful traits for P acquisition under low P and organic farming conditions. The marginal differences between the cultivars compared with more pronounced differences between the sources suggest exploring an alternate phosphorus source in organic production.

Key words: Phosphorus, Organic production, Sweet corn.

4.1 Introduction

Growing producer and consumer interest in food, agriculture and the environment is leading to organic agriculture gaining momentum. While the high input vegetable production systems indicated high extractable P levels (chapter 5), changes in nutrient status during conversion to organic vegetable production on low P content soils would be uncertain and would depend, to a large extent, on management strategies employed and the type of organic fertilisers used. One such strategy investigated in this thesis is exploring genotype x environmental interaction, with P source as environmental factor.

The lack of detailed knowledge about corn (*Zea mays* L.) cultivar performance under organic farming conditions limits the development of specific varieties which perform better under organic conditions and constrains the choice of cultivars and quality of seed available to producers (Lammerts van Bueren et al. 2003). Organic standards require that crops be grown from organic seed or plant propagation materials, but, if suitable organic material is not available in sufficient quality and quantity, a derogation rule allows the use of non-organic, but not genetically modified material (Zeijden 2002). Currently there is a lack of readily available, high quality organic-specific cultivars globally, and for varieties suited to Australian conditions. It cannot be assumed that cultivars developed for one management regime will be reasonably well suited to other management regimes, and further investigation is needed before adoption (Cooper et al. 2001).

Several reports from Australia and other countries, highlighted the important limiting role of P in a range of organic farming systems (Penfold 2000). Organic production prohibits the use of synthetic and readily soluble P fertilisers, and so relatively insoluble rock phosphate (RP) is often used as a source of P in Australian broad-acre organic farming (Evans et al. 2006). Rock phosphate is less commonly used in vegetable growing, where manures, compost and blood and bone can be affordable alternative sources of P (McCoy and Parlevliet 2001). Earlier research on poultry manure versus green leaf manure (*Senna sp.*) in acidic soil revealed that poultry manure was more efficient than other amendments, producing higher

maize yields which were associated with increases in pH, Ca, Mg and P, and a decrease in Al (The et al. 2006).

Regardless of the farming system, the efficient use of P fertilisers is hindered by fixation and immobilisation in the soil (Ayaga et al. 2006). The overall efficiency of plant use of applied P fertiliser (P uptake/unit of fertiliser) has been estimated as <10% (Baligar et al. 2001), and methods are needed for improving P availability (Richardson 2001). Where availability is low, it has been shown that plants can acquire P by changing root physiology and morphology (Schachtman et al. 1998) for example by increasing root biomass and lateral root length (Liu et al. 2004). Increasing roots biomass is achieved by increased root hair proliferation, root hair elongation, proteoid root formation and increased association with mycorrhiza fungi (Raghothama 1999). Acquisition of P under deficit conditions were also achieved by modifying the rhizosphere and excreting organic acids (Hinsinger 2001; Marschner et al. 2007). High P use efficiency can involve adaptive traits that allow plants to produce more dry matter for each unit of applied P, and to take up P at lower concentrations in the soil solution and from less available sources (Marschner 2002).

Varieties that are P efficient need to be identified. In third world tropical and subtropical countries, where field corn (*Z. mays* L.) is typical a staple, the issue of P efficiency is even more significant, particularly from traditional and less soluble fertiliser sources such as organic manures and rock phosphates. Variation in adaptive traits for P uptake within corn cultivars under different management conditions has been reported (Ciarelli et al. 1998; Liu et al. 2004; Machado and Furlani 2004; Nielsen and Barber 1978; Ramirez and Lizaso 2006). Nielsen and Barber (1978) observed differences between corn cultivars in P uptake and emphasised the importance of root parameters in acquiring P, especially from low solubility P sources such as rock phosphate (RP). The most favourable characteristics for P uptake and P use efficiency of parental genotypes were observed in the derived hybrids suggesting that P-efficiency characters are heritable and under genetic control (Ciarelli et al.

1998). Machado and Furlani (2004) identified genotypes adapted to low P status conditions. After studying the cultivar differences in corn for RP application, Ramirez and Lizaso (2006) concluded that cultivars that combine high uptake efficiency (P uptake/root length) and high conversion efficiency (biomass production/P uptake) would improve RP utilisation by corn.

This study was aimed at investigating the genotype x environment interaction, where P source is the environmental variable. It focussed on the possibility that traditional organic sweet corn varieties might perform comparatively better in low P status soils (such as those fertilised with RP where available P is relatively low) relative to modern hybrid corn. This investigation examined the short term (upto 28 days after sowing) differences in plant growth and P uptake of traditional sweet corn cultivars in comparison with modern hybrid corn in a P deficient soil fertilised by RP (low P availability, organically approved), poultry manure (moderate P availability, organically approved), and single superphosphate (high P availability, not organically approved).

4.2 Materials and methods

4.2.1 Experimental design and setup

A glasshouse experiment was conducted consisting of a two way factorial design comprising five corn cultivars (four traditional sweet corn varieties and one hybrid) and four P fertiliser treatments. The sweet corn varieties were Balinese, Golden Bantam, Hawaiian, Jolly Roger and a Hybrid corn (high yielding and widely cultivated cultivar) in Eastern Australia (NSW Department of Primary Industries 2006; Pacific Seeds 2005) was used a control. All the traditional varieties are certified organic and commercially available for cultivation in New South Wales and Queensland (Greenpatch Organic Seeds 2003). The P treatments included single superphosphate (SP), organically approved poultry manure (PM) and rock phosphate (RP) and a zero P (control) treatment. All treatments were replicated four times and pots were randomly arranged on benches in the glasshouse. The temperature of the glasshouse was maintained between 16 °C and 33 °C.

The glasshouse experiment was conducted using a P responsive sandy loam soil, Grey Chromosol – (Isbell 1996) derived from granitic parent material collected on a University owned farm 10 km north west of Armidale, NSW. The surface 10 cm was collected, air dried and passed through 5 mm sieve before the experiment. Selected characteristics of the soil used in this study were measured (Table 4.1) using standard laboratory methods (Lisle et al. 2006).

Each treatment except the control received 50 kg P/ha (80 mg Total P/pot); chosen part way up a previously determined P response curve using corn as test crop, which is linear up to 50 kg P/ha (Appendix 1). Rock phosphate (Bourcrag, 15.9 % total P) and SP (8 % total P) were ground to fine powder of less than 106 μm and PM (3.8 % N, 2.0 % P and 1.6 % K) was applied in the form of pellets (< 2 mm).

Phosphorus sources were thoroughly mixed through 1.75 kg of soil and soil solution samplers (Menzies and Guppy 2000) were placed vertically in the middle of each pot. Soil was brought to field capacity and incubated for one week in a glasshouse before sowing. Basal N (200 kg/ha) as NH_4NO_3, S (50 kg/ha) as Na_2SO_4 and K (50 kg/ha) as KCl were applied to all pots except those receiving PM, where the balance of nitrogen and potassium nutrients not supplied in the PM was added. Seeds of each variety were germinated in moist sand in separate trays and four seedlings per pot were planted and thinned to one plant per pot after emergence. Soil moisture was adjusted to field capacity (17%) daily by weight.

Table 4.1. Selected physical and chemical properties of the test soil, a Grey Chromosol from Armidale NSW

Characteristic	Value	Unit
Texture	sandy loam	
Field capacity	0.17	kg/kg
pH (1:5 water)	5.40	
Cation exchange capacity	3.42	$cmol_c$ (+)/kg
Total N	0.15	%
Total C	1.30	%
Nitrate-N	7.30	mg/kg
Ammonium-N	8.50	mg/kg
Colwell P	13.0	mg/kg
KCl 40-Sulfur	3.40	mg/kg

4.2.2 Solution sampling, biometric measurements and plant analysis

Soil moisture was adjusted to 100 mL more than field capacity the night before the solution sampling. This was done taking into consideration of approximate fresh weight of plants at each sampling date. Soil solution samples were collected on 0, 7, 14, 21 and 28 days after sowing of corn as described by Menzies and Guppy (2000). Soil solution phosphorus was estimated using the methods of Motomizu *et al.* (1984) and soil solution ammonium and nitrate were estimated with the methods of Adamsen *et al.* (1985). Plants were harvested 29 days after sowing. The roots were washed gently and root volume was measured using the displacement technique with a beaker and measuring jar. The fine roots, coarse roots and shoots were separated and oven dried at 70 °C for 48 hours to determine the dry biomass.

After estimating dry biomass, shoots were ground to fine powder of <0.5mm size using a mechanical grinder. Fine and coarse roots were pooled before grinding (to get enough material to pass through the grinder) and ground to <0.5mm size. Shoot N and root N concentration were estimated using a Carlo Erba NA1500 solid sample analyser. Shoot P and K concentration were estimated using the sealed chamber acid digestion method described by Anderson and Henderson (1986) and analysed using an Inductively Coupled Plasma – Optical Emission Spectrometer (Varian Vista Radial MPX).

4.2.3 Statistical Analysis

Results were analysed using analysis of variance using the R statistical package (R Development Core Team 2006) and P values <0.05 were considered significant results. Diagnostic plots were used to check the normality of the data and homogeneity of variance for each response variable and necessary transformations were made using the `boxcox` procedure. Phosphorus use efficiency (PUE) was calculated by subtracting P uptake in the control from P uptake in the other fertiliser treatments and dividing by the applied P rate (Torres-Dorante et al. 2006). Soil solution ammonium and nitrate concentration were

represented as splines plotted using the `loess` procedure. Soil solution P concentrations were plotted as `xyplot` and were not fitted due to a lack of clear trend (except for SP).

4.3 Results

4.3.1 Shoot dry matter

Hybrid produced the highest dry matter yield regardless of P sources (Table 4.2). The dry matter yield of traditional corn cultivars was lower than Hybrid corn depending on P source (with Hybrid corn producing 36 to 100 % higher drymatter yield than other tradiational cultivars. The traditional cultivar Jolly Roger had the lowest shoot biomass for all P fertiliser treatments except PM whereas Hawaiian was lower in shoot biomass accounting for the significant (P=0.02) interaction between cultivars and P fertilisers (Table 4.2). Among the traditional varieties, Golden Bantam had between 8 and 88% higher yield than the other three varieties in all the treatments supplied with P. However where no P was applied Balinese had 20% higher yield than Golden Bantam. Of the organically approved P fertiliser treatments, the highest yields were obtained by PM irrespective of cultivars, being approximately three times greater than RP.

4.3.2 Root dry matter

Traditional cultivars produced lower root dry matter yield in all treatments than did Hybrid (with Hybrid producing 37-60% higher root drymatter than other cultivars). Of particular interest was the lower root biomass production of Golden Bantam relative to other traditional cultivars, particularly given the generally higher shoot growth (Table 4.2). Among organically approved treatments, PM produced twice the root biomass of RP, and the control treatment yield was the lowest irrespective of cultivars. There was a slight difference in coarse root mass from total and fine roots in which Hybrid had more coarse root than fine roots when treated with SP and PM, unlike other cultivars which had more fine roots than coarse roots resulting in the significant interaction of cultivars and P fertilisers in coarse root production (P<0.001) (Table 4.2). Increasing the percentage of root biomass in the fine fraction by

investing less carbon was a technique used by almost all the varieties under P stressed conditions (control and RP) (Table 4.2). The higher production of fine roots in Balinese (similar levels to the Hybrid) may have increased shoot biomass to the same level as that of Golden Bantam (Table 4.2). Root volume showed similar trends to that of total root biomass and is not reported.

4.3.3 Root:shoot ratio

The root:shoot ratio increased with decreasing availability (or lability) of P (SP<PM<RP<Control). Golden Bantam produced the lowest root:shoot ratio irrespective of P sources (Table 4.2). With SP, Hybrid and Jolly Roger produced a 18 and 27% higher root shoot ratio respectively than Hawaiian. However, where PM was applied, root:shoot ratio did not differ, accounting for the significant interaction between P source and cultivar (Table 4.2).

4.3.4 Phosphorus uptake

Shoot P concentration in corn cultivars ranged from 0.12 to 0.23 %. The low shoot P concentration compared to critical P concentration in corn shoots (Reuter and Robinson 1997) in all treatments was expected because the experiment was conducted below the optimum dose on a P response curve for this soil (Appendix 1).

Table 4.2. Effect of phosphorus fertilisers and cultivars on shoot, root dry matter, nutrient uptake and phosphorus use efficiency of corn (pooled standard errors are presented in bold, with P values from the ANOVA for cultivars, sources and their interaction respectively under each parameter)

Variety	Control	Rock phosphate	Poultry manure	Super phosphate	**Standard error**	Control	Rock phosphate	Poultry manure	Super phosphate	**Standard error**
	Shoot biomass (g/ pot)					Root biomass (g/ pot)				
Hybrid	1.9	3.8	12	15	**1.40**	0.8	1.2	3.2	3.9	**0.35**
Balinese	1.8	3.3	9.0	12	**1.12**	0.7	1.3	2.9	3.0	**0.28**
Golden Bantam	1.5	3.5	10	14	**1.28**	0.3	0.7	1.8	2.1	**0.20**
Hawaiian	1.0	2.8	7.9	13	**1.22**	0.5	1.1	2.4	2.9	**0.27**
Jolly Roger	0.8	2.3	8.8	11	**1.14**	0.4	1.0	2.6	3.1	**0.30**
Standard error	**0.17**	**0.23**	**0.38**	**0.30**		**0.07**	**0.09**	**0.15**	**0.16**	
P value			*<0.001, <0.001, <0.05*					*<0.001, <0.001, NS*		
	Coarse root (g/pot)					Fine root (g/ pot)				
Hybrid	0.33	0.49	1.68	2.12	**0.20**	0.51	0.74	1.57	1.74	**0.15**
Balinese	0.20	0.46	1.22	1.36	**0.15**	0.50	0.84	1.69	1.69	**0.15**
Golden Bantam	0.08	0.20	0.73	0.82	**0.08**	0.24	0.52	1.09	1.26	**0.12**
Hawaiian	0.19	0.39	0.88	1.20	**0.11**	0.32	0.72	1.55	1.71	**0.16**
Jolly Roger	0.20	0.38	1.04	1.24	**0.12**	0.24	0.60	1.55	1.86	**0.18**
Standard error	**0.03**	**0.05**	**0.09**	**0.11**		**0.04**	**0.05**	**0.08**	**0.08**	
P Value			*<0.001, <0.001, <0.001*					*<0.001, <0.001, NS*		
	Root-shoot ratio					Root P (%)				
Hybrid	0.46	0.32	0.28	0.26	**0.329**	0.07	0.08	0.10	0.10	**0.004**
Balinese	0.39	0.41	0.32	0.25	**0.342**	0.12	0.12	0.14	0.15	**0.007**
Golden Bantam	0.20	0.19	0.18	0.15	**0.181**	0.10	0.11	0.13	0.13	**0.006**
Hawaiian	0.55	0.39	0.30	0.22	**0.367**	0.09	0.11	0.12	0.13	**0.005**
Jolly Roger	0.57	0.42	0.29	0.28	**0.391**	0.08	0.10	0.12	0.13	**0.007**
Standard error	**0.035**	**0.014**	**0.021**	**0.012**		**0.005**	**0.005**	**0.005**	**0.005**	
P value			*<0.001, <0.001, <0.001*					*<0.001, <0.001, NS*		
	Shoot P uptake (mg/ pot)					Root P uptake (mg/ pot)				
Hybrid	2.5	5.6	17.4	25.6	**2.4**	0.65	1.04	3.28	3.73	**0.37**
Balinese	2.6	5.0	16.9	24.0	**2.3**	0.84	1.58	3.95	4.62	**0.46**
Golden Bantam	2.2	5.6	16.8	26.3	**2.5**	0.34	0.86	2.36	2.72	**0.29**
Hawaiian	1.3	4.3	12.1	26.8	**2.6**	0.47	1.30	2.85	3.94	**0.37**
Jolly Roger	1.0	3.7	15.1	25.8	**2.6**	0.39	1.02	3.21	4.13	**0.42**
Standard error	**0.25**	**0.33**	**0.52**	**0.58**		**0.08**	**0.13**	**0.19**	**0.23**	
P value			*<0.001, <0.05, <0.05*					*<0.001, <0.001, NS*		
	Total P uptake (mg/ pot)					Phosphorus use efficiency (%)				
Hybrid	3.18	6.67	20.66	29.29	**2.74**		4.03	19.28	29.90	**3.22**
Balinese	3.43	6.62	20.87	28.60	**2.71**		3.17	18.61	27.78	**3.17**
Golden Bantam	2.60	6.52	19.14	28.98	**2.73**		4.42	18.86	31.18	**3.38**
Hawaiian	1.74	5.59	14.93	30.75	**2.92**		3.92	14.03	33.17	**3.71**
Jolly Roger	1.37	4.70	18.34	29.91	**2.98**		3.52	18.40	32.22	**3.59**
Standard error	**0.32**	**0.41**	**0.58**	**0.62**			**0.37**	**0.56**	**0.82**	
P value			*<0.01,<0.001, NS*					*NS, <0.001, <0.05*		

The shoot P uptake followed a similar trend as that of shoot biomass in terms of variation between sources irrespective of cultivars (Table 4.2). Comparing individual source response among cultivars, shoot P uptake by organic cultivars (Golden Bantam, Hawaiian and Jolly Roger) was similar to Hybrid corn with SP application. Though shoot P uptake of Golden Bantam and Hybrid were similar, the processes adopted for P uptake were different,

as indicated by the low root volume of Golden Bantam versus higher root volume of Hybrid corn. Shoot P uptake of Balinese and Golden Bantam were similar to Hybrid when treatment was with PM and RP application. Shoot P uptake of Hawaiian treated with PM and RP was 44% and 30% lower than Hybrid. Shoot P uptake of Balinese and Hybrid were similar for the control. Shoot P uptake of Golden Bantam in the control treatment was similar to that for Hybrid and Balinese. In the control, shoot P uptake of Hawaiian and Jolly Roger were approximately two times lower than other cultivars. The lowest shoot P uptake and total P uptake of Jolly Roger among all cultivars under low P status (control and RP) indicate that this cultivar is not suited to a low P environment. Among traditional cultivars, Hawaiian which had the highest shoot and total P uptakes when treated with SP had the lowest shoot P uptake and total P uptake when treated with PM, indicating that Hawaiian did not respond well to organically approved P fertilisers.

Root P uptake of Golden Bantam was significantly lower than all other varieties irrespective of P sources (Table 4.2). The lower root shoot ratio and higher translocation efficiency of Golden Bantam was reflected in root P uptake as more P is translocated to the shoot compared with Balinese which retained most of the P in root.

4.3.5 Phosphorus use efficiency

Phosphorus use efficiency (PUE) includes plant P derived from both applied fertiliser and soil P made available by better root growth as a result of P application. The organically approved sources, PM and RP, had PUEs one to two times and six to eight times respectively less than SP (Table 2) illustrating the PUE problem faced by organic growers. None of the traditional cultivars had significantly higher PUEs than Hybrid when organically approved P sources were used.

4.3.6 Nitrogen and potassium

Shoot N concentration of the corn cultivars ranged about 2 to 4 % and were higher than the critical concentration (Reuter and Robinson 1997). Shoot K concentration was deficient (Reuter and Robinson 1997) in all cultivars when provided SP (1-1.4%), yield in SP could be limited by K deficiency.

4.3.7 Soil solution P, ammonium and nitrate concentrations

The soil solution P data were presented on a log scale in figure 4.1. Considering the magnitude of variation among the replication data, statistical analyses were not performed. Only the general trends are reported and discussed. The highest soil solution P concentrations were observed initially in the SP treatment, but these decreased markedly in all cultivars due to uptake as the plants grew (Figure 4.1). In the PM treatment, the soil solution P was mainly present in low concentration (<0.01 mg/L) and decreased further over time to be below the detection limit by 28 DAS. Soil solution P concentrations for the RP and control treatments were very low (<0.001 mg/L) at 1 and 7 DAS and below the detection limit in all other days of sampling.

Soil solution ammonium and nitrate data are presented in the original scale. Here again the magnitude of variation among the replication data is significant and thus statistical analyses were not performed. Soil solution ammonium and nitrate concentrations tended to be higher in the RP and control compared with the SP and PM treatments, particularly towards the end of the experiment (Figure 4.2 and 4.3). The concentration of soil solution nitrate and ammonium in the PM treatment tended to be lower than for the SP treatment (Figure 4.2 and 4.3). The maximum concentrations (peak) of ammonium and nitrate in the soil solution varied between fertiliser treatments for each variety. Soil solution ammonium for the SP and PM treatment peaked at 7 DAS and started decreasing irrespective of cultivars, except cultivar Jolly Roger with PM, where the peak was at 14 DAS. Soil solution ammonium in RP and

control were highest on 14 DAS and started declining. Soil solution ammonium ranged ≤200 mg/L (Figure 4.2). Soil solution nitrate concentration of SP and PM followed similar trends as that of ammonium with a peak at 7 DAS. Soil solution nitrate in the control and RP treatments did not show a clear peak. Soil solution nitrate ranged ≤550 mg/L (Figure 4.3).

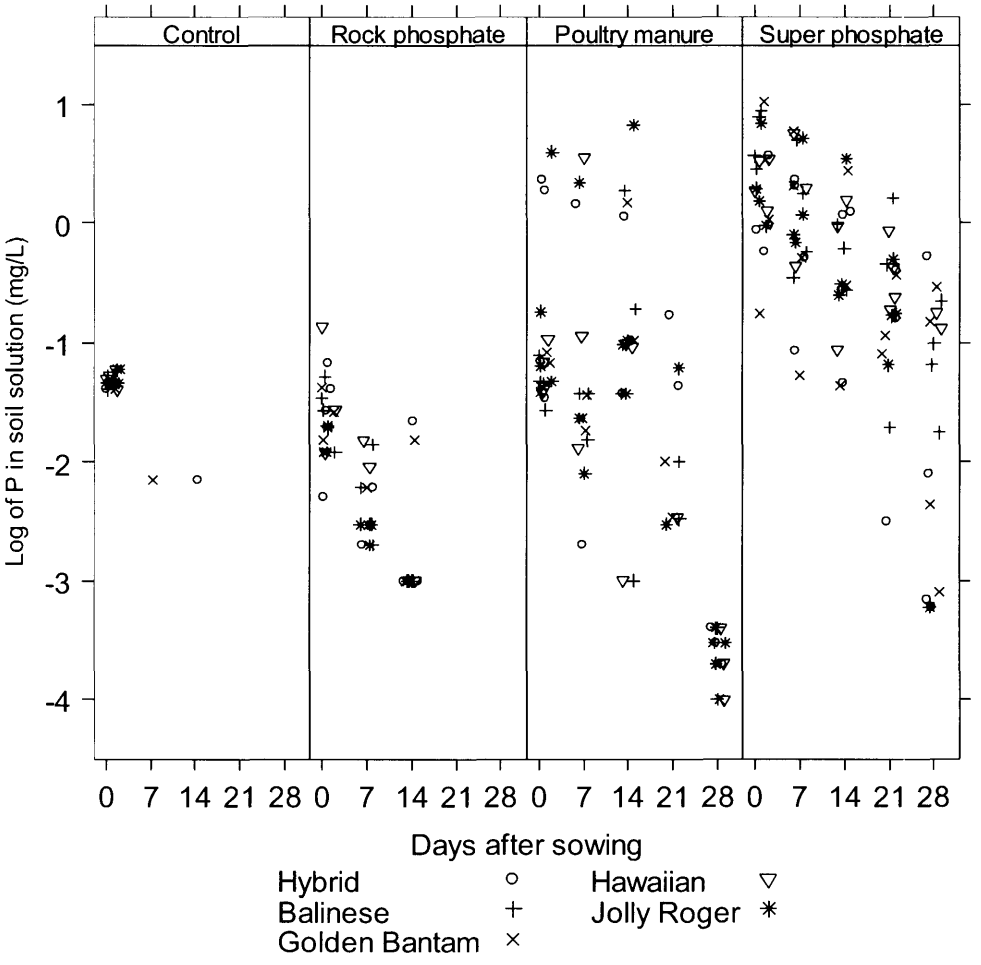

Figure 4.1.Effect of phosphorus fertilisers on soil solution phosphorus concentration (mg/L). Data points (detectable concentrations) are presented on a log scale and are indicated by the symbols in the key above

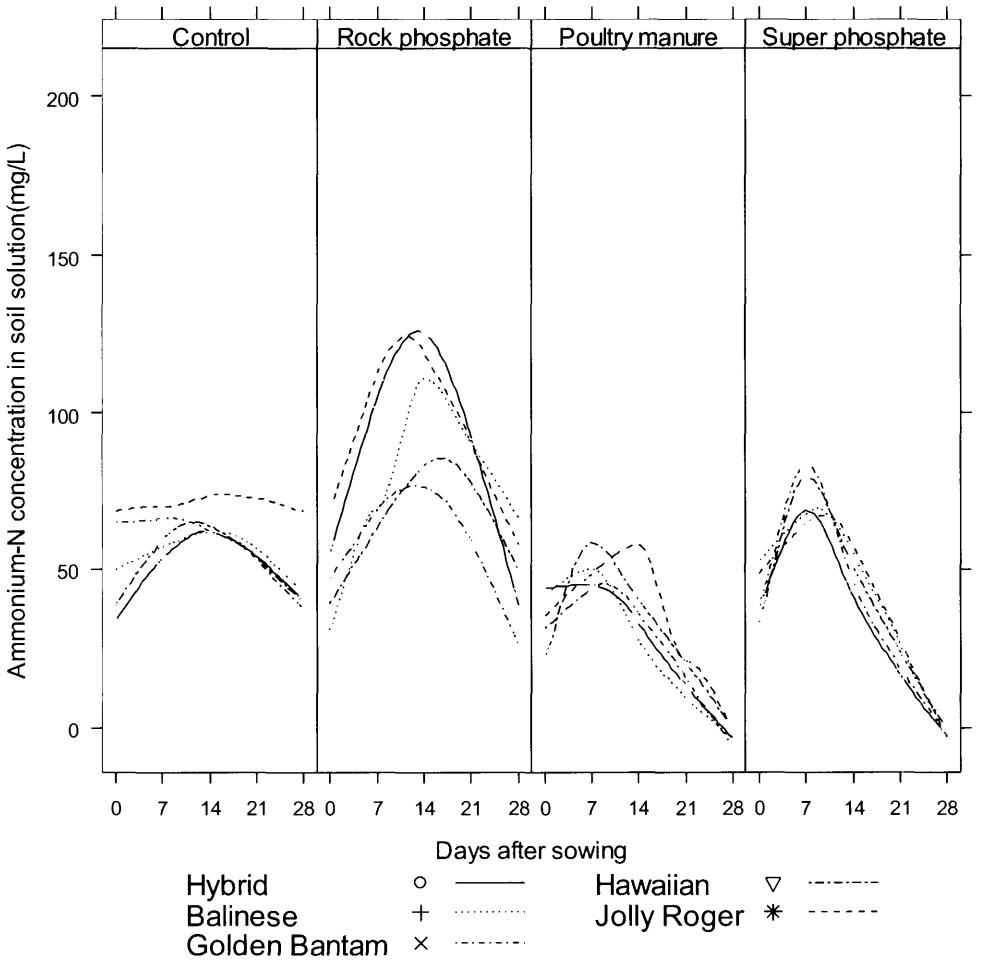

Figure 4.2. Effect of phosphorus fertilisers and cultivar on soil solution Ammonium-N concentration (mg/L). Data points (detectable concentrations) and trend lines (`loess` curves) for cultivar means are indicated in the key above

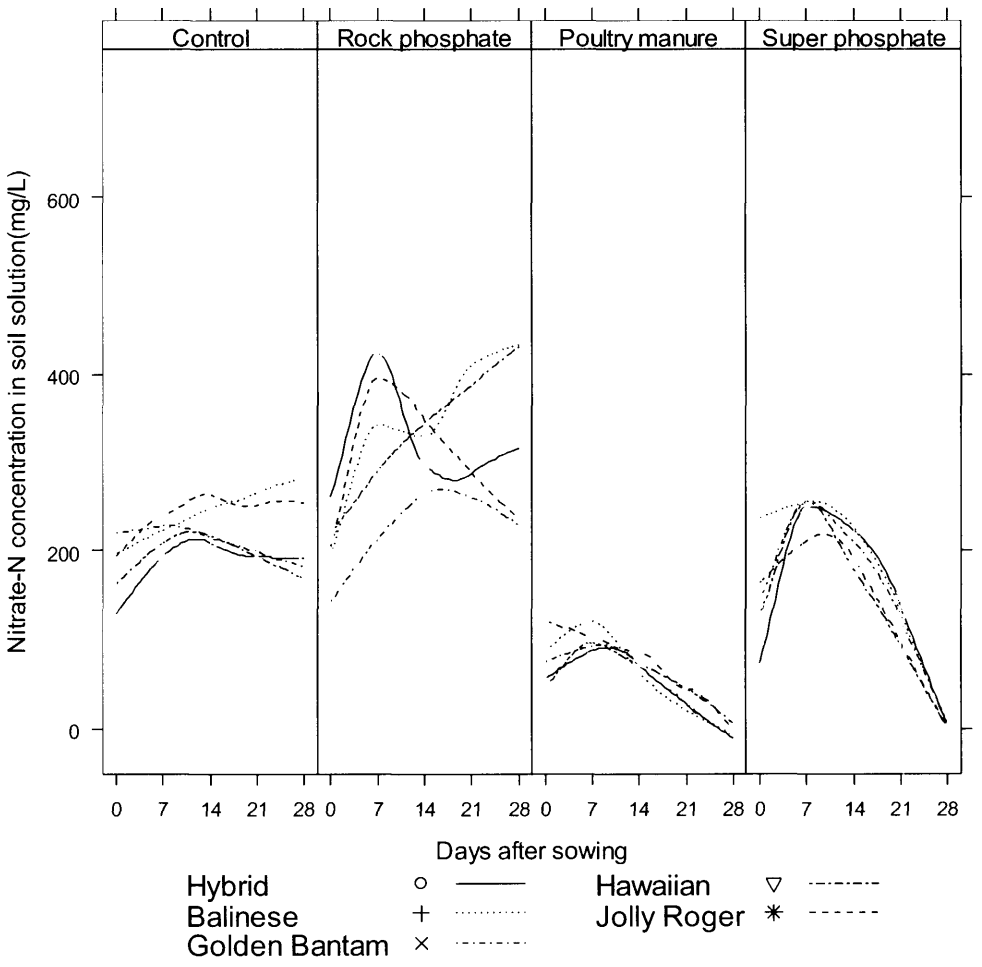

Figure 4.3. Effect of phosphorus fertilisers and cultivar on soil solution Nitrate-N concentration (mg/L). Data points (detectable concentrations) and trend lines (loess curves) for cultivar means are indicated in the key below

4.4 Discussion

As the experiment was focussed on the possibility that traditional organic sweet corn varieties might perform comparatively better in low P status soils (such as those fertilised with RP where available P is relatively low) relative to modern hybrid corn, the results of the experiment indicate that there are marginal differences in relative growth of the corn cultivars grown in soil fertilised with P sources of different solubility (Table 4.2, shoot dry matter data), but there was no difference in phosphorus use efficiency (Table 4.2, PUE data) for the cultivars in the short period, the trial was conducted. The tested cultivars appear to have

achieved similar P acquisition by different methods specific to P acquisition under unique limiting conditions.

The significant interactions found between cultivar and P source for shoot dry matter production, root:shoot ratio, shoot P concentration, uptake and PUE (Table 4.2), are evidence of at least some specificity of cultivar response to different P inputs. Although Duvick and Donald (2005) have suggested that genetic differences and management interact to produce higher P efficiencies, the relative magnitudes of the P responses observed in our pot study suggest that P source is the main contributor to P use efficiency, with cultivar playing only a minor role. It should be noted however that our study examined only five cultivars and the variation between cultivars might be higher were others considered and would also likely be if the plants were grown longer period.

Although there was an interaction between inputs and cultivars for shoot dry matter, none of the traditional cultivars yielded more than Hybrid even in the absence of a P input (control) or with a poorly available P fertiliser (RP) (Table 4.2). The observed interactions are attributed to: (1) the lower shoot dry matter of Jolly Roger under all applied P treatments except PM (where it is 11% higher than Hawaiian); and (2) Golden Bantam had the highest shoot biomass of the four traditional cultivars where P was applied, but not in the absence of P (Table 4.2).

Root:shoot ratios were higher under low P status (control and RP) than under high P status (PM and SP) treatments, as greater root development is necessary to compensate for the low fertility conditions and relatively less above ground biomass is able to be produced per unit soil volume explored when P availability is low (Mollier and Pellerin 1999). Higher root:shoot ratio and lower total root biomass under low P status may reflect an initial enhancement of root growth and decline in shoot growth due to P stress, followed by reduced root growth as carbohydrate production becomes limited (Mollier and Pellerin 1999). Among the cultivars and environmental factor (P source) investigated, the findings do not support the

view that varities should be selected for organic production as the performance of investigated cultivars were similar with soluble and organic permitted sources.

Increased production of fine fraction of roots is a strategy that maximises P acquisition while minimising total root biomass production, and this was observed in all varieties under severe P stress (control or RP). Under moderate stress (SP and PM) fine root fraction of root biomass was up to 50% greater than coarse roots, compared with 200% greater than coarse root fraction where P was low (RP and Control) (Table 4.2). All the cultivars modified root morphology in response to P level, a finding consistent with previous studies (Liu et al. 2004; Mollier and Pellerin 1999). The contrasting root morphology of Hybrid and Golden Bantam provides an example of differences in root morphology being observed, but still resulting in the same shoot P uptake. Golden Bantam increased the uptake and translocation of P per unit of root, while Hybrid had increased the root biomass and volume indicating that cultivars take up similar amounts of P by different mechanisms. Earlier studies have also identified wheat and corn cultivars that have adapted to low P environments by increasing root length density (Egle et al. 1999) and root length when supplied with polyphosphate (Torres-Dorante et al. 2006). Other researchers observed that crops combining high uptake (P uptake / unit root length) and high conversion efficiency (biomass production / unit P uptake) could improve utilisation of RP (Ramirez and Lizaso 2006). Golden Bantam indicated a high translocation efficiency (shoot P uptake/g root) as well when supplied with RP, however this is not sufficient to produce higher shoot P uptake than modern hybrid. The higher translocation efficiency of this cultivar could be explored further to check is there any benefits related to P acquisition under low P levels. The detailed examination of the root morphology of cultivars may advance the understanding of PUE over time.

Variation in PUE amongst cultivars was not significant (Table 4.2), though there was a relative difference in shoot dry matter among cultivars (Table 4.2). A significant interaction between source and cultivar was observed with the results showing that the interaction was

due to the lower PUE of Hawaiian compared with other varieties, when supplied with PM and RP, but the highest PUE with SP. This suggests Hawaiian is better suited to use with inorganic fertilisers rather than in organic production. This again suggests the findings are against the hypothesis that traditional cultivars are suitable for organic production. It must be noted that PUE as determined in this paper is a compound function of both fertiliser availability (solubility and mineralisation rates) and the priming effect of improved P availability in plant use of native soil P (Damodar Reddy et al. 1999). Six to eight times higher PUE when SP was used as compared to RP in this trial is in contrast with the earlier findings of Bolland and Gilkes (1997) who examined the relative effectiveness of RP as opposed to SP in a field trail conducted over several seasons. It is clear that P availability from RP differs significantly over the short term and long term.

Soil solution dynamics are critical to understanding the processes associated with P release and plant nutrient availability. The solution extracted by hollow-fibres used in this method is typical of that which would be available for extraction by plant roots (Menzies and Guppy 2000). Soil solution P levels in this study confirm that less P was immediately available for plants from organic permitted sources (RP and PM) irrespective of cultivars. Earlier studies comparing the relative P uptake from organic (PM) and inorganic source (triple superphosphate) revealed similar P uptake in plants among the sources (Sikora and Enkiri 2005). However the studies involving RP compared to inorganic P (either mono calcium phosphate or SP) has shown relative effectiveness of less than 0.1 (Kumar et al. 1993) and 0.06 to 0.82 (Bolland et al. 2001a) over 30 days. RP effectiveness on yield and solubilisation was found to be <0.25 of SP (Table 4.2). A similar study in western Australia using sandy soil revealed that RP was 77%, 67% and 29% effective as SP in first, second and third crops respectively (Bolland et al. 1997).

Strategies adopted by cultivars to mobilise or solubilise P in low P soils include producing special roots (McCully 1999; Raghothama 1999), exudation of organic acids and

root acid phosphatase (APase) and changing the rhizosphere pH (Hinsinger 2001; Hocking 2001; Liu et al. 2004). The lack of differences in P solubilisation and utilisation for the RP treatment indicates that the cultivars tested differed little if any in their net use of these strategies to mobilise P to meet crop requirements in the 28 day growing period.

Lower soil solution ammonium and nitrate in the first sampling was probably due to the heterogeneous distribution of applied fertilisers which were applied on the same day of sampling. The decrease in soil solution ammonium concentration (Figure 4.2) from 7 DAS to harvest is due to nitrification, crop uptake and volatilisation. Ouyang *et al.* (1999) observed similar results in a growth chamber study in field corn. The pattern of decrease in nitrate concentration in soil solution over the crop growth stages for all cultivars was similar (Figure 4.3) and expected, as corn prefers N as nitrate (Zhou et al. 2000). The pattern of decrease of soil solution nitrate was also similar between PM and SP in this study (Figure 4.3). This trend was also observed in a field experiment on organic (poultry litter) and inorganic fertiliser (ammonium nitrate) application to corn in USA (Cooperband et al. 2002). The increase in nitrate concentration in the control and RP treatments can be explained by nitrification being accompanied by decreased crop demand as a result of poor vegetative growth. The reduced crop demand in control and RP was reflected in the peak concentration (of nitrate and ammonium in soil solution) at 14 days after sowing against 7 days after sowing for SP and PM. The lower soil solution concentration of ammonium and nitrate in the PM treatment compared to the SP treatment (Figures 4.3 and 4.4) was probably due to the difference in N source. 200 kg N/ha as ammonium nitrate was applied to the SP treatment, compared to 105 kg N/ha of ammonium nitrate in the PM treatment which supplemented 95 kg N/ha from the PM itself which may not have fully mineralised at the time of measurement. The results on N dynamics in the soil and plant indicate that application of 200 kg N/ha enabled crop growth without N deficiency and the results are likely due to P differences.

None of the traditional cultivars investigated in this trial appears to be well suited to organic corn production as they have not performed better than Hybrid in P utilisation and yield within permitted sources. However, the traditional cultivar Hawaiian performed better only when supplied with SP and does not appear to be suitable for organic production.

The PUE of plants supplied with PM and RP were two to three times and six to eight times lower than those supplied with SP. Because soluble fertilisers are not permitted in organic production (Scholefield et al. 1999), PM could be one of the options to manage P. Despite earlier attempts to improve P solubilisation rates from existing permitted P sources, e.g. penicillium, elemental sulphur (Evans et al. 2006; Wakelin et al. 2004), soil P availability remains a major limitation to soil fertility management in organic production (Penfold 2000).

4.5 Conclusion

The ranking of most cultivars in this short term experiment with respect to plant growth and uptake parameters (shoot biomass, shoot P uptake, etc.) was similar under high and low P status, though the magnitude of the variation differed between P sources. The lack of interaction between cultivars and sources for some of measured parameters and the existing interaction for shoot biomass, shoot P uptake and PUE indicated that none of the traditional cultivars were capable of performing better than Hybrid under the deficient and sufficient P conditions. While this experiment provided no support for the hypothesis that traditional cultivars were adapted for better P acquisition under low P status or with organic permitted sources, further research should focus on exploring the root morphology and exudates of cultivars such as Golden Bantam to identify the reasons for high translocation efficiency under controlled conditions and to verify if it contains any useful traits for P acquisition under low P and organic farming conditions. The marginal differences between the cultivars when compared with more pronounced differences between the sources suggest exploring an alternate phosphorus source in organic production.

While more pronounced differences between the sources suggests exploring an alternate P source for organic production in chapter 4, the findings of Chapter 3 also reveals that vegetable farms receiving manure and compost have high extractable soil P levels, irrespective of farming system. This has occurred possibly due to manure or compost application to meet the N requirement. This suggests that alternate options to manage P in organic production, while meeting the N requirement, are needed.

Research on N management using leguminous cover crops on for subsequent crops (section 2.5) has indicated the possible P benefits of winter legumes to nutrient cycling. Chapter 5 therefore includes experiments on evaluating the potential of winter legumes to provide phosphorus benefits to subsequent crops, and Chapter 6 uses an isotopic tracer experiment to further explore the movement of phosphorus from winter legume residues to a subsequent corn crop in a low P status.

Statement of Originality:

All the work contained within this paper is the original research of the PhD candidate, Gunasekhar Nachimuthu.

Candidate:

Principal Supervisor:

Statement of Contribution by Others:

This paper has been prepared by the PhD candidate, Gunasekhar Nachimuthu. All coauthors are either PhD supervisors or provided technical assistance and have only contributed to this paper to the extent that would normally be expected of such roles. All coauthors have given their consent for having their contributions to this paper included in the thesis and accept the student's contribution as indicated in the Statement of Originality.

Candidate:

Principal Supervisor:

CHAPTER FIVE

Evaluation of the potential of two legumes to enhance P nutrition in a following crop under organic production in Australia

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Evaluation of the potential of two legumes to enhance P nutrition in a following crop under organic production in Australia

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Abstract

Incorporation of legumes into cropping rotations is a successful technique to manage N under organic production, though less is known about potential benefits for phosphorus (P) nutrition. A number of published reports have indicated a potential for using leguminous cover crops to improve P cycling and availability. Two pot experiments were conducted to investigate the potential benefits of two legumes; faba beans (FB) or field peas (FP), as part of a green manure rotation or as a harvested crop, on the P nutrition of subsequent crops. The first experiment measured P accumulation in shoots and roots of FP and FB to determine the optimum time for incorporation if used purely as a green manure, or as a root P source for subsequent crops following harvest of economic produce. The second experiment investigated the effect of different P sources (zero P (control), rock phosphate (RP), poultry manure (PM) and single superphosphate (SP)) on FB and FP in two contrasting soil types (sandy loam, pH = 5.4 and clay, pH = 7.8) to determine if the legumes were able to utilise organically approved P sources efficiently. Phosphorus accumulation by FB and FP during pod initiation and harvest stage was similar in the vegetative parts (shoot and root), however this needs further verification under field conditions. Maximum P input of both legumes, if used as a green manure, occurred any time after pod initiation. The two legumes accumulated 5-13 % of the applied P and would supply only 2.5 to 6.5 kg P/ha to subsequent crops if supplied with 50 kg P/ha. Winter legumes cannot be recommended as the sole P source in organic production, but may provide supplemental P after the harvest of their economic produce. Field peas acquired similar P from PM and SP sources, although neither was effective in mobilising P from RP in either the acidic or alkaline soils used. There is little evidence that either temperate legume

provides a more efficient means of specifically improving the availability of P to subsequent crops outside of their known benefits as green manure sources of N in organic agricultural systems.

5.1 Introduction

Phosphorus (P) application is essential to improve agricultural production in many farming systems (Bolland et al. 2001b; Heming 2006). Efficient use of P fertilisers is often hindered by fixation and immobilisation in the soil (Ayaga et al. 2006) and the overall efficiency of applied P fertiliser is reported to be less than 25% (Baligar et al. 2001; Heming 2006). Consequently, methods to increase the P uptake by crops are sought. (Richardson 2001). The issue of P efficiency (P uptake/unit of fertiliser) is particularly significant where traditional and less soluble source such as rock phosphate is used (chapter 4). While methods to enhance P availability from different sources permitted in organic production may not meet crop demand (Ryan et al. 2004), some reports suggest that manure and compost applications to meet N requirement might lead to accumulation of P in soil (chapter 3). This suggests exploring alternate options to meet N and P requirement simultaneously. One option being employed successfully to manage N (Stopes et al. 1996) and needs further research on P management in organic producton is using leguminous cover crops as a tool to enhance P nutrition to subsequent crops (Hens and Hocking 2004).

Legumes are generally known for N fixation and N benefits in crop rotations (Herdina and Silsbury 1989; Joachim 2004; Mayer et al. 2003; Ridley et al. 2004; Rochester et al. 1998; Rochester et al. 2001; Unkovich and Pate 2000). Herdina and Silsbury (1990) concluded that N derived from legume roots could not meet the succeeding cereal crop if shoots are not incorporated. However, there are reports that suggest legumes enhance soil fertility by decreasing soil pH and enhancing nutrient availability, particularly P (Jemo et al. 2006; Nuruzzaman et al. 2005a; Nuruzzaman et al. 2005b). Under tropical conditions P accumulating plants such as *Tithonia, Crotalaria, Indigofera, Sesbania and Tephrosia* are

successfully used in crop rotations to enhance P as well as N supply to the succeeding crop (Kwabiah et al. 2003b; Kwabiah et al. 2001; Mapfumo et al. 2005). Under temperate and sub tropical conditions where these species are not native, alternative plants are sought that can supply P.

Winter legumes such as faba bean (FB) and field peas (FP) are major sources of protein in human and animal nutrition, playing a key role in crop rotations in most parts of the world. In Australia, a survey revealed the rotational benefits of pea on subsequent cereal yield increase (Peck and McDonald 2001). Such effects could be of particular benefit for organic production in Australia, where the natural abundant P is low, for example by using winter legumes in a crop rotation. Information on P benefits of winter legumes grown with organic sources could be a boon to organic farmers.

Earlier studies on P benefits of legume–cereal crop rotations (Nuruzzaman et al. 2005a; Nuruzzaman et al. 2005b) investigated the effect of inorganic source of applied P on legume-cereal rotation and inter-specific facilitation of nutrient uptake by intercropped maize and legume (Long et al. 2003). However, there is lack of information on the optimum time of incorporation and quantity of P benefits of legumes supplied with less soluble and organically approved sources in contrasting soil types. With this in view, glasshouse experiments were designed to evaluate the potential of two winter legumes (FB and FP) to enhance P nutrition for subsequent crops in an acidic sandy loam and an alkaline clay soil. Rock phosphate (RP) and poultry manure (PM) are commonly used organically approved P sources with respectively low and high P availability, and these were compared with single superphosphate (SP) as a high P availability non-organically approved fertiliser.

5.2 Materials and methods

5.2.1 Experimental design and setup

Glasshouse experiments were conducted using FB and FP to determine the temporal changes in P uptake from various P sources. An initial experiment was conducted on a Grey Chromosol (Isbell 1996) using two P rates (0 and 50 kg P/ha as SP) and two legumes (FB and

FP) with 16 replications. Four replications were harvested 25, 40, 58 and 70 DAS corresponding to early vegetative, floral initiation, pod initiation and harvest growth stages. Shoot and root P content and uptake were determined after each harvest date.

The second experiment was a two way factorial using two soil types (Grey Chromosol and Brown Vertosol (Isbell, 1996)) and four P fertiliser treatments. Both FB and FP were used as test legume species. The Grey Chromosol was collected near Armidale, NSW and the Brown Vertosol from the Liverpool Plains, NSW (Table 5.1). The surface 10 cm was collected and air dried in a glasshouse and passed through a 5 mm sieve prior to use. The P treatments included a readily available inorganic source, single superphosphate (SP), two organic P sources, poultry manure (PM) and rock phosphate (RP), all at 50 kg total P/ha and a zero P control treatment.

Table 5.1. Selected physical and chemical properties of a Grey Chromosol from Armidale NSW and a Brown Vertosol from the Liverpool Plains, NSW.

Characteristic	Grey Chromosol	Brown Vertosol
Texture	sandy loam	medium clay
Field capacity (kg/kg)	0.17	0.52
pH (1:5 water)	5.4	7.8
Total N (%)[A]	0.15	0.15
Total C (%)[A]	1.30	1.46
Nitrate-N (mg/kg)[B]	7	107
Ammonium-N (mg/kg)[B]	9	4
Colwell P (mg/kg)[C]	13	10

[A]Carlo Erba NA1500 solid sample analyser; [B]2M KCl extract (Page et al. 1982); [C]Colwell (1963)

Each treatment except the control received 50 kg total P ha^{-1} (62 mg P/pot) and was mixed thoroughly through the 1.5 kg of Chromosol and 1 kg of Vertosol soil. This P rate was determined by a preliminary P response experiment to be roughly halfway up the P response curve. Rock phosphate (Bourcrag, 15.9 % total P) and SP (8 % Total P) were ground to fine powder (<106 μm) and PM (3.8 % N, 2.0 % P and 1.6 % K) was applied in the form of pellets (< 2 mm). Following P addition, each soil was brought to field capacity and incubated for one week in a glasshouse before planting. Basal N (200 kg/ha) as NH_4NO_3, S as Na_2SO_4 (50 kg/ha) and K as KCl (50 kg/ha) were applied to all pots in both experiments except those

receiving PM in experiment 2, where the balance of N and K not supplied in the PM (105 kg N/ha) was added. Seeds of FB (var. Barkool) and FP (var. Green Feast) were germinated in sand and four seedlings per pot were planted and thinned to one plant per pot after emergence. Soil moisture was adjusted to field capacity daily by weight. All treatments were replicated four times and pots were randomly arranged on glasshouse benches. The glasshouse was maintained between 10 °C and 25 °C throughout the experiment.

Plants were harvested 48 days after planting. The roots and shoots were separated and oven dried at 70 °C for 48 hours to measure the dry biomass. After measuring dry biomass, the shoots and roots were finely ground (<0.5mm) using a mechanical grinder. Shoot N content was estimated using Carlo Erba NA1500 solid sample analyser. Shoot and root P and K content were estimated using the sealed chamber acid digestion method described by Anderson and Henderson (1986) and analysed using an Inductively Coupled Plasma – Optical Emission Spectrometer (Varian Vista Radial MPX). Fertiliser use efficiency was calculated by subtracting the total P uptake in the control from total P uptake in the other fertiliser treatments and dividing by the applied P rate (Torres-Dorante et al. 2006).

Soil samples were collected at harvest using a core sampler on 48 days after planting of FB and FP. Soil samples were air dried and passed through 2 mm sieve. Soil pH was measured using 1:5 0.01 M $CaCl_2$. Soil nitrate and ammonium content were estimated by extraction with 2 M KCl and analysed using auto analyser following the method of Page *et al.* (1982). Soil available P content was estimated using a resin extraction method (Guppy et al. 2000).

5.2.2 Statistical Analysis

Results were analysed using analysis of variance using R (R Development Core Team 2003) and P value < 0.05 were considered significant. Diagnostic plots were used to check the normality of the data for each response variable and homogeneity of variance and necessary transformations were made using *boxcox* procedure. The results were reanalysed after

transformation for some response variables. Temporal changes in P uptake by legumes were represented as splines plotted using `loess` with mean and standard errors. In experiment 1, we applied two P rates: 0 kg P/ha and 50 kg P/ha and both rates were significantly different. The data from 0 kg P/ha does not contribute to the conclusion of the chapter, and the data were separately analysed for each legume species.

5.3 Results

5.3.1 Temporal changes in P accumulation

Root P uptake and total P uptake of FB and FP differed significantly across the growth stages ($P<0.05$). Root P uptake and total P uptake of FB increased three-fold between 40 and 58 DAS (Figure 5.1; Figure 5.2). The pods of FB plants failed to develop as the flowers were shed at about 50 DAS. In FP, root P uptake and total P uptake steadily increased from 25 to 58 DAS (Figure 5.1; Figure 5.2), but root P uptake and total P uptake were similar at 58 DAS and 70 DAS ($P>0.05$) despite the partitioning of P to the developing pods. In FP, pod development (indicated by mature seeds) was complete at 70 DAS. Maximum P accumulation in both legume species was reached at about 58 DAS.

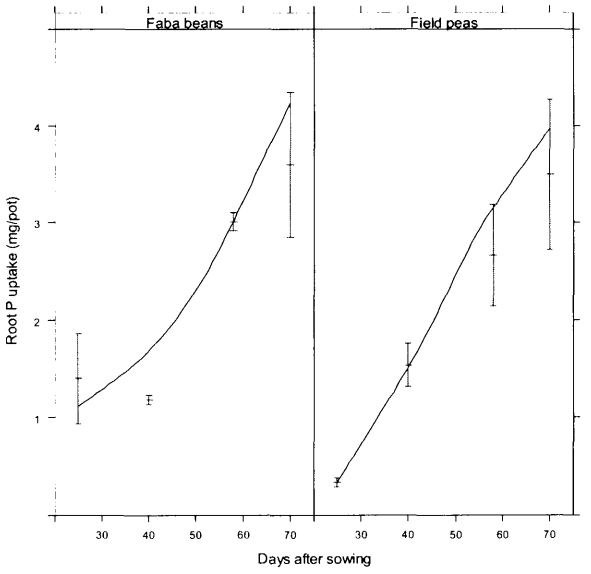

Figure 5.1. Temporal changes in root P uptake of faba beans (*Vicia faba* L.) and field peas (*Pisum sativum* L.) supplied with 50 kg P/ha (means and standard errors are given and the solid line indicates the trend over time based on loess, a non-parametric smoothing function)

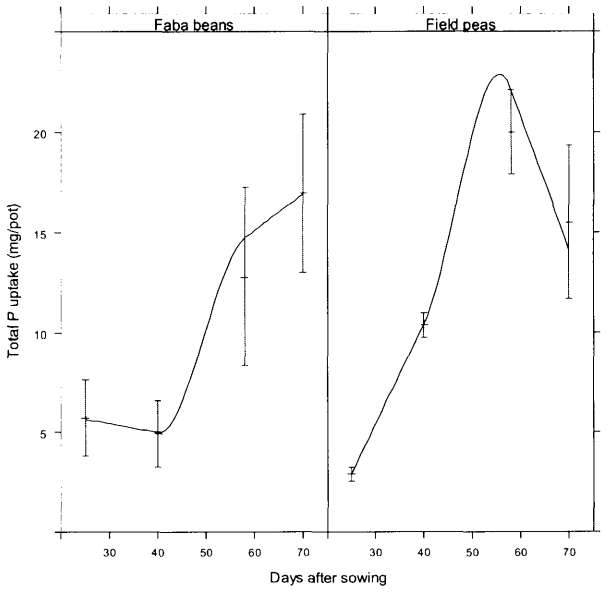

Figure 5.2. Temporal changes in total P uptake of faba beans (*Vicia faba* L.) and field peas (*Pisum sativum* L.) supplied with 50 kg P/ha (means and standard errors are given and the solid line indicates the trend over time based on loess, a non-parametric smoothing function)

5.3.2 Plant dry matter

In the second experiment, P application increased FB shoot dry matter significantly (Nil<RP<PM<SP) ($P<0.001$) irrespective of soil type (Table 5.2). Shoot dry matter of FB in the Vertosol was >55% higher than the Chromosol for all P sources (Table 5.2). Shoot dry matter of FP followed a similar trend as that of FB (Nil=RP<PM<SP) (Table 5.3).

Root dry matter of FB supplied with PM and SP was similar in the Chromosol, but SP application resulted in 53% more roots in the Vertosol (Table 5.2). In contrast, FP supplied with PM produced 41% higher root dry matter than SP in the Chromosol, but similar root growth in the Vertosol (Table 5.3). Root dry matter of FP in control and RP treatment in both soils does not differ significantly (Table 5.3). In general, root dry matter of FB and FP in the Vertosol was 0.5 to 4 times higher than the Chromosol irrespective of P sources (Table 5.2 and Table 5.3).

Table 5.2. Effect of phosphorus fertilisers and soil type on shoot, root dry matter and nutrient uptake of faba bean (*Vicia faba* L.) (Pooled standard errors are presented in bold, with *P* values of ANOVA for sources, soil type and their interaction in order respectively under each parameter). SE=standard error.

Variety	Control	Rock phosphate	Poultry manure	Super phosphate	SE	Control	Rock phosphate	Poultry manure	Super phosphate	SE
	Shoot biomass (g/pot)					Root biomass (g/pot)				
Chromosol	0.63	1.20	1.11	1.92	**0.15**	0.11	0.30	0.59	0.59	**0.07**
Vertosol	1.00	1.32	1.73	2.97	**0.21**	0.60	0.73	1.05	1.61	**0.12**
SE	**0.08**	**0.13**	**0.15**	**0.29**		**0.11**	**0.12**	**0.12**	**0.22**	
P value	**<0.001, <0.001, NS**					**<0.001, <0.001, NS**				
	Shoot P (%)					Root P (%)				
Chromosol	0.20	0.14	0.28	0.18	**0.02**	0.27	0.23	0.28	0.28	**0.02**
Vertosol	0.14	0.14	0.12	0.18	**0.01**	0.15	0.14	0.14	0.18	**0.01**
SE	**0.02**	**0.01**	**0.04**	**0.01**		**0.04**	**0.02**	**0.03**	**0.03**	
P value	**<0.001, <0.001, <0.001**					**<0.001, <0.001, <0.001**				
	Total P uptake (mg/pot)					Root P uptake (mg/pot)				
Chromosol	1.54	2.3	4.62	5.33	**0.54**	0.30	0.67	1.68	1.74	**0.24**
Vertosol	2.43	2.7	3.53	8.11	**0.66**	0.98	0.92	1.44	2.94	**0.24**
SE	**0.23**	**0.22**	**0.30**	**0.95**		**0.14**	**0.10**	**0.24**	**0.39**	
P value	**<0.001, =0.09, =0.05**					**<0.001, <0.001, <0.001**				

Table 5.3. Effect of phosphorus fertilisers and soil type on shoot, root dry matter and nutrient uptake of field peas (*Pisum sativum* L.) (pooled standard errors are presented in bold, with *P* values of ANOVA for soil type, sources and their interaction in order respectively under each parameter). SE=standard error

Variety	Control	Rock phosphate	Poultry manure	Super phosphate	SE	Control	Rock phosphate	Poultry manure	Super phosphate	SE
	Shoot biomass (g/pot)					Root biomass (g/pot)				
Chromosol	0.54	0.57	2.34	2.56	**0.28**	0.18	0.17	0.76	0.54	**0.07**
Vertosol	2.32	2.18	2.98	4.01	**0.24**	0.81	0.82	1.09	1.15	**0.06**
SE	**0.40**	**0.31**	**0.22**	**0.41**		**0.14**	**0.13**	**0.08**	**0.13**	
P value	**<0.001, <0.001, NS**					**<0.001, <0.001, NS**				
	Shoot P (%)					Root P (%)				
Chromosol	0.10	0.11	0.30	0.22	**0.02**	0.10	0.10	0.23	0.20	**0.02**
Vertosol	0.30	0.25	0.29	0.26	**0.01**	0.27	0.23	0.28	0.26	**0.01**
SE	**0.04**	**0.03**	**0.01**	**0.01**		**0.03**	**0.03**	**0.02**	**0.01**	
P value	**<0.001, <0.001, <0.001**					**<0.001, <0.001, <0.001**				
	Total P uptake (mg/pot)					Root P uptake (mg/pot)				
Chromosol	0.74	0.82	8.61	6.87	**1.01**	0.18	0.18	1.70	1.12	**0.19**
Vertosol	9.05	7.29	11.82	13.42	**0.87**	2.23	1.90	3.11	3.00	**0.20**
SE	**1.78**	**1.25**	**1.06**	**1.57**		**0.44**	**0.34**	**0.34**	**0.41**	
P value	**<0.001, <0.001, <0.001**					**<0.001, <0.001, <0.001**				

5.3.3 Phosphorus uptake

The lower concentration of shoot P (Table 5.2 and Table 5.3) than the critical P concentration for FB (0.32%) and FP (0.53%) shoots (Reuter and Robinson 1997) in all treatments was expected because the experiment was conducted under sub-optimal soil P levels to ensure that differences in the P availability of the respective sources could be detected.

Root P uptake of FB and FP was significantly higher in the Vertosol rather than the Chromosol, irrespective of P sources (Table 5.2; Table 5.3). The only exception occurred where PM was applied, as FB removed similar amounts of P in both soils (Table 5.2). With respect to P sources, root and total P uptake of FB with PM and SP application was similar in the Chromosol, but in the Vertosol SP resulted in more than twice as much P accumulated (Table 5.2). In contrast, the root and total P uptake of FP with PM application was >52% higher than SP application in the Chromosol, but did not differ significantly in the Vertosol (Table 5.3).

5.3.4 Phosphorus use efficiency

Phosphorus use efficiency (PUE) includes plant P derived from both applied fertiliser and native soil P made available by P stimulated root growth. Phosphorus use efficiency (PUE) was calculated by subtracting P uptake in the control from P uptake in the other fertiliser treatments and dividing by the applied P rate (Torres-Dorante et al. 2006). SP had 4 and 14 times higher PUEs than PM and RP in the Vertosol when FB was the test species (Table 5.6). When FP were used, the PUE of PM and SP was similar in the Chromosol, but PM was only half as efficient in the Vertosol (Table 5.6). Rock phosphate was not accessible to the FP (Table 5.6).

5.3.5 Nitrogen and potassium

Shoot N concentrations in all treatments were higher than the marginal limit for FB (2.2-3.8%) and FP (2.9-6.7%) (Reuter and Robinson 1997). Shoot K concentration was ~1% in all treatments in the Chromosol for FB and FP which was not deficient (Aini and Tang 1998; Reuter and Robinson 1997).

5.3.6 Post harvest soil nutrient status

Labile, resin extractable P, and available N was twice to three times higher post harvest in the Vertosol than Chromosol (Table 5.4; Table 5.5). In the Chromosol, soil ammonium and nitrate concentration where PM was applied to FP was significantly lower than all other

treatments; due to partial supply of N in organic form. However, in the Vertosol, the high initial soil nitrate content and inherent buffering capacity resulted in little correlation between post harvest soil N status and either crop uptake or P source.

There was no difference in soil pH between the applied sources of P in either soil type for FB (Table 5.4). However FP supplied with PM in the Chromosol resulted in a significantly lower soil pH post harvest (Table 5.5), perhaps due to either crop growth or root exudation of H^+ ions. In the Vertosol, the soil pH of control and RP applied pots was significantly lower than PM and SP applied pots (Table 5.5).

Table 5.4. Effect of phosphorus fertilisers and soil type on post harvest soil nutrient status of faba bean (*Vicia faba* L.) (pooled standard errors are presented in bold, with P value of ANOVA for sources, soil type and their interaction in order respectively under each parameter). SE=standard error.

Variety	Control	Rock phosphate	Poultry manure	Super phosphate	SE	Control	Rock phosphate	Poultry Manure	Super phosphate	SE
	Soil Nitrate (mg/kg)					Soil Ammonium (mg/kg)				
Chromosol	104	48	35	46	**7**	62	51	34	54	**3.6**
Vertosol	307	315	312	404	**23**	4	7	4	8	**0.9**
SE	**40**	**52**	**58**	**74**		**11.7**	**8.5**	**5.8**	**9.1**	
P value	**<0.01, <0.001, <0.05**					**<0.001, <0.001, <0.001**				
	Soil pH					Resin P (mg/kg)				
Chromosol	4.60	4.50	4.50	4.55	**0.02**	0.3	4.0	7	14	**1.5**
Vertosol	7.05	6.98	7.05	6.93	**0.02**	18	19	19	27	**1.7**
SE	**0.47**	**0.47**	**0.48**	**0.45**		**3.7**	**2.8**	**2.4**	**3.8**	
P value	**NS, <0.001, NS**					**<0.001, <0.001, <0.001**				

Table 5.5. Effect of phosphorus fertilisers and soil type on post harvest soil nutrient status of field peas (*Pisum sativum* L) (pooled standard errors are presented in bold, with *P* values of ANOVA for soil type, sources and their interaction in order respectively under each parameter). SE=standard error.

Variety	Control	Rock phosphate	Poultry manure	Super phosphate	SE	Contr ol	Rock phosphate	Poultry Manure	Super phosphate	SE
	Soil Nitrate (mg/kg)					Soil Ammonium (mg/kg)				
Chromosol	55	71	8	41	**7**	59	64	12	46	**6.3**
Vertosol	239	339	324	208	**50**	5	6	5	7	**0.6**
SE	**44**	**54**	**107**	**49**		**11.2**	**10.9**	**1.6**	**8.6**	
P value	**<0.01, <0.001, <0.05**					**<0.001, <0.001, <0.001**				
	Soil pH					Resin P (mg/kg)				
Chromosol	4.50	4.53	4.41	4.54	**0.02**	1	6	3	13	**1.2**
Vertosol	7.03	7.01	7.13	7.14	**0.03**	13	16	14	29	**2.6**
SE	**0.48**	**0.47**	**0.51**	**0.53**		**2.5**	**2.1**	**2.3**	**5.7**	
P value	**<0.001, <0.001, <0.001**					**<0.001, <0.001, <0.001**				

Table 5.6. Effect of phosphorus fertilisers on P use efficiency (%) of faba bean (*Vicia faba* L.) and field peas (*Pisum sativum* L.) (pooled standard errors are presented in bold, with *P* value of ANOVA for sources, soil type and their interaction in order respectively under each parameter). SE=standard error.

Variety	Rock phosphate	Poultry manure	Super phosphate	SE	Rock phosphate	Poultry manure	Super phosphate	SE
	Faba beans				Field peas			
Chromosol	1.3	5.0	6.1	**0.98**	0.15	12.7	9.9	**1.90**
Vertosol	0.6	1.8	9.2	**1.22**	0.0	4.9	7.0	**1.27**
SE	**0.32**	**0.70**	**1.40**		**0.06**	**1.90**	**1.49**	
P value	**<0.001, NS, <0.05**				**<0.001, NS, <0.05**			

5.4 Discussion

5.4.1 Experiment 1

The first experiment was designed to examine the differences in P input resulting from incorporating winter legumes at different growth stages. The results of this study suggest that incorporating FB or FP as green manures without harvesting grain provides the maximal legume accumulated P any time after pod initiation (Figure 5.2). Previous studies have correctly assumed maximal P input from incorporated legumes at harvest (Nuruzzaman et al. 2005a), however this study suggests earlier incorporation does not reduce total P input (Figure 5.2). Herdina and Silsbury (1990) reported that maximum N concentration in vegetative parts of FP and FB occurred during early grain filling. Hence, incorporation at pod-filling may provide the highest N as well as P inputs. However, considering the high variability among the replicates (as indicated by error bars in figure 5.2), further research is required in field conditions to confirm the findings of this study.

Growers, who take the opportunity to harvest legumes for seed, necessarily rely only on accumulated P from roots or residual trash to provide a supplemental organic P source to following crops. Legume species that retain a significant portion of accumulated P in roots may provide greater benefits. Previous research has suggested that legumes such as FB and FP do retain P in roots (Nuruzzaman et al. 2005a; Nuruzzaman et al. 2005b). The observed P uptake and PUE (5-13%) of supplying organically approved P sources (50 kg P/ha) to legumes in this study indicates that only 2.5 and 6.5 kg P/ha is potentially supplied through decomposition of root inputs (Table 5.2; Table 5.3). Earlier studies on the P release from applied medic residues resulted in 5.4% of medic P recovered in subsequently grown wheat with 22-28% accumulating in microbial biomass, and the remainder remaining in the soil (McLaughlin et al. 1988a; b; c). Despite these small additions of legume derived P to subsequent crops, P availability from residual fertiliser or soil P and from legume residues together may increase the P available to a subsequent crop. Maize grown after maize utilised

8% of previously applied RP against 11% utilisation by maize grown after legume (velvet bean) (Pypers et al. 2007). However, it is not recommended that growers in organic systems rely on P released from legumes to solely meet the P demands of following crops. Particularly as the residual benefit of the applied P sources may be higher than that supplied through decomposing legume roots.

Further caution is warranted considering the known higher P requirements of legumes over cereals (Marschner 2002). Ventura and Ladha (1997) examined the long-term efficiency of a legume green manure in rotation with rice with respect to P and N inputs. They observed that legume growth declined much faster than rice growth due to P deficiency, resulting in lower inputs of fixed N to the rice system. Hence, in some lower P input organic farming systems, relying on legumes to provide adequate N (disregarding benefits to P cycling) may result in poorer cereal performance over time.

In this study, FB displayed a typically complex phenology (Adisarwanto and Knight 1997), particularly as the flowers failed to develop into pods or grain. Earlier studies on FB also reported similar problems of pod shedding under irrigated conditions for reasons unknown (Turpin et al. 2002). Given these phenological complexities, firm conclusions regarding the optimal incorporation time (pod initiation vs harvest) of FB must await trials that maintain flowers and pods through to harvest. The study was conducted in glasshouse controlled conditions and results would be different in terms of kg P/ha accumulated by legumes if conducted under field conditions. The duration of different growth stages is longer under field conditions compared with controlled environmental conditions.

5.4.2 Experiment 2

The poor performance of RP as a P source (Table 5.2; Table 5.3) for both legumes suggests that P solubility from RP is not sufficient to meet plant demand, even in an acidic Chromosol; despite recommendations of RP for soils with pH <5.5 (Zapata and Roy 2004), a result confirming the finding in chapter 4 while corn was used as test crop. Certain legume

species are known for altering P chemistry in the rhizosphere to mobilise sparingly soluble P sources (Kamh et al. 1999; Pypers et al. 2006). However, this study suggests FB and FP are not amongst them, particularly as post-harvest soil changes were minimal (Table 5.4; Table 5.5). Using these temperate legumes to increase the P availability to subsequent crops from organically approved RP sources is unlikely to succeed

In contrast, accumulation of P from PM was equal to or greater than that provided by SP for FP in both soil types (Table 5.3). Availability of manure P and its subsequent effect on soil P is usually distinct from mineral fertiliser sources (Griffin et al. 2003). Previous comparisons of PM with mineral fertiliser revealed that only ~4 kg P/ha from PM was required to raise soil test Mehlich-3-P by 1 mg/kg compared with ~17 kg P/ha from mineral fertiliser (Lucero et al. 1995). Similarly, ~6 kg P/ha and 18 kg P/ha from PM or mineral fertiliser respectively was required to increase soil test Olsen P by 1 mg/kg in a soybean-wheat rotation (Damodar Reddy et al. 1998). Some authors have suggested that increased organic acid concentration resulting from manure decomposition was responsible for reduced P sorption and increased P availability from swine manure compared with inorganic P (Laboski and Lamb 2003). This may have contributed to better P availability to FP from PM in the Chromosol. Lower soil pH and organic acids may also have increased the solubility of any calcium P present in PM, which may have constituted ~30% of P within PM, but was not specifically measured in this study (Cooperband and Good 2002; He et al. 2007; He et al. 2006). Given the minimal solubilisation of RP in the Chromosol, organic acid production from PM is more likely than lower soil pH to have potentially increased P availability. Accounting for the significantly lower pH in the Chromosol following FP growth with PM is problematic, particularly as post-harvest nitrate and ammonium concentrations were similar (Table 5.5), implying roughly equivalent uptake of both N sources. However these changes are marginal at best and may simply be the result of greater provision of N in an ammoniacal form where PM was supplied (see methods) as also observed by Hinsinger (2001) using

soybeans. It should also be noted that P uptake of FB was similar to FP, yet no change in pH with PM as the P source was observed (Table 5.4). The lack of a measurable pH change in the Vertosol was not unexpected due to the much higher buffering capacity of this montmorillonite rich soil.

There is little evidence that rhizosphere processes such as organic acid exudation of FP or FB significantly affected P availability or post harvest soil properties. Although post-harvest resin P increased in soils where P was applied, even RP, this is unlikely to be due to root induced solubilisation of P sources without subsequent uptake by the legumes, or even changes in soil pH. Resin P is measured in a dilute soil: solution ratio relative to that experienced by plant roots, and the observed increases are more likely associated with dilution induced dissolution of added P sources. Some authors have observed specific root exudation effects on P availability and uptake, be they through H^+ exudation (Gahoonia et al. 1992) or organic acid exudation (Jemo et al. 2006) however the results presented here can not sensibly be attributed to residual legume root induced changes in P availability.

5.5 Conclusion

This experiment suggests legumes accumulate 5-13 % of applied P and supply only 2.5 to 6.5 kg P/ha to subsequent crop if supplied with 50 kg P/ha. Winter legumes cannot be recommended as sole P source in organic production, but may provide supplemental P after the harvest of their economic produce. Pod initiation stage could be the optimum time for incorporation of both the legumes as green manure to maximise the P supply if used as supplemental P source, however this needs further verification under field conditions. Field peas acquire similar P from organic (PM) and inorganic (SP) sources, although neither was effective in mobilising P from RP in either acidic or alkaline soils. There is little evidence that either temperate legume provides a more efficient means of specifically improving the availability of P to subsequent crops outside of their known benefits as green manure sources of N in organic agricultural systems. Further investigation using radio-tracer technique is

required to quantify the rotational P benefits of incorporating the winter legumes on subsequent crop.

While the findings of this conventional study in this chapter (chapter 5) reveal that the two legumes did not contribute much P to the subsequent crops, an isotopic tracer study investigating the movement of P from P-fertilisers and legume residues to subsequent corn in a low P status soil was conducted to verify and quantify the results of this chapter. This experiment is described in chapter 6.

Statement of Originality:

All the work contained within this paper is the original research of the PhD candidate, Gunasekhar Nachimuthu.

Candidate:

Principal Supervisor:

Statement of Contribution by Others:

This paper has been prepared by the PhD candidate, Gunasekhar Nachimuthu. All coauthors are either PhD supervisors or provided technical assistance and have only contributed to this paper to the extent that would normally be expected of such roles. All coauthors have given their consent for having their contributions to this paper included in the thesis and accept the student's contribution as indicated in the Statement of Originality.

Candidate:

Principal Supervisor:

CHAPTER SIX

Isotopic tracing of phosphorus from ^{33}P labelled legume residues and ^{32}P labelled fertilisers to subsequent corn in legume-corn rotation

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Isotopic tracing of phosphorus from ^{33}P labelled legume residues and ^{32}P labelled fertilisers to subsequent corn in legume-corn rotation

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Abstract

In low input farming systems (e.g. organic) where soil phosphorus (P) fertilisers such as superphosphate are not used, maintaining sufficient available soil P for plant growth can be a major challenge. The use of P accumulating cover crops may have the potential to increase P availability for subsequent crops. We hypothesised that P release from organic residues of legumes (faba bean (*Vicia faba*) and field peas (*Pisum sativum*)) could supply adequate P to meet the needs of subsequent crop in a low fertility soil, and that the P availability from organic residues increases with the amount of residues applied increases. A dual labelling isotope study was conducted to determine the contribution of P by legume green manure to subsequent corn using ^{33}P labelled legume residues along with ^{32}P labelled inorganic fertiliser (KH_2PO_4). The treatments imposed included two rates of P application, (a) 10 kg P ha^{-1} as legume root and shoot residues or as inorganic fertiliser with and without a carbon source, and (b) 38 kg P ha^{-1}as a combination of residues of root and shoot of each legume or a combination of root and inorganic fertiliser and inorganic fertiliser alone. An absolute control (zero P) was also used. Corn was planted 10 days after incorporation of residues. Shoot dry matter, P uptake and P source (residues or fertilisers) of total P in corn were measured at harvest. Soil solution P content was measured on 4, 7, 11, 16, 24 and 46 days after incorporation of residues. Some of the P released from legume residues to the soil solution remained in an organic form throughout most of the experiment. Faba bean and field pea residues alone or in combination with fertilisers contributed up to 10% and 5% of the total P uptake by corn respectively, compared with up to 54% by inorganic fertilisers. Incorporation of legume residues with P concentrations higher than field conditions may still not always

lead to net P release to subsequent crops. These results inform future research into nutrient cycling in field conditions of Australia, where native soils are inherently poor in P status.

Key words: Australia, corn, dual labelling, faba bean, field peas, phosphorus.

6.1 Introduction

Phosphorus (P) is an essential macronutrient for plants (Fletcher et al. 2006). In Australia, plant available P is a limiting factor in broad-acre organic farming due to the low total P cotents (Penfold 2000; Ryan and Ash 1999; Ryan et al. 2000) and slow rate of release from organic-certified fertilizers (Chapter 4). Hence P nutrition in organic farming systems remains unresolved and new approaches should be explored. One possibility is to incorporate P accumulating crops into organic crop rotations (Chapter 5). P accumulating crops have been defined as those which contain more than 0.3% P in their plant tissue (Kwabiah et al. 2003b)..

Legumes are an important component of crop rotations, particularly in organic farming (IFOAM 2002). There are reports suggesting that tropical legumes such as *Tithonia, Crotalaria, Indigofera, Sesbania* and *Tephrosia* have been successfully incorporated in cropping sequence to enhance P availability as well as N to the succeeding crop (Kwabiah et al. 2003b; Kwabiah et al. 2001; Mapfumo et al. 2005). Although there are also reports suggesting that most of the P from applied tropical green manures is not available immediately (Bah et al. 2006). Indirect P benefits of faba bean and field peas were observed in legume-cereal rotation in short term glasshouse experiments, particularly rhizosphere induced changes in soil properties (Hens and Hocking 2004; Nuruzzaman et al. 2005a; Nuruzzaman et al. 2005b). However, study on evaluating the potential of two legumes to enhance P nutrition in a following crop provided little P benefits to subsequent crop (Chapter 5), this needs further verification on the exact quantity of P available to the subsequent crop immediately after application of faba bean and field pea residues.

While P availability to most crops is essential in the early phase of growth, a better understanding of the exact amount and form of P available from green manures or residues

from a previous crop will help in P fertilisation strategies, irrespective of farming systems. This quantification is possible using P isotopes to study soil and plant nutrition. The dual-labelling technique in plant nutrition is a valuable tool to quantify the proportion of P uptake from green manures and fertilisers or soil (Dorahy et al. 2007; McLaughlin and Alston 1986; McLaughlin et al. 1988a; b; c).

With this is in mind, a dual-labelling glasshouse experiment was designed to quantify the P contribution by faba bean and field pea residues to subsequent corn. It was hypothesised that P release from organic residues of legumes could supply adequate P to meet the needs of subsequent crop in a low fertility soil, and that the P availability of organic residues increases as the amount of residues applied increases.

6.2 Materials and methods

6.2.1 Experimental design and setup

Glasshouse experiments were conducted in nutrient solution and soil culture to quantify the P contribution from legume residues of faba beans (*Vicia faba* L.) (FB) and field peas (*Pisum sativum* L.) (FP) to subsequent corn. Faba beans and FP were grown in solution culture labelled with ^{33}P (Section 6.2.2). Subsequent corn was grown in a P responsive Grey Chromosol (Isbell 1996) from Armidale, NSW (Table 6.1) (Section 6.2.3).

Table 6.1. Selected physical and chemical properties of a Grey Chromosol (0-10cm) from Armidale NSW.

Soil characteristics	Grey Chromosol
Texture	sandy loam
Field capacity (kg kg^{-1})	0.17
pH (1:5 water)	5.4
Total N (%)[A]	0.15
Total C (%)[A]	1.30
Nitrate-N (mg kg^{-1})[B]	7
Ammonium-N (mg kg^{-1})[B]	9
Colwell P (mg kg^{-1})[C]	13

[A]Carlo Erba NA1500 solid sample analyser; [B]2M KCl extract (Page et al. 1982);
[C]Colwell (1963)

A range of treatments were used to investigate the rate, type of P source, effect of carbon and presence or absence of plants on P solubilisation (Table 6.2) in a completely randomised design. Each treatment was replicated four times. The treatments imposed included two rates of P application, (a) 10 kg P ha^{-1} as legume root and shoot residues or as inorganic fertiliser with and without a carbon source, and (b) 38 kg P ha^{-1}as a combination of residues of root and shoot of each legume or a combination of root and inorganic fertiliser and inorganic fertiliser alone. An absolute control (zero P) was also used. The chosen addition rate (38 kg P ha^{-1}) was roughly half-way up a previously determined P response curve for legumes grown in this Grey Chromosol to ensure plants were actively seeking P, allowing differences in source availability to be identified not only though isotopic composition but also through yield differences.

Table 6.2. Inorganic fertiliser and legume residue treatments applied to corn planted 10 days after fertilisation in a sandy loam soil and grown for 35 days upto planting.

Abbreviation	Treatment details
Con	Control- Zero P
IP38	[a]Inorganic P 38 kg P ha^{-1}
IP10	[a]Inorganic P 10 kg P ha^{-1}
IP10C	[a]Inorganic P 10 kg P ha^{-1} plus carbon[b]
FBRT10	[c]Faba bean roots 10 kg P ha^{-1}
FPRT10	[c]Field peas roots 10 kg P ha^{-1}
FB38	[c]Faba bean root 8 kg P ha^{-1} plus [c]shoot 30 kg P ha^{-1}
FP38	[c]Field peas root 8 kg P ha^{-1} plus [c]shoot 30 kg P ha^{-1}
FB8IP30	[c]Faba bean root 8 kg P ha^{-1} plus inorganic P[a] 30 kg P ha^{-1}
FP8IP30	[c]Field peas root 8 kg P ha^{-1} plus inorganic P[a] 30 kg P ha^{-1}
FBST10	[c]Faba bean shoots 10 kg P ha^{-1} with plants
FPST10	[c]Field peas shoots 10 kg P ha^{-1} with plant
FBSTC10	[c]Faba bean shoots 10 kg P ha^{-1} without plants
FPSTC10	[c]Field peas shoots 10 kg P ha^{-1} without plants

[a]labelled with ^{32}P; [b]starch, [c]labelled with ^{33}P; except FBSTC10 and FPSTC10 all other treatments had growing corn plants

6.2.2 Preparation of ^{33}P labelled legume residues

Germinated seeds of FB (var. Barkool) and FP (var. Green Feast) were grown in a nutrient solution culture (Lisle et al. 2000) in plastic trays each containing 7.5 L of solution placed in a steel tray lined with polythene (for isotope containment in the event of spillage). Nitrogen was supplied as nitrate to minimise falls in solution pH; pH was maintained near 7 using dilute NaOH. Phosphorus (KH_2PO_4) was labelled with ^{33}P to provide a specific activity of greater than 50 kBq mg^{-1} of P during each application applied on days 3, 8 and 15 after planting @ 20%, 30% and 50% of the final total P applied in solution. This labelling protocol aimed to ensure uniform specific activity of plant parts of various ages, minimising the possibility that isotope was exclusively taken up as inorganic P and stored in leaf tissue prior to harvest. The time intervals were chosen using several preliminary experiments to match the rate of growth of FB and FP in soil and nutrient solution culture. Plants were harvested at 23 days after planting in order to get sufficient crop biomass with many half-lives available for the soil incubation experiment. Roots were separated and rinsed with deionised water. Plant materials were dried at 80°C for 48 hours and ground to <1 mm sieve. The concentrations of nutrients and specific activity of the residues corrected to Day 0 were measured (Table 6.3) as described in the solution and plant analysis section below. The nutrient concentration of both legume residues obtained under solution culture was higher than those observed in field conditions (Reuter and Robinson 1997).

Table 6.3. Nutrient concentrations and specific activity of root and shoot residues of faba beans (*Vicia faba*) and field peas (*Pisum sativum*) grown in solution culture for 23 days.

Plant parts	N%	P%	K%	C%	C:N	C:P	Specific activity*
Faba bean shoot	4.0	0.75	1.6	42	10.3	56	26
Field peas shoot	4.2	0.49	1.7	44	10.3	89	30
Faba bean root	4.8	1.41	2.5	37	7.7	26	53
Field peas root	4.8	1.14	2.5	36	7.4	31	55

*kBq ^{33}P mg^{-1} of P in residues corrected to Day 0

6.2.3 Residue and fertiliser application and corn growth

All pots were lined with polythene bags and corn (*Zea mays* L.) was grown in a total of 1000 g of soil. Ten days before sowing of corn, residues and fertilisers were added according to the treatment schedule in Table 2. Treatments with residue additions were mixed end-over-end for 2 minutes in bags with 700 g of air dried soil. Where inorganic P was applied as solution, it was banded on the surface of the 700g of soil already placed in each pot. Phosphorus was applied as KH_2PO_4 labelled with ^{32}P with a specific activity of 110 kBq mg^{-1} of P. Another 300 g soil was added to each pot and soil moisture was brought to field capacity (17%). Polyacrylo-nitrile hollow fibre soil solution samplers (Menzies and Guppy 2000) were placed in the middle of each pot to ensure solution was extracted from both the residue fertilised layer below the band and the inorganic P labelled band one-third of the way down the pot. The soils in the pots were subject to two wetting and drying cycles prior to sowing at 10 days after fertiliser application (DAF). The pots were nutrient balanced with ammonium nitrate at the rate of 200 kg N ha^{-1} (or 190 mg N pot^{-1}) and potassium chloride at the rate of 75 kg K ha^{-1} (or 71.3 mg K pot^{-1}) basally before sowing and another 100 kg N ha^{-1} (or 95 mg N pot^{-1}) and 75 kg K ha^{-1} (or 71.3 mg K pot^{-1}) 21 days after sowing. Micronutrients were added basally to avoid the effect of micronutrient deficiency.

Seeds of corn (Hybrid 424 (Pacific Seeds 2005)) were germinated 7 DAF in moist tissue paper in separate trays and one seedling per pot was planted on 10 DAF. Soil moisture was adjusted to field capacity (170 g kg^{-1} soil) daily by weight.

Plate 6.1. Winter legumes grown for preparation of labelled residues (faba beans on left and field peas on right).

6.2.4 Soil solution analysis, harvest and plant analysis

Soil solution samples were collected at 4, 7, 11, 16, 24 and 46 DAF. Moisture in the pots was adjusted to contain 270 g water pot^{-1} and approximately 4 mL of soil solution was collected into 10 mL evacuated blood sampling vials via a double sided needle. Samples were prepared for scintillation counting and stored at <10 °C until analysis. One mL of the soil solution was mixed with 17 mL of scintillant and analysed for ^{33}P and ^{32}P activity using liquid scintillation counting (LSC) technique (Till et al. 1984). ^{33}P and ^{32}P standards were prepared by adding 3 mL each of 10%, 5% and 1% solution of original stock used for labelling along with 17 mL of scintillation fluid (Till et al. 1984). ^{33}P and ^{32}P mixed standards of 10%, 5% and 1% were prepared similarly and counts in two windows, 5-100 keV (^{33}P) and 100-1700 keV (^{32}P) were recorded. Samples were counted for 30 minutes and corrected (^{32}P in 5-100 keV, and ^{33}P in 100-1700 keV) using simultaneous equations. Specific activity of the solutions was calculated as described below. Inorganic soil solution P was measured for all the samples using malachite green colorimetric analysis (Motomizu et al. 1984). Due to insufficient volume of solution samples following LSC and inorganic P analysis, total P was only measured for FB38, FP38, IP38, FB8IP30 and FP8IP30 on all time periods and FBRT8 and FPRT8 on 7 and 11 DAF. Total P was measured following persulfate digestion of solution samples. Two mL of 1:1 dilution of soil solution or standards were mixed with 0.3

mL of 3 M H_2SO_4 and digested with 7.7 mL of 26 mM $K_2S_2O_8$ (final conc. 20 mM) in an autoclave at 120 °C and 100 kPa for 60 mins. After digestion, phosphorus concentration in solution samples were measured using malachite green (Motomizu et al. 1984).

Shoots were harvested 35 days afters planting (45 days after residue addition) and dried at 80°C for 48 hours and ground to <1 mm. Tissue P concentrations were determined using the sealed chamber acid digestion method described by Anderson and Henderson (1986) and analysed using Inductively Coupled Plasma – Optical Emission Spectrometer (Varian Vista Radial MPX). The specific activity of the plant tissue was measured using LSC technique (Till et al. 1984) using the acid digested samples of 3 mL each along with 17 mL scintillant as described for soil solution samples but in the appropriate background matrix to account for sample quenching in acid. Specific activity of all the plant and solution samples reported were corrected back to day 0; the day of inorganic fertiliser application for ^{32}P and initial day of labelling the nutrient solution with ^{33}P for legume residues.

Plate 6.2. Soil solution extraction during incubation of residues before the corn is planted

6.2.5 Contribution of P sources to P in plant and soil solution

The contributions by ^{33}P labelled legume residues and ^{32}P labelled inorganic fertilisers to tissue P in subsequent corn were determined. Calculations were done as per International Atomic Energy Agency manual (IAEA 2001). The percentage of P derived from inorganic fertiliser ($\%P_{inorg}$) was calculated as

$$\%P_{inorg} = \left(SA_{plant} / SA_{inorg}\right) \times 100 \qquad 1$$

where SA_{plant} and SA_{inorg} are the specific activity of plant tissues and the inorganic fertiliser respectively.

The proportion of P derived from the legume residues ($\%P_{residue}$) was calculated as

$$\%P_{residue} = \left(SA_{plant} / SA_{residue}\right) \times 100 \qquad 2$$

where $SA_{residue}$ is the specific activity of the legume residues.

The percentage of soil-derived P ($\%P_{soil}$) was estimated as

$$\%P_{soil} = 100 - \left(\%P_{inorg} + \%P_{residue}\right) \qquad 3$$

Phosphorus use efficiency (*PUE*) was determined as

$$PUE = \left(\%P_{inorg} + \%P_{residue}\right) \times \left(P_{uptake} / P_{applied}\right) \qquad 4$$

Where P_{uptake} is the amount of P accumulated in the corn shoots (mg pot^{-1}), and $P_{applied}$ is the amount of P applied as residue or inorganic fertiliser (mg pot^{-1}). The proportion of P in soil solution derived from inorganic fertilisers or legume residues was calculated using equations 1 and 2 by substituting SA_{plant} for $SA_{solution}$, the specific activity of the soil solution. $SA_{solution}$ for FB38, FP38, IP38, FB8IP30 and FP8IP30 was calculated by taking counts per gram of Total P and for other treatments as counts per gram of inorganic P (however due to incomplete digestion of samples, the data on specific activity is not presented). Unaccounted P (data not presented) was calculated by deducting the applied activity from the ^{32}P and ^{33}P recovered in soil solution at harvest and the recovery in plant.

6.2.6 Statistical analysis

Results were analysed using analysis of variance using the statistical program R (R Development Core Team 2006) and *P* values ≤ 0.05 were considered significant. Diagnostic plots were used to check the normality of the data and homogeneity of variance for each response variable and necessary transformations were estimated using the boxcox procedure. Variability was described by the standard error of treatment means (Webster 2007).

6.3 Results

6.3.1 Plant dry matter and P uptake

Shoot dry matter was significantly different between the treatments (P = 0.04), and ranged between 0.3 to 0.8 grams pot^{-1}. The FPRT10 treatment was significantly lower than all other treatments except control (Table 6.4 and Table 6.5). Shoot P uptake was significantly different among treatments ($P < 0.05$) with legume residue treatments at low (10 kg P ha^{-1}) and high rate (38 kg P ha^{-1}) of P application (FBRT10, FBST10, FPST10, FB38, FP38) acquiring a similar amount of P. Phosphorus uptake by the corn in FPRT10 was constrained due to plant mortality before harvest. Phosphorus uptake by corn in IP10 and IP10C were similar suggesting that carbon addition, and consequent transformation into microbial biomass pools, had no effect on P uptake in this trial. Corn P uptake in all treatments supplied with inorganic fertilisers (either partially or fully @ 38 kg P ha^{-1}: IP38, FB8IP30, FP8IP30) was 2-3 times higher than other treatments.

6.3.2 Phosphorus use efficiency

The PUEs in treatments with legume residues alone (FBRT10, FPRT10, FBST10, FPST10, FB38 and FP38) were at least 10 times lower than in those with inorganic and a combination of organic and inorganic P sources (IP10, IP10C, IP38, FB8IP30 and FP8IP30) (Table 6.4 and Table 6.5), and unaccounted P (applied P not recovered in plant or soil solution = 100 – PUE) ranged from 96.6 to 99.9%.

6.3.3 Contribution of legume residues and fertilisers to corn P uptake

The contribution of inorganic fertilisers to corn P uptake ranged from 19% (IP10C) to 54% (IP38) as against contributions from FP of 5-10% and FB residues of 1-4% (Table 6.4 and Table 6.5). Plants supplied with organic residues alone derived nearly all (90-99%) of their P from soil.

Table 6.4. Effect of inorganic fertilisers and residues of faba beans (*Vicia faba* L.) and field peas (*Pisum sativum* L.) (@ 10 kg P ha^{-1} application) on shoot dry matter; P uptake and P use efficiency (PUE) of corn (*Zea mays* L.) and percent contribution to P uptake from inorganic fertilisers (P_{inorg}), legumes residues ($P_{residue}$) and soil (P_{soil}) at 35 days after planting

Treatment	Shoot biomass (g pot^{-1})	P uptake (mg pot^{-1})	PUE (%)	P_{inorg} (%)	$P_{residue}$ (%)	P_{soil} (%)
Con	0.38±0.10^{ab}	0.60±0.04^{b}		0±0	0±0	100±0^{d}
IP10	0.55±0.20^{a}	0.74±0.18^{a}	2.15±0.87^{a}	27±4.5		74±4.5^{a}
IP10C	0.54±0.07^{a}	0.85±0.09^{a}	1.90±0.83^{a}	19±6.8		81±6.8^{a}
FBRT10	0.47±0.01^{a}	0.57±0.07^{abc}	0.26±0.02^{b}		5±0.5	96±0.5^{b}
FPRT10	0.33±0.06^{b}	0.68±0.07^{ab}	0.07±0.02^{c}		1±0.3	99±0.3^{c}
FBST10	0.47±0.07^{a}	0.53±0.02^{c}	0.26±0.08^{b}		5±1.3	96±1.3^{b}
FPST10	0.49±0.07^{a}	0.58±0.06^{bc}	0.19±0.06^{b}		3±0.6	97±0.6^{b}

^{a,b,c,d}Data without common superscript letters are statistically different as separated by the standard error of treatment means

Table 6.5. Effect of inorganic fertilisers and residues of faba beans (*Vicia faba* L.) and field peas (*Pisum sativum* L.) (@ 38 kg P ha^{-1} application) on shoot dry matter; P uptake and P use efficiency (PUE) of corn (*Zea mays* L.) and percent contribution to P uptake from inorganic fertilisers (P_{inorg}), legumes residues ($P_{residue}$) and soil (P_{soil}) at 35 days after planting

Treatment	Shoot biomass (g)	P uptake (mg pot^{-1})	PUE (%)	P_{inorg} (%)	$P_{residue}$ (%)	P_{soil} (%)
IP38	0.70±0.12^{a}	1.97±0.29^{a}	2.96±0.79^{a}	54±7.8		46±7.8^{a}
FB38	0.45±0.04^{b}	0.53±0.04^{b}	0.14±0.02^{b}		10±1.4	90±1.4^{b}
FB8IP30	0.79±0.26^{a}	1.77±0.35^{a}	2.85±0.94^{a}	50±6.8	5±0.9	45±7.4^{a}
FP38	0.39±0.07^{b}	0.51±0.08^{b}	0.04±0.01^{c}		3±0.3	97±0.3^{b}
FP8IP30	0.73±0.09^{a}	1.83±0.36^{a}	2.75±0.68^{a}	49±2.9	4±0.3	47±2.5^{a}

^{a,b,c}: Data marked with superscript of different alphabets are statistically different as separated by the standard error of treatment means

6.3.4 Soil solution P dynamics

Soil solution inorganic P concentrations were below the detection limit (0.0001 mg L^{-1}) in treatments supplied with 10 kg P ha^{-1} as legume residue (FBRT10, FPRT10, FBST10 and FPST10). Total P analysis of the selected treatments revealed that some of the P in soil solution supplied with legume residues was in an organic form. However, due to incomplete digestion of solution samples as described in Section 6.2.4, the total P results reported are minimum values. Total P content in soil solution samples of FBRT10 and FPRT10 on 7 and 11 DAF indicate that at least 0.2 to 0.5 mg L^{-1} of organic P was present in soil solution as against 0.01 to 0.07 mg L^{-1} as inorganic P suggesting at least 3 to 50 times higher organic P

concentrations in soil solution. Inorganic soil solution P concentrations of FBSTC10 and FPSTC10 were similar to FBST10 and FPST10 and ranged from 0.01 to 0.04 mg L^{-1}.

Total P and inorganic P concentrations of soil solution of FB38 and FP38 (Figure 6.1) indicate that some organic P was present in soil solution over the 42 days of sampling. Inorganic P in the soil solution for FB38 ranged from 0.01 to 0.73 mg L^{-1} as against at least 0.03 to 1.2 mg L^{-1} of organic P. Similarly, in FP38, the values are between 0.05 to 0.26 mg L^{-1} and at least 0.05 to 1.1 mg L^{-1} for inorganic and organic P respectively. Some of the P released from green manures was in an organic form and not available for corn growth during the growing period, a result supported by low $\%P_{residue}$ values in Table 6.4 and Table 6.5.

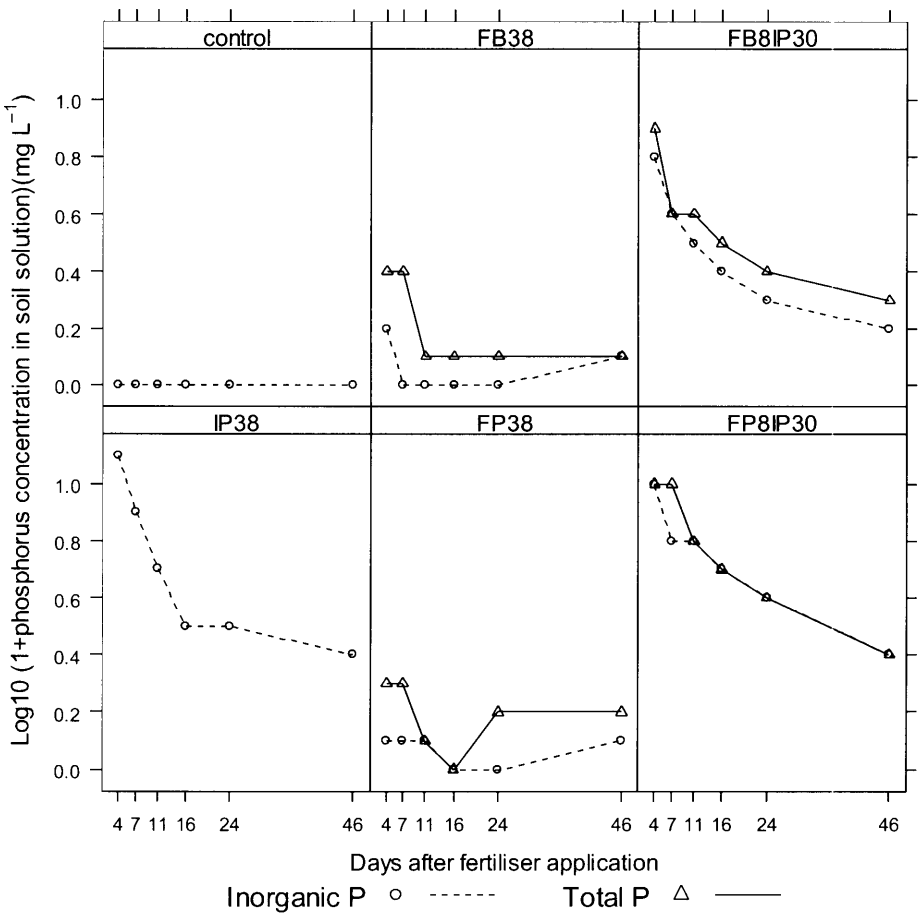

Figure 6.1.Soil solution inorganic and total P dynamics in a sandy loam soil planted with corn 10 days after fertilisation in control and treatments supplied with 38 kg P ha^{-1} of inorganic P fertiliser and/or faba bean (*Vicia faba* L.) and field pea (*Pisum sativum* L.) residue applications.

The contribution of legume residues to soil solution P was unable to be determined due to incomplete digestion. However, the calculated percentage derived from residues exceeded 100% in most instances strongly indicating that most of the P in soil solution in legume residue applied treatments was derived from residues. The percentage contribution of inorganic fertiliser (IP38) to soil solution inorganic P ranged from at least 83% 4 DAF to at least 50% 46 DAF (Figure 6.2). However 16 DAF, the fertiliser contribution was higher (at least 88% of P was derived from fertiliser) and it is not clear what has caused this increase in P solubilisation on this time period.

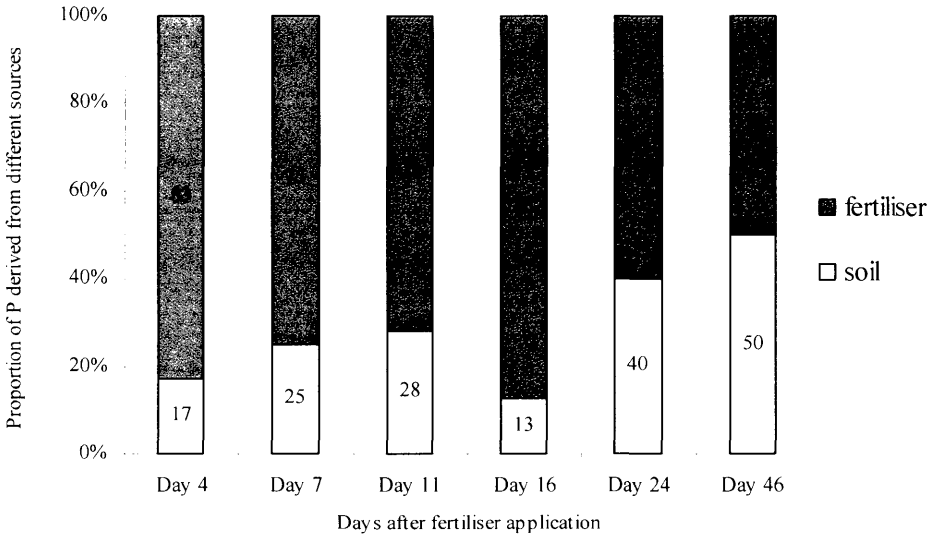

Figure 6.2. Percentage of phosphorus in soil solution P derived from soil and applied fertilisers (38 kg P ha^{-1} as KH_2PO_4) at different days after fertiliser application in a sandy loam soil planted with corn 10 days after fertilisation

6.4 Discussion

This study investigated two hypotheses concerning the relative availabilities of P from both inorganic and organic (legume residues), namely; (a) P release from organic residues of legumes could supply adequate P to meet the needs of subsequent corn crop in a low fertility soil, and (b) that the P availability of organic residues increases as the amount of residues applied increases. The study used legumes, known to be effective in low-input rotations for their N benefits, and sought to quantify any soil fertility benefits arising from P cycling. Using

labelled P sources, the results demonstrated that P utilisation by corn is higher when supplied with inorganic sources, irrespective of the rate of P application. Tracing the P supplied by ^{33}P labelled legume residues indicated that the majority of the P used by corn for growth is derived from soil (minimum 90% P_{soil}) (Table 6.4 and Table 6.5) irrespective of rate of application or legume species compared with a minimum of 45% P_{soil} in the inorganic treatments. Hence, in the important early growth phase of a corn crop, relying only on organic P sources for P nutrition is unlikely to supply adequate P, and merely results in further drawdown in native soil P reserves. In the long term, this could lead to mining of soil nutrient reserves (Gosling and Shepherd 2005; Penfold et al. 1995). Phosphorus from plant residues may be considered available in the longer term (Ryan et al. 2007; Zhang and MacKenzie 1997a), a hypothesis not tested in this experiment. The ten times higher PUE of inorganic fertiliser (<3%) than residue additions at the same rates of P (< 0.3%) illustrates the magnitude of P stress to crops when supplied with organic sources. While this study was conducted in controlled conditions and all the nutrients other than P were supplied in sufficient quantity, under field conditions, deficiency in other nutrients might further limit plant growth and P uptake.

The corn hybrid (Hybrid 424 (Pacific Seeds 2005)) used in this study was found to have similar PUE as that of four traditional organic cultivars when supplied with a range of organic and inorganic sources in an earlier investigation (Chapter 4). This earlier study categorically demonstrated that P mobilised from some organic permitted sources (RP) was low and not sufficient to meet crop demand. Given the examination of legume rotations as a method to increase P mobilisation and availability in this experiment, current methods of relying on mineral fertilisers for supplying P in low-input organic systems are unlikely to provide adequate P to crops. While the critical levels of inorganic P in soil solution samples extracted using the method of Menzies and Guppy (2000) for corn growth is yet to be established, it should be noted that the entire experiment was conducted under conditions of P stress as the

applied fertiliser or residue in this trial was a maximum 38 kg P ha^{-1} chosen to be part way up a previously determined P response curve of this soil, which is linear up to 50 kg P ha^{-1}. Under P stress, strategies adopted by plants to mobilise or solubilise P in low P soils include producing special roots (McCully 1999; Raghothama 1999), exudation of organic acids and root acid phosphatase (APase) and changing the rhizosphere pH (Hinsinger 2001; Hocking 2001; Liu et al. 2004). The hybrid corn could have adopted some of those strategies, but the net P release from legume residues may not have been sufficient. The similar soil solution inorganic P concentration in the treatments with and without corn supplied with similar amount of legume residues (FBST10, FBSTC10 and FPST10, FPSTC10) suggest that presence of living roots has negligible effect on the legume residue decomposition and subsequent P availability (Hinsinger 2001).

An earlier study investigating the potential of FB and FP in enhancing P availability by soil induced changes from native soil P or inorganic source (Nuruzzaman et al. 2005a) revealed indirect benefits from soil induced changes. Later studies along similar lines using P sources permitted in organic agriculture (Chapter 5) revealed that both the legumes provided little evidence in improving the availability of P by changes induced by legumes in soil. However, even if a reduction in soil pH enhances, P availability (Hinsinger 2001; Nuruzzaman et al. 2005b), addition of legumes residues will tend to increase the soil pH after application and will neutralise the effect induced by legumes before harvest due to return of organic anions (Yan et al. 1996) from legume residues.

Two rates of green manure were investigated in this study to simulate a farmer (a) incorporating the legumes as a green manure at floral initiation period (maximum biomass stage) or (b) harvesting the economic produce from the legume crop and use the plant residue root P of the harvested legumes for subsequent crop. The results of this study do not support the hypothesis that P utilisation by corn increased with higher rate of legume green manure addition. However, the amount of P released from any of the legume residue added treatments

was not sufficient (0.01 to 0.07 mg L^{-1}) to meet the crop demand. The results of this study have implications for interpreting previous glasshouse studies reporting indirect P benefits of FB and FP to a subsequent wheat crop through rhizosphere acidification and constant P mobilisation or P mineralisation from root residues (Nuruzzaman et al. 2005a; Nuruzzaman et al. 2005b). These previous studies considered the indirect benefits by soil induced changes rather than direct benefits of quantity and P concentrations of residues incorporated. Because soil pH dynamics with oven dried legume residue application is similar to fresh residue application (Yan and Schubert 2006), our results could be extrapolated to field conditions. The following paragraph focuses on the soil solution P dynamics.

Although, exact quantification of total soil solution P was not possible due to incomplete persulfate digestion and insufficient solution, the results suggested some organic P present in the soil solution of FB38 and FP38 (Figure 6.1). It is assumed that the organic P in soil solution was completely or primarily derived from legume residues, the $P_{residue}$ of these samples hence reflected 200-400% of P in solution from added legume residues (data not shown). The staggered application of ^{33}P resulted in uniformly labelled plant tissue, hence it is unlikely that a flush of inorganic P moved out of the leaves following drying, and that incorporation into the soil through microbial immobilisation is responsible for the high concentration of organic P observed in soil solution. These results provide a cautionary note to earlier reports suggesting very rapid release of P from pasture legume residues (>40%) (Jones and Bromfield 1969), and young FP residues within 15 days after application (~35 mg resin P kg^{-1} soil on day 5 to ~45 mg resin P kg^{-1} soil on day 15 in young field residues applied soils as against <1 mg resin P kg^{-1} soil in mature pea residue applied soils) (Ha et al. 2007). Our study revealed that P release from decomposition of the residues was very slow, remaining in organic forms in solution and hence resulting in minimal utilisation by subsequent maize crops (<0.3% P) (Table 6.4 and Table 6.5). One possible reason for the slower than previously reported mineralisation may be accumulation of residue P by

saprophytic fungi or other micro-organisms (Barak and van Rijn 2000; McGrath and Quinn 2000; Salas et al. 2003; Solaiman et al. 1999).

While one may expect that measured organic P in solution would increase with complete digestion of soil solution samples, the inorganic P content will remain unaltered (Figure 6.1), hence P availability also remains unchanged. The observed decreases in the solution P derived from inorganic fertilisers (P_{inorg}) over time (Figure 6.2), due to either sorption or immobilisation, suggest that mineralised organic P in solution would not necessarily increase P lability to crops. Hence it is unlikely, given the known similarity in P cycling between organic and conventional farming practices (Stockdale et al. 2002), that incorporation of legume residues will dramatically change the availability of P to subsequent crops. Further evidence that stimulation of soil bio-cycling of P does not result in improved P availability is found where a C source was supplied with the inorganic P, not increasing P availability in any way (Table 6.4).

Higher P utilisation from FB residues than FP residues was correlated with difference in P concentration of the residues, as the P concentration of the tissue is increasingly stored as inorganic phosphate. This was not related to the difference in C:P ratio of the residues as both the legume residues had C:P ratios <100 (Lupwayi et al. 2007; Nuruzzaman et al. 2005a; Umrit and Friesen 1994). Despite the P concentration of both the legume residues (0.5 to 1.4%) being considerably higher (Table 6.3) than those observed in field conditions (Lewis and Hawthorne 1996; Reuter and Robinson 1997), the maximum contribution of P from FB and FP residues to subsequent corn P uptake were 10% and 5 % respectively as against 54% (Table 6.4 and Table 6.5) from inorganic fertilisers suggesting that P release in the field will be further constrained. A similar finding was recently reported using residues of *Aschynomene afraspera* and *Crotalaria micans* (Somado et al. 2007).

The corn P uptake and PUE of the combined inorganic fertilisers and legume residues (FB8IP30 and FP8IP30) was similar to the inorganic fertilisers alone (IP38) (Table 6.5). The

amount of P derived from legumes residues ($P_{residue}$) in these treatments were 5% and 4% for FB and FP respectively (Table 6.5) and not significantly different from additions of these legume resides alone at the same rate (Table 6.4 and 6.5). Hence the presence of inorganic P neither increased or decreased the availability of P from legume residues and suggests that a combination of the two in farming systems may result in a net increase in P availability over time, but not necessarily within the same season.

While earlier studies have reported a positive effect of legume rotations in mobilising the residual and fertiliser P (Hens and Hocking 2004; McLenaghen et al. 2004), these studies have used soils with considerable P fertility, such as those in eastern Australian vegetable farms (Chapter 3, Appendix 5). It can therefore be concluded that for soils with low P fertility such as those in Australian broad-acre farms, legume rotations would provide little P benefit outside their known N benefits. This suggests that alternative sources of P should be integrated along with legume residues to overcome the P limitation in organic production especially in low fertility Australian farms.

6.5 Conclusion

While this study did not support the hypotheses that P release from organic residues of legumes could supply adequate P to meet the needs of subsequent crop in a low fertility soil, and that the P availability of organic residues increases as the amount of residues applied increases, there is potential to integrate legume residues with other available sources. Some of the soil solution P from the legume residues remained in an organic form for the entire period of corn crop growth. Faba bean and field pea residues alone or in combination with fertilisers contributed a maximum 10% and 5% respectively of the total P uptake by corn as against <54% by inorganic fertilisers. Incorporation of legume residues with P concentrations higher than field conditions may still not always lead to net P release to subsequent crops. These results inform future research into nutrient cycling in field conditions of Australia, where native soils are inherently poor in P status.

The findings of this chapter further confirm the results of the previous chapter (chapter 5) that the legume residues investigated in this thesis do not contribute significantly to the P requirement of subsequent crop. The next chapter (chapter 7) includes integration of the findings from individual chapters, along with some possible implications for farmers and researchers.

Statement of Originality:

All the work contained within this paper is the original research of the PhD candidate, Gunasekhar Nachimuthu.

Candidate:

Principal Supervisor:

Statement of Contribution by Others:

This paper has been prepared by the PhD candidate, Gunasekhar Nachimuthu. All coauthors are either PhD supervisors or provided technical guidance and have only contributed to this paper to the extent that would normally be expected of such roles. All coauthors have given their consent for having their contributions to this paper included in the thesis and accept the student's contribution as indicated in the Statement of Originality.

Candidate:

Principal Supervisor:

CHAPTER SEVEN

Conclusions and future research

Conclusions and future research

7.1 General conclusions

This chapter summarises the key research findings and suggests a number of avenues for future research. The findings of the literature review were that various chemical and biological methods of enhancing P nutrition had been shown not to meet crop demand in broad-acre organic farming systems where the level of organic inputs was considerably lower than in horticulture. In contrast, the sparse literature on organic vegetable production have indicated an excessive extractable P due to use of manures and compost to meet N demand. However, this could not be generalised to a range of contrasting soil types and climate conditions, highlighting the need for further investigation of the soil fertility status of commcercial organic vegetable farms under different environmental conditions of soil type and climate.

Within the broad objectives of the project, the field investigation of this thesis revealed substantial use of organic inputs in conventional vegetable farms at all localities. Soil extractable P pools were medium to high across farm types (average Colwell P >100 mg kg^{-1}) irrespective of location. While the application of manure and compost to meet N demand could be a possible reason for medium to high P levels the results of our study confirm the earlier work on organic vegetable production in Australia and generalises those findings to a range of contrasting soil types and climate conditions The implications of these results to vegetable growers and researchers are discussed in section 7.2 and 7.3 below.

However, the systems described in chapter 3 have a history of high inputs of soil nutrients especially phosphorus, where as in low P soils, changes in nutrient status during conversion to organic vegetable production would depend to a large extent on management strategies employed and the type of organic fertilisers used. One such strategy evaluated here (chapter 4), selecting corn (*Zea mays* L.) genotypes for specific adaptation to low P soil conditions and organic-approved P fertilizers was investigated in glasshouse experiments conducted to

explore the genotype x environment (P source) interaction. This work revealed that the traditional corn cultivars investigated did not perform better than Hybrid under deficient or sufficient soil P conditions. The marginal differences between cultivars compared with the more pronounced differences between P sources, highlights the importance of exploring an alternate P source in organic production (section 7.3).

In organic production, the use of legume cover crops is a successful N management option, but the simultaneous P benefits of such legumes needs to be quantified. Hence, investigations were conducted using series of glasshouse experiments, including an isotopic tracer study to evaluate the potential of two winter legumes (FB) and (FP) as part of a green manure rotation or as a harvested crop, on the P nutrition of subsequent crops. The findings (Chapter 5 and 6) suggests that there was no evidence that either legumes provided a more efficient means of specifically improving the availability of P to subsequent crops beyond their known benefits as green manure sources of N in organic agricultural systems. Winter legume residues cannot therefore be recommended as the sole P source in organic production, although they may provide some supplemental P after the harvest of their economic produce.

7.2 Implication for farmers

The findings of the field investigation implies that crop management in vegetable production in eastern Australia (whether organic or conventional) should include the measurement of soil nutrient status to avoid over-fertilisation and possible adverse off-farm environmental effects.

While the findings suggest that GxE interaction has little evidence of over coming P stress under limiting conditions, the investigation on legumes suggest integrating these cover crops with supplemental P source to over come P limitation.

7.3 Limitations of current research and implication for researchers

A limitation of the survey work was the lack of comprehensive quantitative nutrient budgets and economic analysis (including fertiliser inputs and crop outputs). A more-detailed, longitudinal investigation over several years of similar management on the same farms could

provide a better understanding of long-term temporal changes in soil nutrient status in these intensive vegetable production systems. Once this is understood, then the ways of improving P management and focus on alternate inputs which are most likely to provide benefits with respect to soil P fertility and plant nutrition could be explored. Nevertheless, the data presented provide a long-term 'snap shot' that was consistent between years and within locations, giving confidence that the current findings are valid.

This study demonstrated that plant-based P management strategies, such as the appropriate choice of cultivars and legume rotations with P-accumlating cover crops, were not capable of providing sufficient P to growing crops in the soils and crop varieties tested. This suggests that further research should focus on developing or identifying better organically approved P sources, designed to meet the immediate nutrient requirement of crops in low input organic systems.

The findings of several investigations in this thesis suggests that generalisations about organic farming being sustainable, or is not sustainable, based on changes in nutrient status, are unwarranted unless the fertiliser regime is specified.

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Appendix 1 Screening phosphorus responsive soils and assessing the optimal rate for screened soils through pot trials

A series of pot experiments were conducted to screen the phosphorus (P) responsiveness of soils (9.1) used in later trials and to determine the optimal rate for P application in three soil types screened (9.3). In addition, a twoway factorial experiment was conducted with and without N and P to study the NP interaction in a Vertosol from the Liverpool Plains (9.2).

9.1 Screening P responsive soils

A pot experiment was conducted to screen the P responsiveness of soils from the vegetable growing regions sampled in Chapter 3, and some locally available samples of similar soil types in the New England and Liverpool Plains region. The results were used for further study on effect of P fertilisers and cover crops on corn growth.

9.1.1 Materials and methods

Five soils with high and low soil available P status were used to indentify three P responsive soils for further study. Soil samples were collected from the top 10 cm, air dried in glasshouse and passed through a 5 mm sieve. The initial characteristics of the soils used in this study are presented in Table A1.1.

Table 9.1. Initial characteristics of soils used for screening experiment

S.No.	Soil	Sampling site	Field capacity (%)	pH	Organic Carbon (%)	Total N (%)	Colwell P (mg kg^{-1})
1	Chromosol	Armidale, NSW	17	5.4	1.3	0.15	13
2	Ferrosol	Dorrigo, NSW	35	5.1	7.4	0.60	26
3	Vertosol1	Gatton, QLD	47	7.7	1.6	0.18	95
4	Vertosol2	Gatton, QLD	47	8.7	1.7	0.21	62
5	Vertosol3	Liverpool plains, NSW	52	7.8	1.5	0.15	10

Plastic pots (110 mm diameter) were filled with 750 g of each soil on an air dried basis. Each of the four replicates received Zero P (P_0) or 20 kg P ha^{-1} (P_{20}) in the form of $NaH_2PO_4.2H_2O$ as a nutrient solution and the soil mixed well after fertilizer application. All the pots received 100 kg N ha^{-1} in the form of ammonium nitrate and 50 kg K ha^{-1} in the form of K_2SO_4 both applied as a nutrient solution before sowing. Four corn (*Zea mays* L.) seeds

were sown in each pot and emerged seedlings were thinned to one per pot seven days after sowing. Soil was maintained at field capacity by watering daily. Plants were harvested after a growth period of 35 days, oven dried at 70 °C and measured for shoot dry matter. Results were analysed using analysis of variance (ANOVA) using the statistical program R (R Development Core Team 2006) and *P* value <0.05 were considered significant. Variability between the treatments in measured parameters was described by the standard error of treatment means.

9.1.2 Results and discussion

The results show that there were significant differences in shoot dry matter for P application and soil type ($P<0.001$) and the interaction between soil type and P application ($P<0.05$) (Figure 9.1). Among the five soils used, the chromosol had a significant difference between P_0 and P_{20} treatments ($P<0.001$). While the Ferrosol had a slight response to P application ($P=0.07$). The other soils, all Vertosols did not show a significant response for P application

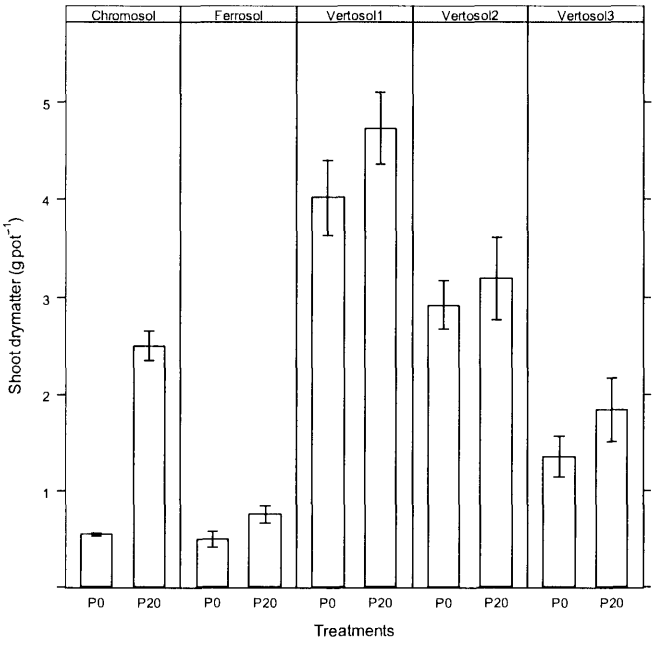


Figure 9.1.Response of corn to phosphorus in different soils.

Vertosol 1 and 2 had very high initial phosphorus levels and the lack of response for applied P was expected based on results from Chapter 3. Vertosol 3, with low Colwell P level, also failed to respond for P application. It suspected that the amount of applied P (20 kg ha^{-1}) was not sufficient to produce a difference in biomass production between the fertilised and control treatment. Also the lack of sufficient N was suspected to be another reason for the lack of difference. Hence another pot trail was designed with and without P and N in two-way factorial design to further investigate the response of Vertosol 3 (Appendix 9.2). It was concluded from this trial that Chromosol and Ferrosol are suitable to for use in further studies and that the suitability of Vertosol 3 is expected to be confirmed based on the results in 9.2

9.2 Study on NP interaction in vertosol

A pot experiment was conducted to investigate the response of vertosol (vertosol 3) to N and P application. The result of the experiment was used for further study on effect of P fertilisers and cover crops on corn growth.

9.2.1 Materials and methods

Vertosol 3 was used for the study and the procedure described in 9.1.1 is followed for initial soil preparation and fertiliser application, except the treatments included a higher P application rate and some N treatments. The initial characteristics of the soil (Vertosol 3) are presented in Table 9.1. Four treatments were imposed: 200 kg N ha^{-1} and 50 kg P ha^{-1} ($N_{200}P_{50}$), 200 kg N ha^{-1} and 0 kg P ha^{-1} ($N_{200}P_0$), 0 kg N ha^{-1} and 50 kg P ha^{-1} (N_0P_{50}) and 0 kg N ha^{-1} and 50 kg P ha^{-1} (N_0P_0). Each treatment was replicated five times. All the pots received 50 kg K ha^{-1} in the form of K_2SO_4 applied in a nutrient solution before sowing. Four corn (Hybrid) seeds were sown in each pot and emerged seedlings were thinned to one per pot seven days after sowing and soil was maintained at field capacity by daily watering for a growth period of 35 days. Plants were harvested, oven dried at 70 °C and measured for shoot dry matter and shoots were analysed for N using mass spectrometry and P and K by sealed chamber digestion with mixture of perchloric acid and hydrogen peroxide (7:3 mixture) and using ICPOES. Results were analysed using analysis of variance (ANOVA) using the

statistical program R (R Development Core Team 2006) and *P* value <0.05 were considered significant. Variability between the treatments in measured parameters was described by the standard error of treatment means.

9.2.2 Results and Discussions

Results (Table 9.3) indicate that there was a significant difference due to P and N application ($P<0.001$) and also interaction between N and P ($P<0.001$). The corn growth is significantly different (atleast 581% higher) from other treatments when N and P were applied together ($N_{200}P_{50}$). Nutrient uptake also followed a similar trend.

The biomass of corn in the previous experiment (9.1) for vertosol 3 when supplied with 100 kg N ha^{-1} and 20 kg P ha^{-1} was 2 g pot^{-1}. When supplied with 50 kg P ha^{-1} along with 200 kg N ha^{-1} the yield is increased by 193% (5.86 g pot^{-1}). The large difference between $N_{200}P_{50}$ and $N_{200}P_0$ (581%) indicates that this soil is highly responsive to P application if other nutrients are available in sufficient amounts. Therefore, this soil was also included for further studies.

Table 9.2 Effect of nitrogen and phosphorus on corn growth and nutrient uptake (means and standard errors are given)

Treatments	Shoot biomass (g pot^{-1})	Shoot N uptake (g pot^{-1})	Shoot P uptake (g pot^{-1})	Shoot K uptake (g pot^{-1})
N_0P_0	0.57 ± 0.046	0.54 ± 0.043	0.05 ± 0.005	2.15 ± 0.154
$N_{200}P_0$	0.86 ± 0.115	2.09 ± 0.228	0.07 ± 0.008	3.61 ± 0.448
$N_{200}P_{50}$	5.86 ± 0.730	11.0 ± 0.814	0.63 ± 0.065	19.3 ± 2.151
N_0P_{50}	0.70 ± 0.073	0.58 ± 0.058	0.15 ± 0.016	2.88 ± 0.272

9.3 Determination of optimal P rate for corn growth in three types of soil

Pot experiments were conducted on three soils (Chromosol, Ferrosol and Vertosol) to determine the suitable phosphorus (P) rates for adequate P supply for corn production after initial applications of P. The results of the experiment were used to establish suitable treatment levels for further studies on effect of P fertilisers and cover crops on corn.

9.3.1 Materials and methods

Chromosol, Ferrosol and Vertosol were used for this study and the procedure described in 9.1.1 is followed for initial soil preparation and fertiliser application. The soils

were supplied with P ranging from 0 to 400 kg ha^{-1} depending on soil types. The exact application rates are shown in Table 9.3. The experimental design was a randomised complete block design with three replications. Four corn (cv. Hybrid 424) seeds were sown in each pot and emerged seedlings were thinned to one per pot seven days after sowing and the soil was maintained at field capacity by daily watering for a growth period of 35 days. Plants were harvested, oven dried at 70 °C and measured for shoot dry matter and shoots were analysed for N using mass spectrometry and P and K by sealed chamber digestion with mixture of perchloric acid and hydrogen peroxide (7:3 mixture) and using ICPOES. Results were analysed using analysis of variance (ANOVA) and linear regression using the statistical program R (R Development Core Team 2006) and *P* value <0.05 were considered significant. Variability between the treatments in measured parameters was described by the standard error of treatment means.

9.3.2 Results and discussions

Results (Table 9.3) indicate that the response for applied P is linear for all the three soils. The shoot biomass response of Chromosol, Ferrosol and Vertosol were linear to applied P up to 50 kg P ha^{-1} (r^2=0.85), 100 kg P ha^{-1} (r^2=0.83), 400 kg P ha^{-1} (r^2=0.96) respectively. Considering that the maximum applied P would be sufficient to produce a difference between treatments, it was decided to adopt a P fertilizer rate of 50 kg ha^{-1} for further trials using the Chromosol. Given the high fixation capacity of Ferrosols, it was decided to use a P fertilizer rate of 200 kg P ha^{-1} in further studies, however further experiments in Ferrosol was not conducted due to revision of research objectives. The Vertosol produced a significant difference for applied 50 kg P ha^{-1} in 9.2. Although the increase of yield in vertosol is linear the rate of increase between 50 and 100 kg P ha^{-1} is same (41%) as that of 25 and 50 kg P ha^{-1} (42%). So it was decided to adopt 50 kg P ha^{-1} for Vertosol for further study.

Table 9.3. Yield and nutrient uptake response of corn to increasing application in three soils at 35 days after sowing. (means and standard errors are given)

	Chromosol			
P applied (kg ha^{-1})	Shoot biomass (g pot^{-1})	Shoot N uptake (g pot^{-1})	Shoot P uptake (g pot^{-1})	Shoot K uptake (g pot^{-1})
0	0.48 ± 0.091	2.12 ± 0.355	0.04 ± 0.008	1.98 ± 0.355
5	0.51 ± 0.020	2.27 ± 0.117	0.04 ± 0.003	2.12 ± 0.102
10	0.65 ± 0.080	2.66 ± 0.272	0.07 ± 0.008	2.85 ± 0.401
25	0.97 ± 0.332	3.75 ± 1.220	0.11 ± 0.033	3.97 ± 1.393
50	2.92 ± 0.197	8.37 ± 0.302	0.38 ± 0.024	10.5 ± 0.316
	Ferrosol			
P applied	Shootbm	ShootNuptake	ShootP uptake	Shoot K uptake
0	0.45 ± 0.109	2.2 ± 0.614	0.04 ± 0.011	1.43 ± 0.324
50	1.04 ± 0.105	4.22 ± 0.42	0.10 ± 0.011	4.25 ± 0.373
100	1.05 ± 0.172	4.00 ± 0.526	0.12 ± 0.012	4.83 ± 0.957
200	2.10 ± 0.219	6.88 ± 0.645	0.20 ± 0.020	9.85 ± 1.151
400	3.65 ± 0.021	10.5 ± 0.348	0.45 ± 0.018	18.1 ± 0.857
	Vertosol			
P applied	Shootbm	ShootNuptake	ShootP uptake	Shoot K uptake
0	1.42 ± 0.163	3.49 ± 0.542	0.18 ± 0.032	7.00 ± 0.999
10	1.84 ± 0.233	4.33 ± 0.715	0.19 ± 0.020	8.37 ± 1.292
20	3.22 ± 0.371	7.17 ± 0.712	0.33 ± 0.019	15.2 ± 1.659
50	4.58 ± 0.633	9.58 ± 0.584	0.55 ± 0.062	20.2 ± 1.668
100	6.46 ± 0.859	11.8 ± 0.661	0.99 ± 0.148	26.5 ± 2.658

Appendix 2 Determination of optimal P rate for faba bean and field peas grown in three types of soil

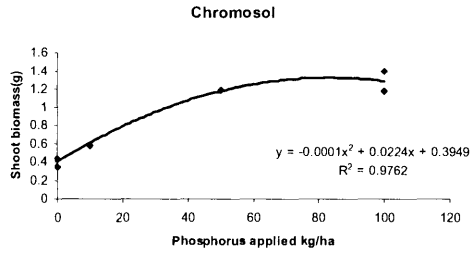
Pot experiments were conducted on three soils (Chromosol, Ferrosol and Vertosol) to determine suitable fertilizer rates for adequate P supply for faba beans and field peas production after initial applications. The results of the experiment were used in further studies on the effect of P fertilisers on cover crops.

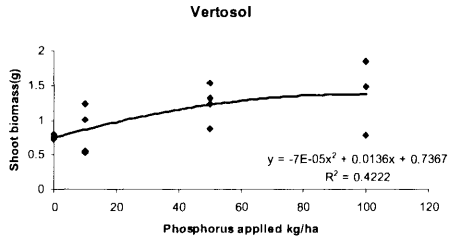
10.1.1 Materials and methods

Chromosol, Ferrosol and Vertosol were used for this study and the procedure described in 9.1.1 is followed for initial soil preparation and fertiliser application. The soils were supplied with P ranging from 0 to 400 kg ha^{-1} depending on soil type. The exact application rates are shown in Table 9.3. The experimental design was a randomised complete block design with three replications. The soil was watered to field capacity (Table A1.1). Faba beans and field peas seeds were pergerminated and sown one seed per pot and soil was maintained at field capacity by daily watering for a growth period of 35 days. Plants were harvested, oven dried at 70 °C and measured for shoot dry matter and shoots were analysed for N using mass spectrometry and P and K by sealed chamber digestion with mixture of perchloric acid and hydrogen peroxide (7:3 mixture) and using ICPOES.

10.1.2 Results and discussions

The results (Figure 10.1 and 10.2) indicate that the shoot biomass response of faba beans and field peas in the chromosol showed a curvilinear trend. Both the crops responded up to 50 kg P ha^{-1}, with no significant increase in growth above that P application rate. Similarly, the shoot biomass responses for both the crops in the vertosol were linear up to 50 kg P ha^{-1} with no significant growth above that rate.





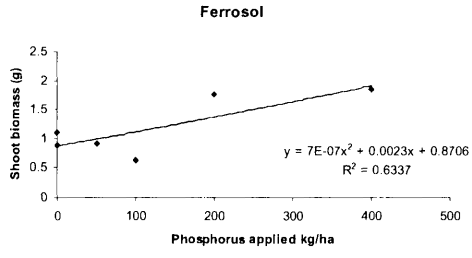
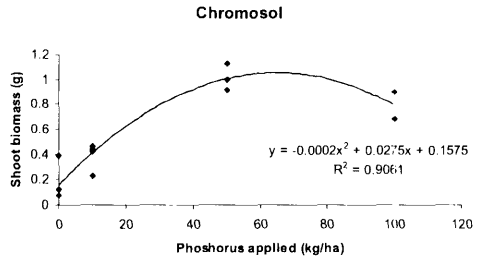
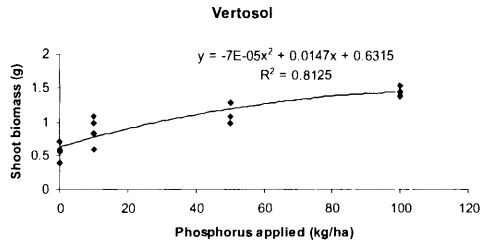


Figure 10.1 Yield (shoot biomass g pot-1) response of faba bean to increasing P application in three soils





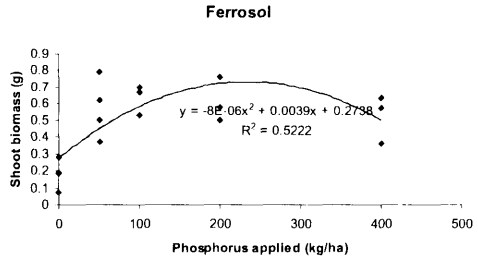


Figure 10.2 Yield (shoot biomass g pot-1) response of field peas to increasing P application in three soil

The shoot biomass response of faba beans in Ferrosol was not clear due to crop failure caused by damping off. The surviving replications indicated no difference between 200 and 400 kg P ha^{-1}. The field pea response was curvilinear, with a maximum response at 200 kg P ha^{-1}. Based on the response of faba beansand field pea to applied P, it was decided to use 50, 50 and 200 kg P ha^{-1} for Chromosols, Vertosols and Ferrosols respectively in further studies on the effect of P fertilisers on cover crops.

Appendix 3 Additional information related to Chapter 4 and 5

Table 11.1. Effect of phosphorus fertilisers and cultivars on growth parameters of corn (the data represent the mean±standard errors)

	Inputs				Inputs			
Variety	Control	PM*	RP*	SP*	Control	PM*	RP*	SP*
	Shoot P (%)				Shoot K (%)			
Balinese	0.15 ± 0.013	0.19 ± 0.007	0.16 ± 0.004	0.20 ± 0.010	4.8 ± 0.18	2.1 ± 0.14	4.0 ± 0.33	1.2 ± 0.05
GB*	0.15 ± 0.008	0.16 ± 0.003	0.16 ± 0.005	0.19 ± 0.008	5.2 ± 0.25	1.9 ± 0.05	3.8 ± 0.25	1.3 ± 0.02
Hawaiian	0.13 ± 0.006	0.16 ± 0.013	0.15 ± 0.009	0.21 ± 0.009	4.3 ± 0.16	2.1 ± 0.20	4.0 ± 0.09	1.3 ± 0.04
Hybrid	0.13 ± 0.006	0.15 ± 0.005	0.15 ± 0.005	0.17 ± 0.002	3.8 ± 0.12	1.5 ± 0.08	3.3 ± 0.19	1.0 ± 0.03
JR*	0.12 ± 0.004	0.17 ± 0.005	0.16 ± 0.010	0.23 ± 0.008	3.5 ± 0.20	1.8 ± 0.10	3.7 ± 0.35	1.4 ± 0.01
	Chlorophyll				Shoot N			
Balinese	29 ± 1.2	41 ± 0.2	37 ± 1.6	38 ± 1.9	3.9 ± 0.17	1.8 ± 0.17	3.9 ± 0.14	2.1 ± 0.07
GB*	36 ± 1.4	47 ± 0.9	40 ± 0.3	43 ± 0.9	4.1 ± 0.09	1.6 ± 0.08	4.1 ± 0.12	2.3 ± 0.04
Hawaiian	31 ± 0.9	42 ± 0.3	37 ± 0.2	41 ± 1.0	4.0 ± 0.17	1.9 ± 0.12	3.9 ± 0.16	2.1 ± 0.04
Hybrid	33 ± 1.3	40 ± 1.0	39 ± 0.6	44 ± 1.6	4.1 ± 0.13	1.6 ± 0.17	3.9 ± 0.04	2.0 ± 0.10
JR*	33 ± 1.5	42 ± 1.1	39 ± 1.0	41 ± 1.1	4.4 ± 0.14	2.1 ± 0.14	4.2 ± 0.15	2.5 ± 0.06
	Shoot N uptake				Root N uptake			
Balinese	6.8 ± 1.23	16 ± 0.6	6.8 ± 1.23	16 ± 0.6	1.4 ± 0.37	3.7 ± 0.45	2.9 ± 0.63	4.6 ± 0.47
GB*	6.3 ± 1.36	16 ± 0.6	6.3 ± 1.36	16 ± 0.6	0.6 ± 0.27	2.4 ± 0.30	1.6 ± 0.71	3.6 ± 0.40
Hawaiian	3.8 ± 0.57	15 ± 0.3	3.8 ± 0.57	15 ± 0.3	1.0 ± 0.16	3.4 ± 0.36	2.7 ± 0.67	4.5 ± 0.54
Hybrid	7.8 ± 1.80	19 ± 1.0	7.8 ± 1.80	19 ± 1.0	1.7 ± 0.42	3.9 ± 0.40	2.3 ± 0.17	4.8 ± 0.38
JR*	3.4 ± 1.10	18 ± 0.7	3.4 ± 1.10	18 ± 0.7	1.1 ± 0.49	3.9 ± 0.11	2.3 ± 0.58	4.9 ± 0.60
	Shoot K uptake				Root K uptake			
Balinese	8.6 ± 1.93	19 ± 0.43	13 ± 1.36	15 ± 0.73	0.6 ± 0.20	1.8 ± 0.27	1.0 ± 0.29	1.7 ± 0.31
GB*	7.8 ± 1.56	19 ± 0.36	13 ± 0.43	17 ± 0.35	0.1 ± 0.07	0.9 ± 0.13	0.3 ± 0.14	0.9 ± 0.11
Hawaiian	4.2 ± 0.66	16 ± 0.42	11 ± 1.32	16 ± 0.21	0.4 ± 0.12	1.4 ± 0.30	0.8 ± 0.25	1.5 ± 0.18
Hybrid	7.2 ± 1.68	17 ± 0.27	12 ± 0.44	15 ± 0.35	0.7 ± 0.19	1.8 ± 0.15	0.7 ± 0.10	1.6 ± 0.16
JR*	2.8 ± 1.04	16 ± 0.46	8.0 ± 1.55	16 ± 0.29	0.6 ± 0.40	1.8 ± 0.03	0.8 ± 0.18	1.8 ± 0.23

Table 11.2. Effect of phosphorus fertilisers and soil type on shoot, root dry matter, nutrient uptake and phosphorus use efficiency of fababean (pooled standard errors are presented in bold, with P value of ANOVA for sources, soil type and their interaction in order respectively under each parameter). SE=standard error.

Variety	Control	Rock phosphate	Poultry manure	Super phosphate	SE	Control	Rock phosphate	Poultry manure	Super phosphate	SE
	Root shoot ratio					Shoot P uptake (mg pot^{-1})				
Chromosol	0.18	0.25	0.61	0.30	**0.06**	1.24	1.66	2.94	3.60	**0.33**
Vertosol	0.58	0.54	0.61	0.54	**0.03**	1.36	1.77	2.10	5.17	**0.41**
SE	**0.09**	**0.06**	**0.09**	**0.05**		**0.13**	**0.13**	**0.20**	**0.57**	
P value	**<0.05, <0.001, NS**					**<0.001, <0.001, NS**				
	Shoot N (%)					Shoot N uptake (mg pot^{-1})				
Chromosol	2.26	2.24	3.77	3.62	**0.28**	14.87	28.07	42.93	67.27	**6.46**
Vertosol	3.66	3.42	3.14	2.64	**0.12**	36.70	43.58	54.23	77.78	**4.38**
SE	**0.35**	**0.39**	**0.19**	**0.24**		**4.79**	**5.16**	**4.55**	**5.69**	
P value	**<0.001, <0.001, <0.001**					**<0.001, <0.001, <0.01**				
	Shoot K (%)					Root K (%)				
Chromosol	1.00	0.93	1.04	1.06	**0.03**	0.37	0.35	0.29	0.25	**0.03**
Vertosol	2.60	2.98	2.74	2.97	**0.10**	3.76	3.47	3.35	4.47	**0.15**
SE	**0.31**	**0.40**	**0.33**	**0.39**		**0.70**	**0.59**	**0.59**	**0.80**	
P value	**<0.01, <0.001, NS**					**NS, <0.001, <0.001**				
	Shoot K uptake (mg pot^{-1})					Root K uptake (mg pot^{-1})				
Chromosol	6.35	11.18	11.45	20.20	**1.62**	0.40	1.03	1.69	1.57	**0.22**
Vertosol	25.83	40.30	47.43	88.83	**7.04**	26.8	25.6	34.9	71.8	**5.78**
SE	**3.75**	**7.13**	**7.12**	**14.23**		**5.76**	**5.64**	**6.44**	**13.70**	
P value	**<0.001, <0.001, <0.001**					**<0.001, <0.001, <0.001**				

Table 11.3. Effect of phosphorus fertilisers and soil type on shoot, root dry matter, nutrient uptake and phosphorus use efficiency of field peas (pooled standard errors are presented in bold, with P value of ANOVA for soil type, sources and their interaction in order respectively under each parameter). SE=standard error.

Variety	Control	Rock phosphate	Poultry manure	Super phosphate	SE	Contr ol	Rock phosphate	Poultry manure	Super phosphate	SE
	Root shoot ratio					Shoot P uptake (mg pot^{-1})				
Chromosol	0.33	0.30	0.32	0.21	**0.01**	0.56	0.65	6.91	5.76	**0.83**
Vertosol	0.36	0.37	0.38	0.29	**0.01**	6.83	5.39	8.71	10.41	**0.69**
SE	**0.02**	**0.02**	**0.01**	**0.02**		**1.34**	**0.91**	**0.75**	**1.18**	
P value	**<0.001, <0.001, NS**					**<0.001, <0.001, NS**				
	Shoot N (%)					Shoot N uptake (mg pot^{-1})				
Chromosol	5.95	6.65	4.25	4.56	**0.28**	32.2	37.5	99.1	117.7	**11.2**
Vertosol	3.93	3.41	3.23	2.87	**0.11**	90.6	73.9	95.0	115.1	**6.3**
SE	**0.41**	**0.62**	**0.21**	**0.34**		**14.1**	**7.0**	**5.1**	**12.4**	
P value	**<0.001, <0.001, <0.001**					**<0.001, <0.001, <0.01**				
	Shoot K (%)					Root K (%)				
Chromosol	1.00	1.00	1.49	1.31	**0.07**	0.13	0.22	0.30	0.23	**0.03**
Vertosol	2.29	2.19	2.73	2.79	**0.12**	4.08	3.81	3.20	2.35	**0.22**
SE	**0.28**	**0.24**	**0.24**	**0.32**		**0.78**	**0.68**	**0.55**	**0.44**	
P value	**<0.01, <0.001, NS**					**NS, <0.001, <0.001**				
	Shoot K uptake (mg pot^{-1})					Root K uptake (mg pot^{-1})				
Chromosol	5.33	5.63	34.98	33.43	**4.18**	0.24	0.39	2.18	1.37	**0.26**
Vertosol	57.0	47.5	81.68	113.6	**8.69**	33.56	30.89	34.53	26.87	**2.10**
SE	**12.15**	**8.20**	**10.43**	**18.47**		**7.36**	**5.87**	**6.16**	**5.18**	
P value	**<0.001, <0.001, NS**					**<0.001, <0.001, <0.01**				

Appendix 4 Comparison of methods for measuring soil microbial activity using cotton strips and a respirometer

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Abstract

In order to develop a method of measuring the level of microbial activity in soil that is suitable for use by farmers, land managers, and other non-scientists, a simple method for determining soil microbial activity was evaluated and compared with two standard techniques. Soils sampled from vegetable farms in south east Queensland were incubated in the laboratory under controlled moisture and temperature conditions. Three methods were used to measure soil microbial activity, a respirometry method and two methods using the cotton strip assay (CSA) technique (image analysis and tensometer). The standard CSA method measured loss of tensile strength over a 35 day incubation period of buried cotton strips using a tensometer. The new CSA technique measured the intensity of staining by microbes using a flatbed scanner to create an image of the cotton strip whose staining percentage was determined using Photoshop® software. The respirometry method used the substrate induced respiration rate (SIR) to determine microbial biomass in the soil at day 12 of incubation. The CSA method using image analysis was the cheapest technique to measure soil microbial activity. The strong correlation between the image analysis method and the tensometer method ($r^2 = 0.81$), a technique used by scientific researchers, suggests that the image analysis method could be used to monitor aspects of soil biological health by general community land-care groups and farmers. The image analysis method uses equipment which is readily available and, while not strongly correlated with more precise measurements of soil biological activity such as microbial biomass ($r^2 = 0.26$), it would detect gross trends in biological health in a soil monitoring program. CSA using image analysis can be a valuable tool in conjunction with other simple indicators of soil physical and chemical health such as slaking and pH to monitor soil amelioration or rehabilitation programs.

Keywords: Microbial activity, microbial biomass, cotton strip assay, decomposition, soil health, image analysis

12.1 Introduction

The role that soil micro-organisms play in decomposing organic matter and in nutrient cycling (Bing-Ru et al., 2006) has important implications for soil fertility management in agriculture. In order to better utilise the functions of soil microbes it is necessary to have reliable measurements of microbial activity and biomass in soil. Such measurements enable the impacts of agricultural activities on soil health or biological processes to be assessed. Measurement of microbial biomass by mineralisation of carbon present in soil microbes was developed by Jenkinson and Powlson (1976), subsequently modified (Anderson and Domsch, 1978b), and has since been widely used to measure microbial biomass in the soil (Fließach and Widmer, 2006). The sophistication of equipment and technical expertise required puts many soil assays beyond the reach of farmers and community land-care groups. A simple yet sensitive tool is required to assess and monitor soil health by lay people (Wagner, 2005) and the use of calico cloth has been recommended to measure soil biological activity, although methods for quantifying the results have been questioned (Tuckombil-Landcare, 2002). Techniques such as the cotton strip assay (CSA) are relatively simple and efficient methods for measuring overall soil biological activity (Correll et al., 1997; Walton and Allsopp, 1977). Woven cotton strips (CS), containing mostly cellulose, are decomposed by microbes when

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buried in soil. The CS lose their tensile strength during incubation and measurement of this loss of tensile strength (LTS) can be a reliable method of measuring CS decay (Knacker et al., 2003; Latter et al., 1988; Obbard and Jones, 1993). This is an indirect method of estimating soil microbial activity but has been well correlated with other established methods (Correll et al., 1997; King et al., 1996). However measuring LTS requires the use of specialised equipment that is often expensive to use and difficult to access.

In addition to reducing the CS tensile strength, microbial growth also produces stains on the strips suggesting the possibility of using image analysis to measure the microbial growth. However, the relationship between LTS and degree of staining of CS has not been examined. Can the staining on the CS after burial be regarded as an indirect measurement of soil biological activity? In this paper, it has been hypothesized that stains produced by micro-organisms on CS incubated in soil can serve as a quantitative measurement of microbial activity in soil. It is also hypothesised that the use of image analysis is cheaper than LTS as an indicator of decay in the CSA.

The use of image analysis in agricultural research has been reported for several applications including weed detection, crop germination, root growth (Alchanatis et al., 2005; Ducournau et al., 2004; Gregory et al., 2003; Ngouajio et al., 1999) and leaf damage by spider mites (Skaloudova et al., 2006). In regard to CSA, Cox and Whelan (1998) measured the undecomposed area of CS rather than extent of staining. Images were captured and processed digitally using NIH Image, a public domain image processing and analysis program. They initially processed the on-screen image by using a computer mouse to draw an outline around the remaining undecomposed CS and then using the software to calculate the enclosed area (J. Cox, pers. comm., 2005).

The aim of the research presented here was to develop a simpler method of quantifying the CSA on intact strips and to evaluate its sensitivity against two standard methods, (a) measuring soil microbial biomass by respirometry and (b) loss of tensile strength of cotton strips using a tensometer. An examination of the cost and practicability of each method was also carried out.

12.2 Materials and methods

12.2.1 Initial soil sampling and characteristics

A bulked soil sample (0-10 cm) was collected at random from paddocks in each of two vegetable farming systems (conventional and organic) in each of two localities in south-east Queensland, Australia. At Gatton (152.28°E, 27.56°S) the soil was a Black Vertosol (clay loam), and at Stanthorpe (151.93°E, 28.66°S) a Bleached Orthic Tenosol (sandy loam) (Isbell, 1996). The main organic farming management practices employed were the use of cover crops, such as dolichos (*Dolichos purpureus*) and lucerne (*Medicago sativa*), and organic manures such as commercial pelletised chicken manure (0-5 t ha^{-1}) and guano (100 kg ha^{-1}). No chemical control of weed, pest and diseases was used. The organic farms at Gatton and Stanthorpe had been under these regimes continuously for 7 and 14 years respectively.

Conventional farms used a range of readily available synthetic fertilisers including urea, single superphosphate, calcium nitrate and potassium nitrate as well as feedlot manure and occasional cover crops. These farms have been under current management in Gatton and Stanthorpe for 50 and 15 years respectively.

Soil samples were collected in February 2005 and stored under aerobic conditions for 45 days with an average temperature ranging from 20-25°C. Key characteristics of the soils are presented in Table 12.1.

[insert table 12.1 here]

12.2.2 Cotton strip incubation

Cotton strips (4.5 x 4.5 cm) were cut from Shirley Soil Burial Test Fabric (Shirley Dyeing and Finishing Ltd., UK), a fabric developed for measuring soil microbial activity (Harrison et al.,

1988). The fabric contains blue and green threads on the cloth to mark areas with standard thread counts, e.g. 36 threads across 1 cm of cloth bounded by two blue threads. This ensures that an equal number of threads are broken in a tensometer during measurement of tensile strength across different soil treatments.

Soil was broken into small clumps, and large invertebrates, stones and roots were removed before the soil was brought to uniform moisture by mass: 30% for Vertosols and 17% for Tenosols. This represented approximately 70% of field capacity, a point where soil respiration is maximised (Kathleen King, unpublished data).

The soil treatments were two soil types (Vertosol and Tenosol) and two farming systems (organic and conventional). Cotton strips were buried in the moistened sample soils for each of the four treatments. A 15 mm layer of soil was spread on the bottom of a plastic container (13 cm x 10 cm x 5.8 cm, 750 ml). Six cotton strips were placed on the soil surface and then covered with another 15 mm layer of soil. The container was filled with alternating layers of cotton strips and soil until a total of 24 strips per container were buried with a final 15 mm soil layer. The container was sealed with a lid and incubated at 25°C. Each soil treatment had three replicate containers (total 288 strips) and all containers were randomly laid out in the incubator. Three cotton strips per container were retrieved at each of eight burial periods: 2, 6, 9, 12, 16, 20, 25 and 35 days. The strips were carefully washed in deionised water, air-dried (20 °C) for 48 hours and subjected to the image analysis method while still intact (Figure 12.1), and then broken in the tensometer which destroyed the strips. Both methods of evaluating the extent of microbial activity in the cotton strips are described below.

12.2.3 CSA image analysis method

This method was developed so that the degree of staining of the cotton strips could be quantified as a measurement of microbial activity. The amount of light reflected in the images of the strips (luminosity) was used as a measure of staining by soil microbes. It was hypothesised that the more microbial activity that occurred on the strips, the darker would be the staining produced and the lower the luminosity. The dried cotton strips were placed on a flat bed scanner (HP Scanjet 5470C) and an image of the strips was obtained by scanning in true colour mode at 300 dpi and saving in TIF format, using Adobe Photoshop® Version 5. The images of each CS were cropped to a standard 3.8 x 3.8 cm size so that each contained a uniform pixel count (299^2). The coloured threads in the cloth were removed from the image using the Magic Wand tool and mean luminosity was estimated using the histogram tool. Luminosity ranges from 0 for a black pixel to 255 for a white pixel. The level of staining was calculated using the formula, staining (%) = [(255- mean luminosity of image)/255] × 100. Images of the cotton strips before and after image processing are presented in Figure 12.1.

[insert Figure 12.1 near here]

12.2.4 CSA tensometer method

After scanning, the LTS of the test strips was measured. Strips were frayed back to 4 cm length using blue threads as a guide on the outside of a strip so that each strip was 4 cm length (138 threads). Strips were placed in the jaws of a tensometer (designed and built by CSIRO Livestock Industries, near Armidale) and subjected to a gradually increasing load until they broke. The load required for breaking (kg) was the measure of tensile strength of the strip. Unburied (control) strips of similar dimensions were also broken as a reference for determining loss of tensile strength using the formula, LTS (kg) = load required to break control strip (kg) – load required to break test strip (kg).

12.2.5 Respirometer method

Soil microbial biomass was determined at a single time point in the incubation period of the cotton strip experiment using the method of Anderson and Domsch (1978a). Separate soil containers were used to those in the CSA incubation, but using the same four soil treatments.

The moistened soil was incubated at 25°C for 12 days and then placed in a respirometer. This corresponded to the half-way point in the CSA experiment.
Eighty grams of the incubated soil were added to a respirometer pot (10.5 cm tall and 6 cm in diameter), using three replications per treatment (total 12 respiration pots). The respiration pots were placed in a 20°C water bath and the CO_2 evolution rate from soil microbial respiratory activity was measured in an electronic respirometry system (Respicond III, Nordgren Innovations AB, Terrangvagen 3A S-903 38 Umea, Sweden). After 48 h in the respirometer, 0.22 g glucose was added to the soil to measure substrate induced respiration (SIR) (mg CO_2/hr/100 g DM Soil) and soil microbial biomass per unit dry matter (DM) soil was calculated by using the equation (Anderson and Domsch, 1978a):
Microbial biomass (mg microbial carbon/100g soil) = (40.4 * SIR) + 0.37.

12.2.6 Economics of three methods

The cost of equipment and the labour involved in each method was calculated in Australian dollars (AUD). The level of skill and the availability of equipment for each technology were estimated qualitatively.

12.2.7 Statistical analysis

Data for staining and LTS changes over time of incubation were fitted to a Weibull function,

$$y = a \times \exp\left(-\left(\frac{x}{b}\right)^{c}\right) \qquad \text{(Equation 1)}$$

where y represents either the staining of cotton strips (%) or LTS (kg), x is the incubation period (days), a represents the asymptote, b is the time at 50 % of a, and c is a shape parameter (Rawlings et al., 1988). Staining and LTS were correlated with microbial biomass using linear regression.
In addition, decay of cotton strips or rate of staining, for soil type and farming system was calculated using the formula, LTS rate (kg day^{-1}) or increase in staining (% day^{-1}) = a*0.5/b, with a and b derived from the Weibull function for each farming systems under each soil type (Table 12.1). Data were statistically analysed using R (Team, 2003).

12.3 Results and discussion

12.3.1 CSA methods

The level of staining of cotton strips obtained by image analysis (Figure 12.2A) showed a non-linear response through time (Equation 1), with 50% of maximum staining occurring at about 4.4 days after burial when averaged across farming system and soil type. In the Tenosol, the organically farmed soil showed faster staining (2.20 % day^{-1}), and thus decomposition, than the conventional soil (0.88 % day^{-1}). This may be related to the carbon content of the Tenosol (1.97 % for organic soil and 1.36 % for conventional soil) (Table 12.1), as biological activity depends on amount of organic matter under specific conditions (Condron et al., 2000; Dumaresq and Greene, 2001; Shannon et al., 2002). In the Vertosol, the difference in rate of staining between the conventional soil and the organic soil was 0.39 % day^{-1} with the latter soil being higher. The lack of consistency between staining and soil carbon levels in the Vertosol treatments may be due to the shorter duration of the organic, management regime (7 years compared with 14 years in Tenosol) and the low intensity lucerne rotation practiced in the organic plots in the previous three years compared with the conventional block.
The interaction of farming systems and soil type in soil carbon levels indicated that farming system differences in Tenosols were greater than for differences in Vertosols. The overall maximum was found in the organic Tenosol and the minimum found in the conventional Tenosol, whereas the soil carbon levels in the Vertosol were intermediate between those extremes and were relatively similar. It is likely that this reduced the contrast between the LTS and staining by micro-organisms in the Vertosol treatments.

The overall rate of staining of strips was higher in the Tenosol than the Vertosol, irrespective of farming system. This is likely to be due to several reasons, including soil carbon, chemical fertility status, management practices and physical properties such as aggregate stability (Milne and Haynes, 2004; van Diepeningen et al., 2006).

The changes in LTS during incubation (Figure 12.2B) were also fitted to Equation 1. Fifty percent of LTS occurred in about 5.6 days. Rate of LTS in all four soil treatments is presented in Table 12.1. The LTS followed the same pattern as the degree of staining of the strips. In the conventional Vertosol, the rate of LTS was 6.9 kg day^{-1}, which is slightly lower than the organic Vertosol (7.4 kg day^{-1}). In a similar way, rate of LTS was higher in the organic Tenosol (8.5 kg day^{-1}) than in the conventional Tenosol which took 8.4 days to reach 50 % decomposition (or 4.0 kg day^{-1})

[insert Figure 12.2 near here]

12.3.2 Comparison of CSA and respirometer methods

Staining and LTS at day 12 were compared with microbial biomass over the same period of incubation using linear regression. Staining was weakly correlated (r^2 = 0.26) with soil microbial biomass measured using SIR. The tensometer method (LTS) and soil microbial biomass were better correlated (r^2 = 0.41) although too low for useful prediction of microbial biomass directly from LTS data alone. Previous attempts to correlate cotton strip decomposition with soil microbial parameters such as biomass (King et al., 1996; Pankhurst et al., 1995; Smith and Maw, 1988; Williamson, 1994) have shown that measurements of LTS can detect differences in microbial biomass among treatments which included different tillage practices, stubble management and crop rotation.

One reason for the weak correlation could be due to the difference in substrates for micro-organisms in the soil. The respirometer method provides glucose as substrate whereas cotton strips provide cellulose as a substrate. De Nobili et al. (2001) tested the effect of different substrates (including glucose and cellulose) on soil microbial activity and found that high molecular weight organic compounds with low solubility such as cellulose do not induce a 'trigger response' in promoting microbial activity, unlike simpler, highly soluble compounds such as glucose. They also note that stimulation of microbial activity will vary for different functional or taxonomic groups within the soil biomass.

It is well known that soil physico-chemical conditions regulate soil microbial activity (Hill et al., 1988). Farmers should employ the CSA at consistent times/seasons in the annual cycle of farm management as would be recommended for analysis of chemical properties of soil.

Other studies have compared results from CSA, used with a tensometer, and chemical and physiological methods for measuring microbial activity and all methods have been shown to detect differences between many different soil treatments. Two relevant studies were carried out in long-term experiments (around 10 years) in NSW (King et al., 1996) and South Australia (Pankhurst et al., 1995). Each study was carried out on similar soil types within each experiment, i.e. clay (King et al., 1996) and sandy loam (Pankhurst et al., 1995), and comparisons made at the same stage of the cropping and rotation cycle in each experiment. King et al. (1996) found general agreement between results obtained using CSA and microbial respiration measured by CO_2 evolution rate, in detecting differences in microbial activity between zero tillage and conventionally tilled sites where stubble had been burned for comparison. Pankhurst et al. (1995) considered both CSA and soil microbial biomass (fumigation-extraction method), to be potentially useful indicators of soil biological health. Changes in soil microbial populations due to both tillage and stubble management practices were detected by these two biological parameters. Penfold et al. (1995) measured soil chemical, physical and biological characteristics of soils in organic, biodynamic and conventional broad-acre farming systems in South Australia. They found no differences in microbial biomass and CSA data between these farming systems after six years due to the

large variability under field conditions; however, they did detect a decline in LTS and microbial biomass with increasing depth due to cooler soil temperatures.

12.3.3 Comparison of CSA methods

The relationship between the two CSA methods was examined using linear regression (Figure 12.3). The correlation over the entire 35 day incubation period was $r^2 = 0.81$. As LTS increased, staining of the strips also increased, due to increased colonisation of the strips by microbes. This colonisation could present itself as rounded colonies on strips and colonies were of different colours. The colonisation of the strips was not correlated with the physico-chemical properties of the soil such as soil organic matter (Table 12.1). The level of correlation between the two CSA methods indicates that the image analysis method is as well suited as the tensometer method for measuring microbial activity in the range of soils tested here. The correlation is higher for Tenosol (organic $r^2 = 0.95$ and conventional $r^2 = 0.90$) than the Vertosol (organic $r^2 = 0.78$ and conventional $r^2 = 0.88$).

[insert figure 12.3 near here]

Although the image analysis method is well correlated with the tensometer method, and while other authors have found similar CSA image analysis methods to be effective (Cox and Whelan, 1998), further refinements of this method can be suggested. Washing the strips may have removed some staining before scanning and a small error may have been introduced into this method here. On some cotton strips, microbial activity could be seen as pale coloured plaques. These plaques would not have increased the level of staining recorded, but the underlying microbial growth presumably would have contributed to LTS. Manual selection and exclusion of these regions is possible, and image analysis techniques could also be used to distinguish the extent of the pale growth on the strips.

Many image analysis techniques used in agriculture have shown good detection ability for weeds, leaves, soil and roots, but minor errors and some false detection rates have been reported due to poor image quality, inappropriate processing techniques or the lack of a sensitive tool (e.g. detection algorithms) for analysing of images (Alchanatis et al., 2005; Ducournau et al., 2004; Gregory et al., 2003; Ngouajio et al., 1999). Image pre-processing and segmentation may be similar when measuring a single characteristic of the whole image (i.e. luminosity) compared to measuring specific features such as area or counting objects, but image quantification is likely to simpler. The manual intervention in the method of Cox and Whelan (1998) increases the time required for image analysis and potentially creates a source of subjective human error in the process. The present method using intact strips prior to disintegration is quick and there is less chance of losing material from fragile fragmented strips.

There are also reported errors in CSA using tensometers where the tensile strength of cotton strip is increased by fungi (Latter et al., 1988) and the breakdown of cotton strips (cellulose) did not relate to specific soil biochemical processes such as nutrient cycling (Howard, 1988). Hence, both the methods of CSA quantification are subject to minor errors. CSA can be adopted for measuring overall biological activity by lay people (Correll et al., 1997; Tuckombil-Landcare, 2002), with scope for further refinement by scientists.

12.3.4 Comparison of costs and practicability of each type of measurement

The cost of measuring soil microbial activity using the three different methods was evaluated (Table 12.2). Time, labour and materials were calculated for the three methods. The two cotton strip methods were about 70% cheaper than the respirometer method used in this experiment. The costs involved in the tensometer method were $6.60 for breaking strips, $10 for labour and $1 for cotton strips, a total of $17.60. The costs for the image analysis method were $15; $14 for the labour charge for scanning and image analysis and $1 for cotton strips. The standard Shirley burial cloth would not be needed for the farmers to test their field activity as unbleached calico cloth could be used as a substitute. The latter is readily available

and 10 times cheaper than the Shirley cloth needed for use with a tensometer. Image correction, to remove the green blue guide strips would also be unnecessary. It is also possible to capture an image of the strips using a digital camera and analyse in the same way.
A number of qualitative criteria were used to compare the three methods (Table 12.2). The CSA image analysis method is feasible for general use compared to other two methods because of the lower level of skills required and greater accessibility (Table 12.2). The respirometer and the LTS methods are mainly suited to scientific researchers while the image analysis method is a better method for non-experts. The usefulness of the CSA, as a simple indicator of soil biological health, could be expanded if used in conjunction with other simply measured indicators of soil physical and chemical condition. An example of this is found in the Northern Rivers Soil Health Card produced by the Tuckombil Landcare group (2002) where land owners are encouraged to measure not only the CSA in a simplified version of Cox and Whelan's (1998) method, but also pH with a simple commercially available pH kit, soil slaking and dispersion tests as recommended in Cass et al. (1996), counting earthworms and several other soil tests which are easily carried out.

12.4 Conclusion

In conclusion, the strong relationship between the simple image analysis method and the LTS method for CSA indicates the value of the image analysis method for monitoring soil biological health by the general community such as land-care groups and organic farmers. It has been shown to be a cheap method, using equipment which is readily available outside laboratories and, while not strongly correlated in these experiments with more precise measurements of soil biological activity such as microbial biomass, it can detect gross trends in biological health in soil monitoring programs. As such, CSA staining measurement can be a valuable tool in conjunction with other simple indicators of soil physical and chemical health such as slaking and pH to monitor soil amelioration or rehabilitation programs.

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Table 12.1 Characteristics of the Vertosol and Tenosol soils from two vegetable production systems (organic and conventional) in south east Queensland, Australia.

Characteristics	Vertosol		Tenosol	
	organic	conventional	organic	conventional
Texture	clay loam	clay loam	sandy loam	sandy loam
pH	7.7	7.9	6.0	7.6
EC[1] (dS/m)	0.22	0.14	0.05	0.13
Total nitrogen (%)	0.18	0.19	0.22	0.15
Total carbon (%)	1.6	1.8	2.0	1.4
Microbial biomass[2]	16.0	23.3	21.9	10.2
Rate of staining (% day^{-1})	2.2	1.8	2.2	0.9
LTS rate (kg day^{-1})[3]	7.4	6.9	8.5	4.0

[1] Electrical conductivity, [2] mg microbial C/100g dry matter soil after incubation for 12 days, [3] Loss of tensile strength

Table 12.2.Cost of measurement, skill and equipment required for measuring soil microbial parameters using the three methods tested.

Criteria	Microbial biomass	CSA loss of tensile strength (tensometer)	CSA staining (scanning method)
Equipment	respirometer	tensometer	flat bed scanner + image analysis software
Accessibility	limited	limited	common
Practicability	in research	in research	general use
Skill required	high	medium	low
Reproducibility and repeatability	high	medium	medium
Cost ($ $sample^{-1}$)	55	17	15

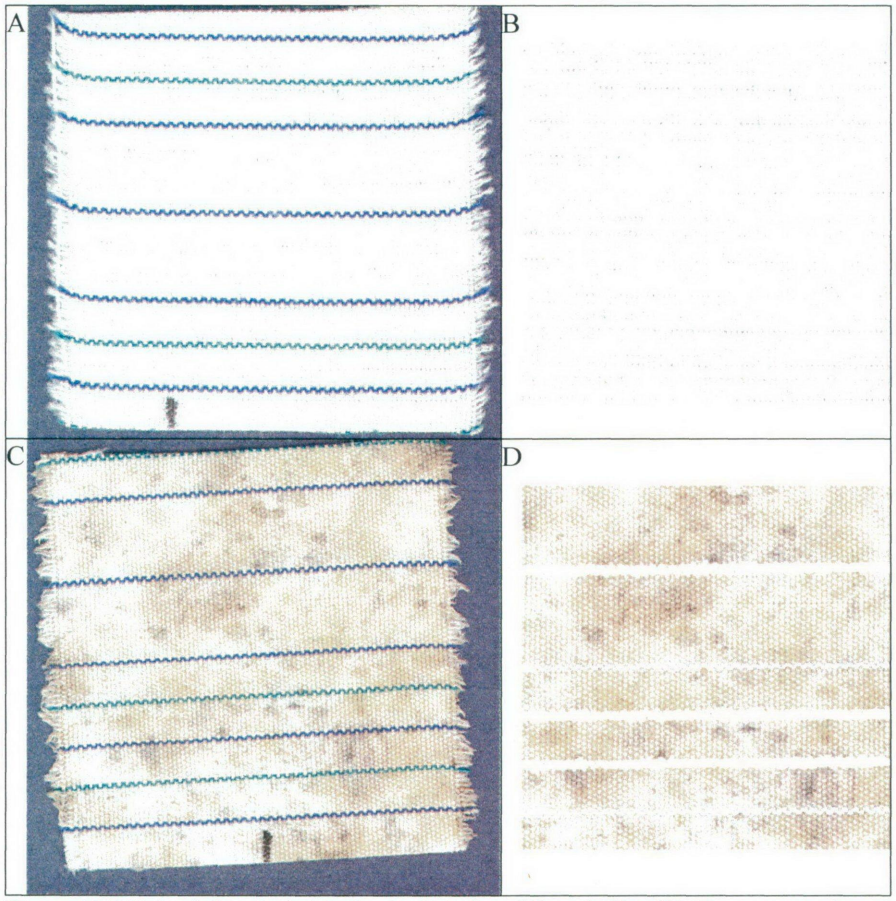
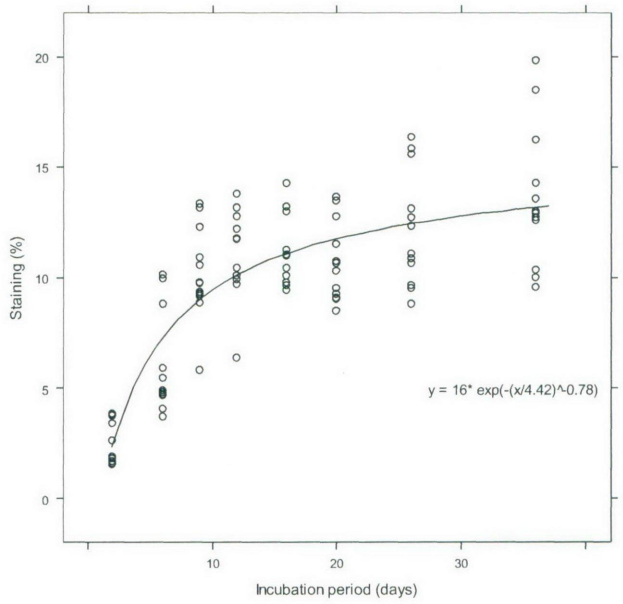


Figure 12.1 Unburied cotton strip (control) before (A) and after (B) image processing, and buried strip before (C) and after (D) image processing.

A



B

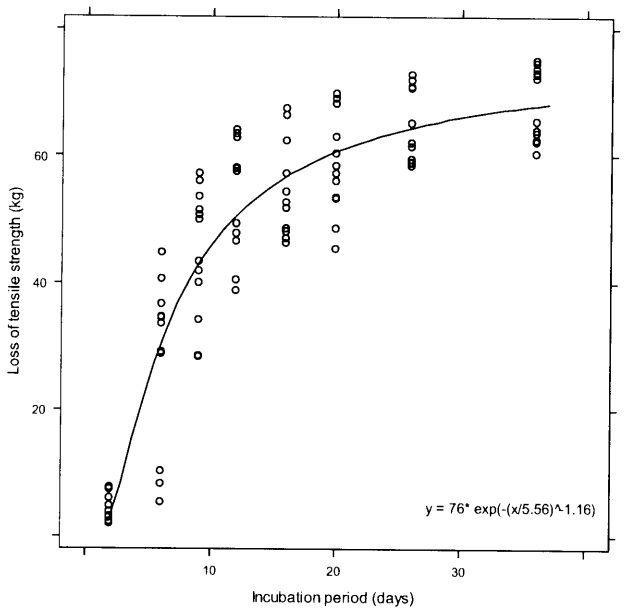


Figure 12.2 Changes in cotton strip staining (A) and loss of tensile strength (B) over a 35 day incubation period. The equation describes the fitted Weibull function (solid line) and the circles represent the individual data points.

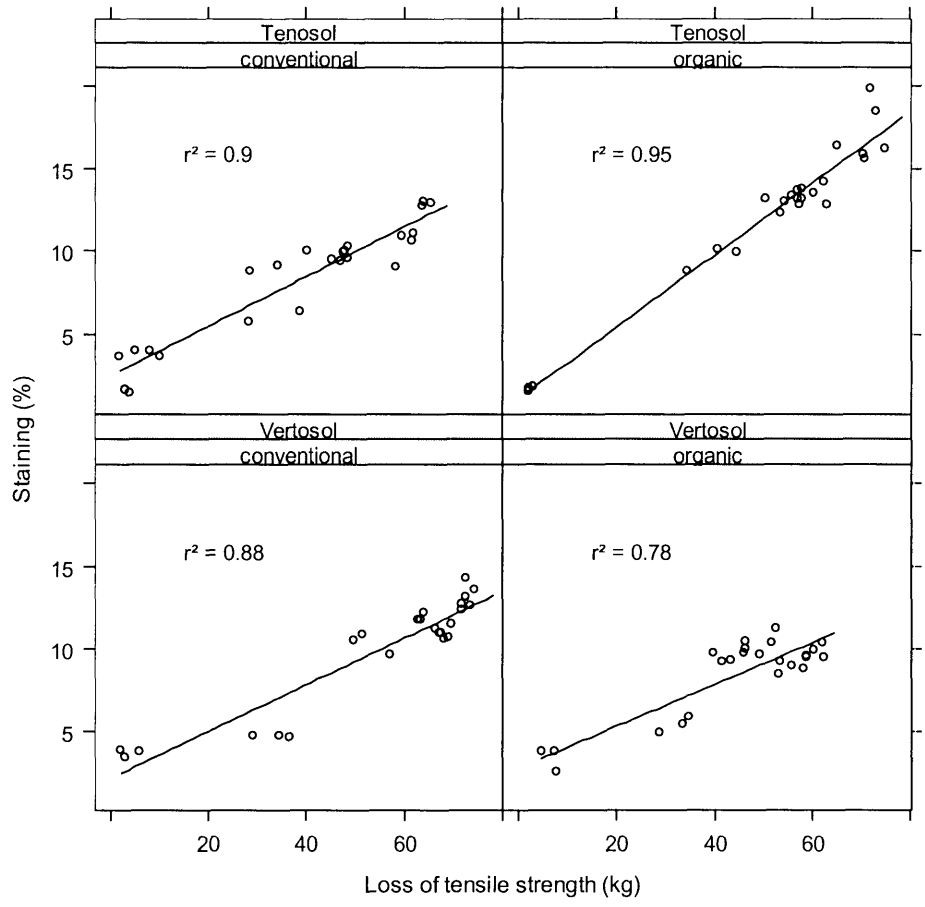


Figure 12.3 Correlation (r2) between staining and loss of tensile strength of cotton strips incubated in two soil types (Tenosol and Vertosol) from two vegetable production systems (conventional and organic). Measurements were recorded over a 35 day incubation period. The solid line represents the linear regression and the circles represent the individual data points.

Appendix 5 Paper published in conference proceedings of QLIF conference. Soil phosphorus status in organic and conventional vegetable farms in Southeast Queensland, Australia

Gunasekhar Nachimuthu[2], Paul Kristiansen[1], Peter Lockwood[1] and Chris Guppy[1]

Key words: organic, vegetable production, phosphorus, soil fertility, sustainability

Abstract

The soil phosphorus (P) status (0-10 cm) of two farming systems (organic (OF) and conventional (CF) vegetable farms) at two locations (Gatton and Stanthorpe) was examined amongst a suite of soil fertility indicators. The P status was similar between farming systems, in contrast to some broad-acre organic systems. Examination of farm management records revealed substantial overlap between P inputs at both localities with CF systems also receiving organic inputs, e.g. green manure and composts. A statistical analysis of the effects of different inputs also indicated that P fertility did not vary significantly between farms. Soil P levels were medium to high across farm types indicating a potential environmental risk for vegetable producers particularly in sandy well drained soils. The three methods of extraction Colwell, Olsen and Resin were well correlated with each other and produced similar results indicating the similar nutrient pools exist between farming system.

13.1 Introduction

Organic farming is considered an alternative to conventional farming, providing sustainable crops with high export demand and less environmental effect (Wood et al., 2006). In Australia, growth in organic production is estimated at 15-25% annually. Growth is expected to continue because of strong domestic demand and expanding markets overseas, especially in Asia (Alexandra and May, 2004). However, Australian organic growers face particular challenges due to infertile soils, high climatic variability and large distances between farms and input sources (Malcolm et al., 1996).

Soil health is a central tenet of organic agriculture and is critical to sustainable agriculture (Widmer et al., 2006). However, fertility management in Australia may not respond well to European organic methods. Of specific concern are the findings of Penfold (2000) and Ryan et al. (2004), who indicated that plant available P is a limiting factor in organic farming due to the low natural abundance of P and slow rate of release from organic-certified fertilisers. Organic farming may deplete soil P built up during conventional management (Gosling and Shepherd, 2005). But recent research in Australia has indicated a positive balance of P in organic vegetable production (Wells et al., 2000), suggesting that the

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problem of P limitations in organic production in Australia are restricted to specific farming systems (e.g. broad-acre enterprises) and specific bio-physical conditions (e.g. high pH soils).

A soil survey was conducted to determine whether the P limitations faced by broad-acre grain growers in southern and western Australia are also a problem for typical vegetable growers in the east of the continent. The survey also sought to identify the different management strategies adopted by vegetable farmers to manage soil fertility.

13.2 Materials and methods

Soil samples (0-10 cm) were collected from two different localities in south east Queensland, Gatton (Vertosols-medium clay) (27.5° S, 152° E, 94 m elevation) and Stanthorpe (Tenosols-sandy loam) (28.6° S, 152° E, 872 m elevation) from organic vegetable farms (OF) and neighbouring conventional farms (CF) on similar soils (Isbell, 1996). In February 2005, five OF and three CF were sampled at Gatton and four OF and four CF at Stanthorpe. Selected properties were resampled in 2006.

Soil samples were air-dried, and sieved (<2 mm) prior to analysis. Labile P was estimated using three common methods: Olsen (Olsen et al., 1954), Colwell (Colwell, 1963) and Resin (Guppy et al., 2000) in 2005. Correlations between the three methods were calculated.

All farmers completed a questionnaire about their farm practices in the last five years regarding the amount and type of green manure or cover crops (GM), bulky manure application (BM), compost application (CT) and synthetic fertiliser application (SF). Since the number of farms sampled was low and the quality of questionnaire responses was variable, the data were classified as "Yes" or "No" indicating if the management practice was used or not. Percentage of farms within each farming system receiving each input were calculated (Table 1).

The data were subjected to a two factor ANOVA using R programming (R Development Core Team, 2003) for each management variable with location as the other factor. Sampling date was not significant for all variables ($P > 0.05$), as was the interaction term ($P > 0.05$), hence the data was pooled across years.

13.3 Results

Soils from Gatton were alkaline (pH from 7.7 to 8.6) and Stanthorpe soils were acidic to neutral (pH from 5.5 to 7.6). No difference in P status was observed between the farming systems ($P > 0.05$) and locations ($P > 0.05$). Sixty and forty percent of CF in Gatton received compost and green manure respectively. Twenty nine, fifty seven and seventy one percent of CF in Stanthorpe received CT, GM and BM respectively (Table 1). Further analysis based on different types of fertilisers revealed no difference in soil P status ($P > 0.05$) irrespective of extraction method. The three methods of available P analysis were well correlated among

each other with R^2 value of 0.72, 0.84 and 0.85 for Colwell:Resin, Colwell:Olsen and Olsen:Resin respectively. The P levels were generally medium to high for vegetable production (Peverill et al., 1999) both in OF and CF.

13.4 Discussion

The similar soil P status between organic and conventional vegetable farms in two locations with contrasting soil types and climatic conditions indicates that organic vegetable farmers are not at a nutritional disadvantage compared to their conventional counterparts, as has been reported for some organic broad-acre cropping systems (Penfold, 2000; Ryan et al., 2004). A survey conducted by van Dienpeningen (2006) also reported no difference in P levels between organic and conventional farms in Netherlands. Also there are reports of positive P balance in different organic farming system such as vegetables in (New South Wales) Australia (yellow earth - *Luvic Ferrasol*) (Wells et al., 2000) and cereal legume crop rotations (*sandy clay loam soil*) in Italy (Marinari et al., 2006). Our survey confirms that many conventional growers include substantial inputs of organic fertilisers, so that the distinction between organic and conventional systems is less well defined than might be expected, a result reported earlier by van Diepeningen et al. (2006)

The medium to high P levels for both CF and OF (Peverill et al., 1999) suggests that all are high input production systems with potential for adverse environmental effects, such as nutrient leaching, especially in sandy soil (e.g. Stanthorpe) (Zhang and MacKenzie, 1997).

Table 13.1: Percentage of farms using particular fertilisation practices and soil phosphorus levels of organic and conventional vegetable farms in Queensland (means and standard errors are given with the range in brackets)

Farm	GM (%)	BM (%)	CT (%)	SF (%)	Colwell P (μg g^{-1})	Olsen P (μg g^{-1})	Resin P (μg g^{-1})
Gatton organic	100	100	100	0	104±14.3 (62-146)	48±5.7 (37-66)	107±10.8 (64-163)
Gatton conventional	40	0	60	100	151±18.9 (126-188)	66±6.3 (55-77)	134±11.6 (110-173)
Stanthorpe organic	67	67	50	0	137±71.4 (24-341)	51±24.3 (12-122)	99±32.5 (13-236)
Stanthorpe conventional	57	71	29	100	163±79.0 (40-395)	51±14.9 (20-92)	95±25.5 (20-183)

GM: green manure, BM: bulky manure, CT: compost, SF: synthetic fertiliser

The good correlation between the three extraction methods and the similar P levels between OF and CF measured by the three methods indicate that soil P pools in organically farmed soils were equivalent to those in conventionally managed soils (Watson et al., 2002).

13.5 Conclusions

Results of the soil survey of organic and conventional vegetables farms in two contrasting locations revealed that soil P was similar between farming systems, partly due to the high use of organic inputs in CF. This finding contrasts with reports of P deficiencies in other Australian organic farming systems. The medium to high P levels in CF and OF confirm that these are high input systems with the potential for adverse environmental effects. The three extraction methods were well correlated and demonstrated that different pools of soil P were similar across farming systems.

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