

NUTRITIONAL CHARACTERISATION OF THE RHIZOSPHERE OF SYMBIOTIC  
COWPEA AND MAIZE PLANTS IN DIFFERENT CROPPING SYSTEM

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Science Studies

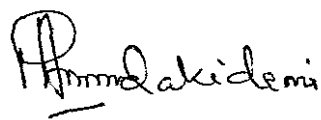
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**Supervisor:** Professor Felix D. Dakora

## Declaration

I declare that this thesis is my own work. It is being submitted for the Doctoral degree in Chemistry in the Department of Interdisciplinary Mathematics and Science Studies, Cape Peninsula University of Technology. It has not been submitted for any degree or examination at any other University.

Signature:

A. Akidemi

## Dedication

I dedicate this thesis to my parents who sent me to school and encouraged me to aim at higher education

## Abstract

A 2-factorial experiment, involving 3 levels of phosphorus (0, 40, and 80 kg P.ha<sup>-1</sup>) as main treatment and 4 cropping systems (mono crop, maize/cowpea inter-row, maize/cowpea intra-row, and maize/cowpea intra-hole cropping) as sub-treatment was conducted in the field for 2 consecutive years in 2003 and 2004 to assess i) the effects of exogenous P supply and cropping system on the concentrations of plant-available nutrients in the rhizosphere of cowpea and maize; ii) the effect of exogenous P supply on tissue concentrations of minerals in nodulated cowpea and maize in mixed plant cultures iii) the effects of exogenous P supply and cropping system on plant growth and N<sub>2</sub> fixation, and iv) the effects of exogenous P supply and cropping system on phosphatase activity and microbial biomass in the rhizosphere of cowpea and maize.

At harvest, it was found that applying 40 or 80 kg P.ha<sup>-1</sup> significantly increased cowpea grain yields by 59-65% in 2003 and 44-55% in 2004. With maize, the increases in grain yield were 20-37% in 2003 and 48-55% in 2004 relative to zero-P control. In both cropping seasons, the number of pod-bearing peduncles per plant, the number of pods per plant, the number of seeds per pod, and seed yield per cowpea plant were significantly increased with the application of exogenous P. In contrast, these parameters were all significantly depressed by mixed culture relative to mono crop cowpea. Intercropping maize with cowpea produced higher total yields per unit land area than the sole crop counterpart.

Supplying 40 or 80 kg P.ha<sup>-1</sup> to cowpea and maize significantly decreased the soil pH, increased soil organic matter and concentrations of extractable P, Ca, Mg, S, Na, Zn, and Fe in rhizosphere soil in 2003 and 2004. However, the concentrations of P, K, Ca, Mg, S, Zn, Cu, Mn, B, and Fe were significantly lower in the rhizosphere soil of cowpea in 2003 relative to maize, while in 2004 only P, K, Mg, Zn, Mn and Fe were decreased in the legume's rhizosphere compared with

the cereal. There was no effect of cropping system on the rhizosphere pH of cowpea and maize, except for *intra-hole* planted cowpea, where pH was higher relative to the other cropping systems.

In both Year 1 and 2, P application significantly increased the concentrations of P, Mg, Ca and K in shoots and pods of cowpea but decreased those of Zn, Cu and B. P supply also increased P, K, S and Na in roots, but decreased Zn, Cu, Mn and B in the two years of study. Similarly, P elevated nodule concentrations of P, Mg and S, but decreased those of Zn, Cu, Mn and Al in Year 1, just as nodule P and S increased in Year 2 with a decrease in Zn concentration. At whole-plant level, tissue concentrations of P, Ca and S rose with exogenous P supply, while those of Zn, Cu and B were decreased. There was also a significant increase in the concentrations of P, Ca, Mg, K, S, Na, Cu, Zn, Mn, Fe and Al in shoots, roots and whole plants of the (cowpea) legume relative to the (maize) cereal in the both years of study.

The cropping system also differentially affected nutrient concentrations and amounts in the test species. For example, shoot levels of P, K, Cu and B in cowpea were significantly greater in monoculture compared with mixed cultures in both years of experimentation. Furthermore, the co-planting of cowpea and maize in one hole increased shoot Fe (Year 1), Mg, Ca and B (Year 2), as well as whole-plant Fe and B (Year 1) compared with either monoculture or the other mixed cultures.

Exogenous supply of P to cowpea plants numerically, but not significantly, increased growth of all organs and whole plants in Year 1. However, in Year 2, shoots, pods, roots, nodules and whole-plants of cowpea were significantly increased with exogenous P application at either 40 kg or 80 kg P.ha<sup>-1</sup> relative to zero-P control. Applying mineral P to maize also significantly

increased growth of shoots and whole plants in both Year 1 and 2 relative to zero-P control. The dry matter yield of organs and whole plants of cowpea was numerically, but not significantly, decreased in mixed culture relative to monoculture in Year 1. However, in Year 2, biomass of shoots, pods, roots, nodules and whole plants were markedly decreased in mixed culture relative to monoculture. But with maize, plant growth was not affected by planting pattern in both Year 1 and 2.

Intercropping increased N concentrations in whole plants of maize relative to sole culture in Year 1. Cowpea was unaffected in Year 1. In Year 2, mixed culture, especially intra-hole planting, significantly increased N concentrations in roots and whole plants of cowpea relative to monoculture. Similarly, intercropping increased N concentrations in shoots and whole plants of maize relative to sole culture.

Isotope analysis showed that the  $\delta^{15}\text{N}$  values of intercropped cowpea shoots, pods, roots, nodules and whole plants were much lower when compared to those in monoculture. The mixed culture, especially intra-hole planting, also markedly decreased the  $\delta^{15}\text{N}$  values of shoots, roots and whole plants of maize relative to those in sole culture. As a result, the Ndfa of shoots, pods and whole cowpea plants were increased with intercropping relative to monoculture. Not only was the % Ndfa in shoots, pods, roots and whole cowpea plants increased by mixed culture relative to sole culture, but also the magnitude of this increase was greater in Year 2 compared to Year 1.

Applying exogenous P to the cropping system decreased the alkaline phosphatase activity in the rhizosphere soil of cowpea and maize in Year 1. The acid phosphatase activity was however not significant for both species in Year 1. The provision of P also decreased both acid and alkaline phosphatase activity in the rhizosphere of both cowpea and maize especially at the 80 kg P.ha<sup>-1</sup> in

Year 2. Although the cropping system had no effect on the acid and alkaline phosphatase activity in the rhizosphere of both cowpea and maize in Year 1, acid phosphatase activity in the rhizosphere of cowpea was significantly increased by mixed culture, especially in the *intra-hole* planting, relative to monoculture in Year 2. Alkaline phosphatase activity was also significantly higher in the rhizosphere of inter-row planted cowpea relative to monoculture.

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CHAPTER 1  
GENERAL INTRODUCTION AND LITERATURE REVIEW

## 1.1 Introduction

Mixed culture (or intercropping) of legumes and cereals is an old practice in tropical agriculture that dates back to ancient civilization. The main objective of intercropping has been to maximise use of resources such as space, light and nutrients (Willey, 1990; Morris and Garrity, 1993; Lie *et al.*, 2003b), as well as to improve crop quality and quantity (Nel, 1975; Izaurrealde *et al.*, 1990; Mpairwe *et al.*, 2002). Other benefits include water quality control through minimal use of inorganic nitrogen fertilisers that pollute the environment (Crew and Peoples, 2004). The current trend in global agriculture is to search for highly productive, sustainable and environmentally friendly cropping systems (Crew and Peoples, 2004). This has resulted into renewed interest in cropping systems research (Vandermeer, 1989).

When two crops are planted together, interspecific competition or facilitation between plants may occur (Vandermeer, 1989; Zhang *et al.*, 2003). For example, studies have shown that mixtures of cereals and legumes produce higher grain yields than either crop grown alone (Mead and Willey 1980; Horwith, 1984; Tariah and Wahua, 1985; Ofori and Stern, 1987a; Lawson and Kang, 1990; Watiki *et al.*, 1993; Peter and Runge-Metzger, 1994; Skovgard and Pats, 1999; Rao and Mathuva, 2000; Olufemi *et al.*, 2001; Mpairwe *et al.*, 2002; Dapaah *et al.*, 2003). In such crop mixtures, the yield increases were not only due to improved nitrogen nutrition of the cereal component, but also to other unknown causes (Nel, 1975; Connolly *et al.*, 2001).

Many of the unknown and less researched processes occur in the rhizosphere of mixtures (Connolly *et al.*, 2001; Zhang *et al.*, 2003 & 2004). The rhizosphere soil is the narrow zone of soil surrounding the roots where soil, micro-organisms and roots jointly play key roles in the ecosystem. Compared with the bulk soil, the rhizosphere has different biological, physical and chemical soil properties. It is rich in root exudates, and, therefore, play a major role in nutrient

mobilisation and microbial activities (Dakora and Phillips, 2002; Dakora, 2003). So far however, little attention has been paid to rhizosphere effects on crops grown in mixtures (Connolly *et al.*, 2001; Zhang *et al.*, 2003; 2004), where interaction between different organisms is maximal.

The major management practices employed in mixed cultures to attain good yield includes the enhancement of microclimatic conditions, improved utilisation and recycling of soil nutrients, improved soil quality, provision of favourable habitats for plants and stabilisation of soil, among others (Juma *et al.*, 1997). These conditions are achieved by manipulating management practices such as planting patterns of the mixtures.

Although monocultural systems involving cereals and legumes are well researched many of the complex mixed systems such as those practised by farmers in Africa have received little attention. For example, many planting patterns for maize and cowpea exist in Africa whose belowground interactions have received little research attention and hence their ecology still explored (Connolly *et al.*, 2001).

Intercropping systems are deliberately designed to optimise the use of spatial, temporal, and physical resources both above- and belowground, by maximising positive interactions (facilitation) and minimising negative ones (competition) among the components (Willey and Osiru, 1972; Willey, 1979; Mead and Willey, 1980; Horwith, 1985; Ofori and Stern, 1986, 1987a&b; Jose *et al.*, 2000; Silwana and Lucas, 2002). An understanding of the biological and chemical processes and mechanisms involved in the allocation of resources in such systems is essential. The complex interactions in cropping systems such as those used by traditional farmers have received little research attention (Connolly *et al.*, 2001; Zhang *et al.*, 2004) because quantitative rhizosphere studies in the field involving complex mixtures are notoriously difficult

and cumbersome. Information from such studies is likely to provide an understanding of plant survival strategies when subjected to stress in mixtures.

## **1.2 Interactions between plants in mixtures**

Plant-to-plant interactions can occur in the above- or below-ground plant compartments. Interactions will occur in the growth process, especially when the component species are exploiting growth resources above- and below-ground (Vandermer, 1989; Willey, 1990; Ong *et al.*, 1996) from the same location or at the same time. In crop mixtures, any species utilizing the same combination of resources will be in direct competition. However, based on differences in phenological characteristics of species in mixtures, the interaction among them may lead to an increased capture of a limiting growth resource (Willey and Osiru, 1972; Willey, 1979; Mead and Willey, 1980; Horwith, 1985; Ofori and Stern, 1986, 1987a&b; Silwana and Lucas, 2002) and then accrue greater total yield than the cumulative production of those species if they were grown separately on an equivalent land area (Mead and Willey, 1980; Horwith, 1984; Tariah and Wahua, 1985; Ofori and Stern, 1987a; Lawson and Kang, 1990; Watiki *et al.*, 1993; Peter and Runge-Metzger, 1994; Myaka, 1995; Asafu-Agyei *et al.*, 1997; Skovgard and Pats, 1999; Rao and Mathuva, 2000; Olufemi *et al.*, 2001; Dapaah *et al.*, 2003). Thus, mixed culture systems between cereals and legumes may experience a complex series of inter- and intra-specific interaction (Izaurrealde *et al.*, 1990; Giller and Cadisch, 1995; Evans *et al.*, 2001; Li *et al.*, 2003c) guided by modifications and utilisation of light, water, nutrients and enzymes. More studies are needed to quantify such interactions in different cowpea/maize mixtures such as those used by farmers in Africa.

### 1.3 Rhizosphere interaction in legume cereal mixtures

Most annual crop mixtures such as those involving cereals and legumes are grown almost at the same period, and develop root systems that explore the same soil zone for resources (Horwith, 1984; Chang and Shibles, 1985a & b, Reddy et al., 1994; Jensen *et al.*, 2003). Under such conditions, below-ground competition for resources such as nutrients is most likely to occur. For example, research has shown that activities in mixed cropping systems involving maize and cowpea occur between the top 30 – 45 cm of soil, and their density decreased with depth (Maurya and Lal, 1981; McIntyre *et al.*, 1997). Because of these interactions, cowpea yields can be reduced significantly relative to that of maize (Watiki *et al.*, 1993). In contrast to some negative effects on yield, root systems in mixtures may provide some of the major favourable effects on soil and plants. These include, amongst others, carbon enrichment through carbon turnover (Ridder *et al.*, 1990; Vanlauwe *et al.*, 1997), release of phenolics, phytosiderophores and carboxylic acids as root exudates by component plants (Dakora and Phillips, 2002; Dakora, 2003). These molecules play a major role in the mineral nutrition of plants. For instance, some studies have shown that, in P-deficient soils, pigeonpea roots use piscidic, malonic, and oxalic acids to solubilise Fe-, Ca- and Al-bound P (Ae *et al.*, 1990). Once mobilised, P and Fe then become available for uptake by the pigeonpea plant as well as by other associated plant species and microflora in the cropping system.

In Al toxic soils, oxalate released by buckwheat roots forms an Al-oxalate complex that renders the Al non-toxic to plants and mutualistic microbes in the cropping system (Ma *et al.*, 1998). In that way, productivity of the cultural system is enhanced. Whether similar processes take place in maize-cowpea mixtures, and the extent to which they affect the below ground activities, need to be established. This is due to the fact that, thus far, research efforts on mixed cultures has centred on the intra- and inter-specific competition for light and water, and research reports on

competition for nutrients in maize and cowpea mixtures are limited (Connolly *et al.*, 2001; Zhang *et al.*, 2003; 2004). It is, therefore, of greater importance to explore how the rhizosphere systems of the associated plant species in mixtures interact under different maize-cowpea cropping systems.

#### **1.4 Rhizospheric pH changes in different management systems in legume/cereal mixtures**

Many plants have the ability to modify the pH of their rhizosphere (Hoffland *et al.*, 1989, 1992; Raven *et al.*, 1990; Degenhardt *et al.*, 1998; Muofhe and Dakora, 2000; Dakora and Phillips, 2002) and enhance nutrient availability such as P, K, Ca, and Mg, which are otherwise fixed in unavailable forms (Vandermeer, 1989; Hauggaard-Nielson and Jensen, 2005). For instance, legumes induce several reactions that modify the rhizosphere pH (Jarvis and Robson, 1983; McLay *et al.*, 1997; Tang *et al.*, 1998, 2001) and affect nutrient uptake (Brady, 1990; Vizzatto *et al.*, 1999). For example, Dakora *et al.* (2000) have shown that due to pH changes in the rhizosphere, *Cyclopia genistoides*, a tea-producing legume indigenous to South Africa, increased nutrient availability in its rhizosphere by 45–120% for P, 108–161% for K, 120–148% for Ca, 127–225% for Mg and 117–250% for boron (B) compared with bulk non-rhizosphere soil. Hence, legumes may take up higher amounts of base cations, and in the process of balancing internal charge, release  $H^+$  ions into the rhizosphere that results in soil acidification (Jarvis and Robson, 1983; McLay *et al.*, 1997; Tang *et al.*, 1998, 2001; Sas *et al.*, 2001; Dakora and Phillips, 2002; Cheng *et al.*, 2004). Other legumes such as alfalfa, chickpea, lupins, and cowpea can release considerable amounts of organic anions and lower their rhizosphere pH (Liptone *et al.*, 1987; Dinkelaker *et al.*, 1989 & 1995; Braum and Helmke, 1995; Gilbert *et al.*, 1999; Neumann *et al.*, 1999; Rao *et al.*, 2002; Li *et al.*, 2004b), a condition conducive for the hydrolysis of organic P and hence improving P nutrition for plants and microorganism in the soil. In the same context, white lupin (*Lupinus albus*) exuded organic acids anions and protons that lowered

rhizosphere pH and recovered considerable amounts of P from the soil and made them more available to wheat than when it was grown in a monoculture (Horst and Waschkes, 1987; Kamh *et al.*, 1999). Similarly, pigeonpea increased P uptake of the intercropped sorghum by exuding piscidic acid anions that chelated  $\text{Fe}^{3+}$  and subsequently released P from  $\text{FePO}_4$  (Ae *et al.*, 1990). In a field experiment, faba bean facilitated P uptake by maize (Zhang *et al.*, 2001; Li *et al.*, 1999 & 2003b; Zhang and Li, 2003). In another comparative study, the ability of chickpea to mobilise organic P was shown to be greater than that of maize due to greater exudation of protons and organic acids by chickpea relative to maize (Li *et al.*, 2004a).

Thus, in mixed cultures, plants such as maize, which do not have strong rhizosphere acidification capacity can benefit directly from nutrients solubilised by legume root exudates. What is, however, not clearly known is the extent of rhizosphere pH changes in mixed cultures involving nodulated cowpea and maize and their influence on other biological and chemical processes in the soil.

### **1.5 Changes in rhizosphere mineral concentration with P supply**

In most agricultural ecosystems, P is the second most limiting plant nutrient after N. Phosphorus deficiency is more critical because only limited quantities mined are returned to the system in crop residues. As a result, extreme phosphorus deficiencies are quite common especially in the tropics (Buresh *et al.*, 1997), where no supplementary sources of this element are applied to soils. Therefore, P supply to crops such as cowpea and maize is reported to increase their rhizosphere concentration, plant growth and yield in deficient soils (Wahua, 1983; Chang and Shibles, 1985a&b; Muleba 1999; Buerkert *et al.*, 2001; Carsky, 2003; Jensen *et al.* 2003). The supply of P to plants from external sources is, therefore, important in reducing the competition that may occur in plants sown in monoculture or mixtures to meet their internal P requirements.



However, when P fertilisers are supplied to replenish soil fertility, about 70–90% of the P fertilisers are adsorbed and retained in soil in various P compounds, some of which may not be available to plants (Kamprath, 1967; Yost *et al.*, 1979). Some plants have evolved mechanisms of acquiring such adsorbed minerals. It is well established that, under conditions of inadequate P supply or during P stress in the soil, plant roots release a variety of organic acid anions into the rhizosphere (Lipton *et al.*, 1987; Hoffland *et al.*, 1992; Jones and Darrah, 1995). Bolan *et al.* (1990) reported that organic acids increase soil P availability by decreasing adsorption of P and increasing dissolution of relatively insoluble P compounds. Other reports (Dinkelaker *et al.*, 1995) suggest that root-released citrate increases the availability of mineral-bound P by solubilising Ca, Fe, and Al phosphates. The amount of exuded citrate may even lead to precipitation of Ca citrate on the root surface (Dinkelaker *et al.*, 1989). It is possible that with adequate P supply, such acid molecules will not be released in larger quantities into the rhizosphere. In some cases, it has been reported that P applications changed soil pH, increased cation exchange capacity and organic matter content of the soil (Sanchez and Uehara, 1980), conditions that may favour the accumulation of some mineral nutrients such as Ca, Mg, Zn, Mn and Fe (Li *et al.*, 2004b). In some cases, P supply can lead to a decrease in soil pH (Lambers *et al.*, 2002; Li *et al.*, 2004b) and an increase in the availability of some nutrients in soil, particularly Al, Fe, Mn, and Zn (Römheld and Marchner, 1986; Marshner, 1995). Added P may also form some complexes with other minerals and render them unavailable to plants. Such complexes may involve Zn, Cu, Mn, Fe and Ca (Handreck, 1991; Zhu *et al.*, 2001 & 2002).

Generally, adequate P nutrition is reported to enhance the uptake of other nutrients through improved overall plant growth, especially root development (Reinbott and Blevins, 1997). It has, however, been shown that increasing soil P availability and uptake can result in lower levels of

available Zn in plants (Lindsay *et al.*, 1962; Christensen and Jackson, 1981; Singh *et al.*, 1988; Gianquinto *et al.*, 2000).

### **1.6 N<sub>2</sub> fixation in legume/cereal mixtures**

Biological nitrogen fixation by grain legume crops has received a lot of attention (Eaglesham *et al.*, 1981; Giller *et al.*, 1991; Izaurralde *et al.*, 1992; Giller and Cadisch, 1995; Peoples *et al.*, 2002) because it is a significant N source in agricultural ecosystems (Heichel, 1987; Dakora and Keya, 1997). However, studies on N<sub>2</sub> fixation in complex cereal/legume mixtures are few (Stern, 1993; Peoples *et al.*, 2002). Intercropping usually includes a legume which fixes N<sub>2</sub> that benefits the system, and a cereal component that depends heavily on nitrogen for maximum yield (Ofori and Stern, 1986; Cochran and Schlentner, 1995). Controlled studies have shown a significant direct transfer of fixed-N to the associated non-legume species (Eaglesham *et al.*, 1981; Giller *et al.*, 1991; Frey and Schüepp, 1993; Stern, 1993; Elgersma *et al.*, 2000; Høgh-Jensen and Schjoerring, 2000; Chu *et al.*, 2004). There is evidence that the mineralisation of decomposing legume roots in the soil can increase N availability to the associated crop (Dubach and Russelle, 1994; Schroth *et al.*, 1995; Evans *et al.*, 2001). In mixed cultures, where row arrangements and the distance of the legume from the cereal are far, nitrogen transfer could decrease. Research has shown that competition between cereals and legumes for nitrogen may in turn stimulate N<sub>2</sub> fixation activity in the legumes (Fujita *et al.*, 1990; Hardarson and Atkins, 2003). The cereal component effectively drains the soil of N, forcing the legume to fix more N<sub>2</sub>.

### **1.7 Soil microbial biomass and phosphatase activity in legume/cereal mixtures**

The microbial biomass is influenced by biological, chemical, and physical properties of the plant-soil system. Generally, soil and plant management practices may have greater influence on the level of soil microbial C (Gupta and Germida, 1988; Dick *et al.*, 1994&1997; Alvey *et al.*, 2003).

For instance, soil microbial C tend to show the highest values in cropland and grassland soils and the lowest in bare cultivated soils (Brookes *et al.*, 1984; Gupta and Germida, 1988). Monocultural systems are expected to contain reduced amounts of microbial biomass and activities in comparison to those in mixed cultures (Moore *et al.*, 2000). Studies have indicated that legumes accumulated greater amounts of soil microbial C in the soil than cereals (Walker *et al.*, 2003). This is attributed to lower C:N ratio of legume than that of cereal (Uriyo *et al.*, 1979; Brady, 1990).

Microbial biomass and enzyme activities could increase after the addition of an energy source. The stimulation of soil microbial biomass and enzyme activity by organic amendments is higher than that induced by organic fertilisers (Bolton *et al.*, 1985; Goyal *et al.*, 1993; Höflich *et al.*, 2000). Soil organic matter content and soil microbial activities, vital for the nutrient turnover and long term productivity of soil, are enhanced by the balanced application of nutrient and/or organic matter/manure (Bolton *et al.*, 1985; Guan, 1989; Goyal *et al.*, 1993; Höflich *et al.*, 2000; Kanchikerimath and Singh, 2001). Under conditions of adequate nutrient supply such as P, the microbial biomass C will be increased due to improved plant growth and increased turnover of organic matter in the soil (Bolton *et al.*, 1985). Whether the management practices in mixed cultures involving cowpea and maize may favour the stimulation of biological soil activity and, thus, result in a higher turnover of organic substrates in the soil that are utilised by micro-organisms is a good subject to be investigated.

Although there is a lot of information that show the relationship between soil management and soil microbial activity, little is known about these effects under mixed cultures such as those practised by farmers in the tropical/subtropical environments (Dick, 1984; Dick *et al.*, 1988; Deng and Tabatabai, 1996). In this context, the measurement of their activities could provide

useful information concerning soil health, and also serve as a good index of biological status in different crop management systems.

Plants have evolved many morphological and enzymatic adaptations to tolerate low phosphate availability. This includes transcription activity of acid phosphatases, which tends to increase under P starvation (Tarafdar and Jungk, 1987; Goldstein, 1992; Duff *et al.*, 1994; del Pozo *et al.*, 1999; Haran *et al.*, 2000; Baldwin *et al.*, 2001; Miller *et al.*, 2001; Li *et al.*, 2002). Phosphatase enzymes in the soil serve several important functions, and are good indicators of soil fertility (Dick and Tabatabai 1992; Eivazi and Tabatabai 1997; Dick *et al.*, 2000). Under conditions of P deficiency, acid phosphatase secreted from roots is increased (Nakas *et al.*, 1987; Chrost, 1991; Hays *et al.*, 1999; Li *et al.*, 1997). Gilbert *et al.* (1999) found that white lupin roots from P-deficient plants had significantly greater acid phosphatase activity in both the root extracts and the root exudates than comparable samples from P-sufficient plants. At different stress levels, these enzymes release phosphate from both cellular (Bariola *et al.*, 1994) and extracellular (Duff *et al.*, 1994) organic compounds. The transcripts and activity of phosphate transporters are increased to optimise uptake and remobilisation of phosphate in P-deficient plants (Muchhal *et al.*, 1996; Daram *et al.*, 1999; Kai *et al.*, 2002; Karthikeyan *et al.*, 2002; Mudge *et al.*, 2002; Versaw and Harrison, 2002). It is thought that these morphological and enzymatic responses to P starvation are coordinated by both general stress-related and P-specific signalling systems.

The amount of acid phosphatase secreted by plants is genetically controlled, and differs with crop species and varieties (Izaguirre-Mayoral and Carballo, 2002) as well as crop management practices (Patra *et al.*, 1990; Staddon *et al.*, 1998; Wright and Reddy, 2001). Some studies have shown that the amount of enzymes secreted by legumes were 72 % higher than those from cereals

(Yadav and Tarafdar, 2001). Li *et al.* (2004) found that, chickpea roots were also able to secrete greater amounts of acid phosphatase than maize. The soil microbial biomass and the activity of acid phosphatases are expected to be higher in biologically managed systems because of higher quantity of organic C found in those systems. In fact, the activity of acid and alkaline phosphatase were found to correlate with organic matter in various studies (Guan 1989; Jordan and Kremer, 1994; Aon and Colaneri, 2001).

It is, therefore, anticipated that management practices in mixed cultures that induce P stress in the rhizosphere, may also affect the secretion of these enzymes. To date, there have been few studies examining the influence of cropping system on the phosphatase activity in the rhizosphere of cowpea and maize. Understanding the dynamics of enzyme activities in these systems is crucial for predicting their interactions as in turn their activities may regulate nutrient uptake and plant growth in the ecosystem.

Thus, the overall objective of the study was to examine the influence of P supply and planting patterns on the performance of cowpea and maize. The specific objectives were:

1. to assess a maize/cowpea cropping system in relation to the effects of exogenous P supply and different planting patterns on yield components of nodulated cowpea and maize in the Western Cape of South Africa.
2. to examine the effects of i) exogenous P supply, ii) planting system, and iii) plant species on the concentrations and uptake rates of plant-available nutrients in the rhizosphere of cowpea and maize.

3. to assess the effect of exogenous P supply on tissue concentrations of minerals in nodulated cowpea and maize in mixed plant cultures.
4. to assess the effect of exogenous P supply and cropping system on the accumulation and partitioning of both major and minor nutrients to organs of nodulated cowpea and maize in sole and mixed cultures.
5. to assess the effects of exogenous P supply and cropping system on plant growth and N<sub>2</sub> fixation in intercropped cowpea with maize in the Western Cape Province of South Africa.
6. to examine the effects of exogenous P supply and cropping system on acid and alkaline phosphatase activity in the rhizosphere of cowpea and maize.
7. to assess the effect of P supply and cropping system on the size of soil microbial C in the rhizosphere soil of cowpea and maize.

## CHAPTER 2

YIELD COMPONENTS OF NODULATED COWPEA (*VIGNA UNGUICULATA* L. WALP.)  
AND MAIZE (*ZEA MAYS* L.) PLANTS GROWN WITH EXOGENOUS P IN DIFFERENT  
CROPPING SYSTEMS

## 2.1 Introduction

Maize and cowpea are major food crops grown by small-scale farmers in many parts of Africa, including South Africa (Ayisi *et al.*, 2000). Their production is, however, limited by low concentrations of soil nutrients, especially phosphorus (P) (Smaling *et al.*, 1997; Sanchez, 2002). Additionally, variation in yields of both legume and cereals is also associated with poor agronomic practices in cropping systems (Chang and Shibles, 1985b; Tariah and Wahua, 1985; Ofori and Stern 1986, 1987a&b). Symbiotic legumes have a high requirement for P (Israel, 1987), which is known to stimulate root and shoot growth in plants as well as influence the efficiency of the rhizobium-legume symbiosis through facilitation of energy transfer reactions involving ATP in nitrogenase activity. Studies have shown that the provision of P to cowpea and maize increased plant growth and grain yield relative to unfertilised control (Wahua, 1983; Chang and Shibles, 1985a&b; Buerkert *et al.*, 2001; Carsky, 2003). There is also ample evidence (Mead and Willey, 1980; Horwith, 1984; Tariah and Wahua, 1985; Ofori and Stern, 1987a; Lawson and Kang, 1990; Watiki *et al.*, 1993; Peter and Runge-Metzger, 1994; Skovgard and Pats, 1999; Rao and Mathuva, 2000; Olufemi *et al.*, 2001; Dapaah *et al.*, 2003) to show that in intercropping the legume/cereal combinations do, on average, yield better than monocultures through the concept of Land Equivalent Ratio (LER).

The spatial arrangement of the legume crop is one of the most important management factors determining the advantages of an intercropping system. As a result, some improved and profitable sole and intercropping practices have been developed for crops such as cowpea and maize (Chang and Shibles, 1985b; Tariah and Wahua, 1985; Ofori and Stern, 1987a; Olasantam, 1988; Watiki *et al.*, 1993; Myaka, 1995; Sing *et al.*, 2003). However, most African farmers still seem to prefer their traditional system (Henriet *et al.*, 1997, van Ek *et al.*, 1997) of planting



intercrops randomly without any defined rows, planting legumes in-between two maize plants along the same row, or planting maize and cowpea in the same hole (Fawusi *et al.*, 1982). Often, the consequence is low plant density and low grain yields.

Both traditional and conventional cropping strategies have always involved varying the component crop species in time and/or space in order to enhance crop productivity and its sustainability. On research stations, the spatial arrangements and plant densities of the component species are generally manipulated to enhance complementarity and reduce inter-species competition in order to maximise agronomic and physiological advantages (Willey and Osiru, 1972; Willey, 1979; Mead and Willey, 1980; Horwith, 1985; Ofori and Stern, 1986, 1987a&b; Silwana and Lucas, 2002). For example, the arrangement of component plant species within and in-between rows generally aims to improve transmission of photosynthetic radiation to the lower legume canopy, so as to enhance plant growth and yield efficiency of the legume (Ofori and Stern, 1987a). In a maize-cowpea intercrop, Myaka (1995) showed that cowpea yield was 57% higher in a 2-to-2 compared with 1-to-1 maize: cowpea rows. In a separate study, Asafu-Agyei *et al.* (1997) also found that a 2-to-2 maize: cowpea rows gave greater yields of maize and cowpea, as well as higher LER and net benefit than 1-to-1 maize cowpea rows.

Although smallholder farmers often intercrop maize and cowpea in the same hole, or along the same row as opposed to alternate row intercropping for reasons such as perceived efficiency in the use of land and labour, as well as ease of crop management, growth performance and grain yield data are scanty on these practices.

This study assesses a maize/cowpea cropping system in relation to the effects of exogenous P supply and different planting patterns on yield components of nodulated cowpea and maize in the Western Cape Province of South Africa.

## **2.2 Materials and Methods**

### **2.2.1 Site location and description**

Field experiments were conducted at the Agricultural Research Council Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa, during the 2003 and 2004 summer seasons. The site lies in the winter rainfall region of South Africa at an elevation of 146 m above sea level. The mean annual rainfall on the farm is 713.4 mm and mean annual temperatures range from 22.6 °C at day to 11.6 °C at night.

The experimental site in 2003 had a previous history of grape cultivation, whereas in 2004 it was under grass fallow. The soil type was sandy loam (Glenrosa, Hutton form), which according to the Soil Classification Working Group (SCWG) is equivalent to skeletal leptosol (SCWG, 1991). Following land preparation, but prior to planting, soil samples were collected and analysed for nutrients.

### **2.2.2 Experimental design**

The experimental treatments consisted of 3 P levels (0, 40, and 80 kg P.ha<sup>-1</sup>) and 4 cropping systems (namely, mono crop, maize/cowpea inter-row, maize/cowpea intra-row, and maize/cowpea intra-hole cropping). The experimental layout followed a split-plot design with P levels as the main plots, and cropping system as the subplots. There were 4 replicates per treatment; and the plots measured 4.5 m x 3.2 m. All maize plots had inter-row spacing of 90 cm,

and intra-row spacing of 40 cm, giving a density of 55,555 plants per hectare. Sole cowpea was sown with inter-row spacing of 60 cm, and intra-row spacing of 20 cm to produce plant density of 166,666 per hectare. The within-row spacing of cowpea in the maize/cowpea inter-row cropping system was 20 cm, resulting in cowpea density of 111,111 plants per hectare. The maize/cowpea intra-row planting distance was also 20 cm, giving a density of 55,555 plants per hectare (identical to that of maize). The intra-hole planting produced a plant density of 55,555 per hectare. The intra-row and intra-hole planting mimicked the practice of traditional smallholder farmers in Africa (Fawusi *et al.* 1982). Planting was done after ploughing, harrowing, and P application to the respective plots. A local maize variety and farmer-selected cowpea variety (Bengpilaa) were used. Three seeds were planted per hole for each species, and later thinned to 2 at 2 weeks after planting. The rhizobial inoculant used in this study was peat-based *Bradyrhizobium* strain CB756, which was applied at the rate of  $10^9$  cells.g<sup>-1</sup> of inoculant. Weeding was done manually with a hoe at 3 and 8 weeks after planting.

### **2.2.3 Plant harvesting and analysis**

At physiological maturity, the plants in the two middle rows of each plot were counted and harvested for assessing grain yield. The border plants within each row were excluded. For cowpea, 10 plants were sub-sampled from each plot to determine the number of pod-bearing peduncles, number of pods per plant and number of seeds per pod. Both cobs and pods were manually threshed and allowed to dry to 13% moisture content. Grain yield was determined for each plot and weight of 100 seeds recorded for each replicate.

#### 2.2.4 Land equivalent ratio

The grain yield of maize and cowpea were used to calculate land equivalent ratio defined as the relative land area that is required when growing sole crop to produce the yield achieved in intercropping (Willey, 1979). According to Mead and Willey (1980) the LER is calculated as:

$$LER = (Y_{ij}/Y_{ii}) + (Y_{ji}/Y_{jj}) \quad (1)$$

Where  $Y$  is the yield per unit area,  $Y_{ii}$  and  $Y_{jj}$  are sole crop yields of the component crops  $i$  and  $j$ , and  $Y_{ij}$  and  $Y_{ji}$  are intercrop yields.

#### 2.2.5 Statistical analysis

Mean replicate values of yield components were analysed statistically using a 2-factorial analysis of variance (ANOVA). These computations were done using the software of STATISTICA programme 1997. Fisher's least significant difference was used to compare treatment means at  $P \leq 0.05$  level of significance (Steel and Torrie, 1980).

### 2.3 Results

#### 2.3.1 Effect of P supply on cowpea yield and yield components

There was a significant response in the yield components of cowpea to exogenous P supply. In both 2003 and 2004, the number of pod-bearing peduncles per plant and the number of pods per plant increased significantly with P application relative to control (Tables 2.1 and 2.2). The grain yield of cowpea, whether measured on individual plant basis or per hectare, also increased with P supply in both cropping seasons (Tables 2.1 and 2.2). On a per-plant basis, cowpea yield was 59-65% greater than control in 2003 and 44-55% higher in 2004.

### **2.3.2 Effect of P supply on maize yield**

Whether measured on per plant or per hectare basis, supplying 40 or 80 kg P. ha<sup>-1</sup> significantly increased grain yield of maize in both growing seasons (Tables 2.1 and 2.2). Compared with zero-P control, the application of 40 and 80 kg P.ha<sup>-1</sup> significantly increased maize yields by 20-37% in 2003, and 48-55% in 2004.

### **2.3.3 Effect of cropping system on yield components of cowpea**

In general, the yield components were higher in the second season compared to the first season. In 2003, the number of pod-bearing peduncles, the number of pods per plant, the number of seeds per pod and seed yield per plant were all significantly greater in the mono crop relative to the other cropping systems (Table 2.1). The number of pods per plant and number of seeds per plant were lowest in the intra-hole cropping system (Table 2.1). Interestingly, 100-seed weight was higher in intra-row and intra-hole planting relative to mono cropping (Table 2.1). Grain yield of cowpea was greater in mono crop, possibly due to greater plant density. The effects of cropping system in the second season were similar to those obtained in the first season. In 2004, the number of pod-bearing peduncles, the number of pods per plant, the number of seeds per pod and seed yield per plant decreased in the mixed cropping system relative to sole crop (Table 2.2). In contrast, 100-seed weight increased significantly with mixed cropping compared with sole crop (Table 2.2).

### **2.3.4 Effect of cropping system on maize yield**

With maize, cropping system showed no effect on seed yield, although sole crop maize numerically yielded more than the rest, followed by the intra-hole cropping system in both 2003 and 2004.

### **2.3.5 Phosphorus x cropping system interaction**

The P x cropping system interaction was significant for only grain yield in 2003 (Figure 2.1A). However, in 2004, P x cropping system interaction was significant for number of pod-bearing peduncles per plant, number of pods per plant, grain yield per plant and seed yield per hectare (Figures 2.1B, C, D, E, F). In general, cowpea yield components were increased by cropping system at higher P levels.

### **2.3.6 Effects of cropping systems on total LER**

The results of this study showed that the yields of maize and cowpea were increased by intercropping practices as demonstrated by the LER values which were all greater than 1 in 2003 and 2004 (Table 2.3). On average, the yield advantage from intercropping was greater in 2003 compared with 2004, as shown by the higher LER values obtained in 2003. Surprisingly, the highest LER value for 2003 was obtained from inter-row cropping of cowpea and maize without P.

## **2.4 Discussion**

In Africa, soil degradation and low nutrient fertility are major constraints to increased crop yields. Of all the nutrients, N and P are generally regarded as the most limiting for plant growth due to their unavailability for uptake by roots (Marschner, 1995; Vance, 2001). In this study, P was, no doubt, a major limiting factor to increased yields of both cowpea and maize in the Western Cape, as applying 40 or 80 kg P.ha<sup>-1</sup> increased cowpea yields by 59-65% in 2003 and 44-55% in 2004. With maize, the yield increases were 20-37% in 2003 and 48-55% in 2004. According to Vance (2003), a maize crop yield of 6-9 t.ha<sup>-1</sup> (as obtained here for 2003) requires crop uptake of 30-50 kg P.ha<sup>-1</sup>, thus suggesting that the applied P in this study was efficiently utilised by the crop species as P recovery is generally low (Vance 2001). Russel (1973) has, in

fact, reported that even with adequate P supply, only 20% or less of the exogenous P is removed by plants in the first year of growth due to retention by soil. Our findings are consistent with those of other workers (Wahua, 1983; Chang and Shibles, 1985a&b; Muleba, 1999; Buerkert *et al.*, 2001; Carsky, 2003; Jensen *et al.*, 2003;) who showed that with adequate P supply, maize growth and symbiotic performance of cowpea was significantly increased, leading to greater grain yield of both crops. Clearly, the application of P as fertiliser is important for increased grain yield of maize and cowpea in the Western Cape. However in Africa at large, where most small-scale farmers are resource-poor, the promotion of fertiliser use in crop production systems is likely to remain a challenge for quite some time.

In addition to the promotive effects of P application on crop yield components, the reproductive function of cowpea, but not maize, was affected by cropping system (Tables 2.1 and 2.2). For example, the number of pod-bearing peduncles, the number of pods per plant, and the number of seeds per plant were all significantly depressed in mixed plant culture relative to mono crop cowpea (Tables 2.1 and 2.2). This negative effect of intercropped maize on its cowpea partner has been reported by several workers (Tariah and Wahua, 1985; Ofori and Stern, 1986, 1987b; Watiki *et al.*, 1993; Dapaah *et al.*, 2003) and attributed to the shading of cowpea by maize plants (Fawusi *et al.*, 1982; Chang and Shibles, 1985a&b; Ofori and Stern, 1987a) which results in competitive advantage of maize over cowpea for light during both vegetative and reproductive stages (Fawusi *et al.*, 1982; Tariah and Wahua, 1985; Ofori and Stern, 1987a&b; Watiki *et al.*, 1993; Myaka, 1995). Our data are however unable to confirm this aspect because the transmission of photosynthetically-active radiation through the canopy was not determined in this study. Interestingly, although the number of seeds per pod were fewer for the intra-row and intra-hole cropping systems, their 100-seed weight values were larger than that of the sole crop, indicating that the low seed numbers per pod were compensated with bigger seed size from

assimilates channelled during ovule formation and seed development, as observed in common bean (Scarisbrick *et al.*, 1977). The seed yield of cowpea, measured as kilograms per hectare, was expectedly higher in the mono crop relative to mixtures as a result of the low cowpea plant density in intercrop (Tables 2.1 and 2.2). The decrease in seed yield on single-plant basis with intercropping was still manifested even when the data were expressed on the basis of equal plant density (Tables 2.1 and 2.2). Taken together, these results clearly indicate that the low grain yield of cowpea in mixed culture was not only due to differences in cowpea plant density, but also to the suppressive effect of the cropping system on cowpea development and reproductive function.

Furthermore, there was a significant interactive effect of P and cropping system on yield components of cowpea. At each level of P application in 2003, the mono crop cowpea consistently out-yielded those in mixed culture, followed by inter-row, and least intra-row and intra-hole planted cowpea (Fig 2.1A). Applying higher levels of P markedly increased grain yield of cowpea in mixed culture relative to zero-P control. The data for 2004 were similar in pattern to 2003 (Fig 2.1B, C, D, E and F). Whether considering the number of pod-bearing peduncles per plant, the number of pods per plant, the grain yield per cowpea plant or grain yield per hectare, the values for sole crop cowpea were generally much higher than those of cowpea in mixed culture. This was closely followed by inter-row planted cowpea, which also performed better than intra-row and intra-hole cowpea (Fig 2.1B, C, D, E and F). In all instances, the yield components of sole cowpea and, to some extent, inter-row cowpea were hugely increased by the provision of 40 and 80 kg P.ha<sup>-1</sup> relative to zero-P control (Fig 2.1B, C, D, E and F). There was, however, no effect of P x cropping system interaction on maize yield (Tables 2.1 and 2.2).

In comparing intercropping with sole cropping, an index of biological advantage is usually used that places yield of component crops on comparable basis. The intercropping of maize and cowpea in this study resulted in improved productivity as shown by the LER values, which were



generally greater than 1. LER values above 1 indicate that the intercropping combinations were more efficient in the use of resources as compared to sole cropping (Mead and Willey, 1980; Ofori and Stern, 1986, 1987a&b; Dapaah *et al.*, 2003). The pooled mean LER value was greater for the inter-row arrangement, followed by intra-row and intra-hole cropping (Table 2.3), indicating that the biological efficiency and productivity were highest for cowpea in intercropping. It is likely that the inter-row arrangement permitted more light transmission into the canopy to support photosynthetic activity of cowpea, and thus resulted in significantly increased yields.

In conclusion, this study has shown that exogenous supply of P increased yield components of both cowpea and maize. Furthermore, at each level of P application, sole cowpea consistently out-yielded intercropped cowpea. The decreased yield of cowpea in mixed culture was due, in part, to differences in cowpea plant density, and to the suppressive effect of the cropping system on cowpea development and reproductive function. Intercropping maize with cowpea produced higher total yields per unit land area than their sole crop counterparts.

Table 2. 1. Yield components of cowpea and maize in response to applied P in different cropping systems in 2003. Values followed by dissimilar letters in the same column (bold type) differ significantly at  $P < 0.05$

Treatments	Cowpea					Maize				
	Plant density	No pod bearing peduncles plant <sup>-1</sup>	No. pods plant <sup>-1</sup>	No. seeds pod <sup>-1</sup>	100-seed wt (g)	Seed yield (g plant <sup>-1</sup> )	Seed yield (kg ha <sup>-1</sup> )	Seed yield equal density basis (kg ha <sup>-1</sup> )	Seed yield (g plant <sup>-1</sup> )	Seed yield (kg ha <sup>-1</sup> )
<i>Main - treatments (kg P ha<sup>-1</sup>)</i>										
P <sub>0</sub>	NA	4.0 ± 0.28b	6.6 ± 0.60b	6.4 ± 0.26a	14.8 ± 0.18a	4.6 ± 0.33b	483 ± 84b	759 ± 56b	118.5 ± 3.9c	6586 ± 216c
P <sub>90</sub>	NA	5.8 ± 0.39a	9.0 ± 1.05a	6.8 ± 0.25a	14.6 ± 0.19a	7.3 ± 1.05a	758 ± 149a	1209 ± 174a	142.3 ± 5.3b	7906 ± 297b
P <sub>180</sub>	NA	6.4 ± 0.48a	8.9 ± 0.82a	7.0 ± 0.26a	14.5 ± 0.21a	7.6 ± 0.73a	807 ± 157a	1258 ± 122a	162.3 ± 7.1a	9014 ± 394a
<i>Sub- treatments (cropping systems)</i>										
Mono (cowpea or maize)	166666	7.2 ± 0.61a	12.8 ± 0.90a	7.6 ± 0.29a	14.1 ± 0.22b	8.7 ± 0.76a	1446 ± 126a	1446 ± 126a	151.2 ± 10.5a	8401 ± 583a
Maize/cowpea inter-row	111111	5.2 ± 0.31b	7.3 ± 0.47b	6.9 ± 0.19ab	14.6 ± 0.18ab	6.0 ± 0.58b	665 ± 65b	998 ± 97b	133.8 ± 6.7a	7436 ± 370a
Maize/cowpea intra-row	55555	4.6 ± 0.37b	7.5 ± 0.53b	6.6 ± 0.25bc	14.8 ± 0.23a	5.7 ± 0.78b	319 ± 43c	956 ± 130b	138.3 ± 5.7a	7683 ± 316a
Maize/cowpea intra-hole	55555	4.7 ± 0.45b	5.4 ± 0.44c	6.0 ± 0.28c	15.0 ± 0.17a	5.4 ± 1.30b	301 ± 72c	903 ± 216b	140.8 ± 9.2a	7821 ± 513a

Table 2. 1. Yield components of cowpea and maize in response to applied P in different cropping systems in 2003. Values followed by dissimilar letters in the same column (**bold type**) differ significantly at  $P < 0.05$

Treatments	Cowpea					Maize				
	Plant density	No. pod bearing peduncles.plant <sup>-1</sup>	No. pods plant <sup>-1</sup>	No. seeds.pod <sup>-1</sup>	100-seed wt (g)	Seed yield (g plant <sup>-1</sup> )	Seed yield (kg.ha <sup>-1</sup> )	Seed yield equal density basis (kg.ha <sup>-1</sup> )	Seed yield (g plant <sup>-1</sup> )	Seed yield (kg.ha <sup>-1</sup> )
<i>Main - treatments (kg P.ha<sup>-1</sup>)</i>										
P <sub>0</sub>	NA	<b>4.0±0.28b</b>	<b>6.6±0.60b</b>	6.4±0.26a	14.8±0.18a	<b>4.6±0.33b</b>	<b>483±84b</b>	<b>759±56b</b>	<b>118.5±3.9c</b>	<b>6586±216c</b>
P <sub>30</sub>	NA	<b>5.8±0.39a</b>	<b>9.0±1.05a</b>	6.8±0.25a	14.6±0.19a	<b>7.3±1.05a</b>	<b>758±149a</b>	<b>1209±174a</b>	<b>142.3±5.3b</b>	<b>7906±297b</b>
P <sub>60</sub>	NA	<b>6.4±0.48a</b>	<b>8.9±0.82a</b>	7.0±0.26a	14.5±0.21a	<b>7.6±0.73a</b>	<b>807±157a</b>	<b>1258±122a</b>	<b>162.3±7.1a</b>	<b>9014±394a</b>
<i>Sub- treatments (cropping systems)</i>										
Mono (cowpea or maize)	166666	<b>7.2±0.61a</b>	<b>12.8±0.90a</b>	<b>7.6±0.29a</b>	<b>14.1±0.22b</b>	<b>8.7±0.76a</b>	<b>1446±126a</b>	<b>1446±126a</b>	<b>151.2±10.5a</b>	<b>8401±583a</b>
Maize/cowpea inter-row	111111	<b>5.2±0.31b</b>	<b>7.3±0.47b</b>	<b>6.9±0.19ab</b>	<b>14.6±0.18ab</b>	<b>6.0±0.58b</b>	<b>665±65b</b>	<b>998±97b</b>	<b>133.8±6.7a</b>	<b>7436±370a</b>
Maize/cowpea intra- row	55555	<b>4.6±0.37b</b>	<b>7.5±0.53b</b>	<b>6.6±0.25bc</b>	<b>14.8±0.23a</b>	<b>5.7±0.78b</b>	<b>319±43c</b>	<b>956±130b</b>	<b>138.3±5.7a</b>	<b>7683±316a</b>
Maize/cowpea intra - hole	55555	<b>4.7±0.45b</b>	<b>5.4±0.44c</b>	<b>6.0±0.28c</b>	<b>15.0±0.17a</b>	<b>5.4±1.30b</b>	<b>301±72c</b>	<b>903±216b</b>	<b>140.8±9.2a</b>	<b>7821±513a</b>

Table 2.2. Yield components of cowpea and maize in response to applied P in different cropping systems in 2004. Values followed by dissimilar letters in the same column (**bold type**) differ significantly at  $P \leq 0.05$

Treatments	Cowpea						Maize			
	Plant density	No.pod-bearing peduncles. plant <sup>-1</sup>	No.pods. plant <sup>-1</sup>	No.seeds. pod <sup>-1</sup>	100-seed wt (g)	Seed yield (g.plant <sup>-1</sup> )	Seed yield (kg.ha <sup>-1</sup> )	Seed yield equal density basis (kg.ha <sup>-1</sup> )	Seed yield (g.plant <sup>-1</sup> )	Seed yield (kg.ha <sup>-1</sup> )
<i>Main - treatments (kg P.ha<sup>-1</sup>)</i>										
P <sub>0</sub>	NA	<b>5.8±0.83a</b>	<b>6.6±0.95b</b>	6.2±0.29a	14.9±0.21a	<b>5.5±0.73b</b>	<b>626±143b</b>	<b>916±121b</b>	<b>58.8±4.4b</b>	<b>3265±242b</b>
P <sub>30</sub>	NA	<b>7.1±0.65ab</b>	<b>10.0±0.99a</b>	7.1±0.30a	15.1±0.16a	<b>8.5±0.99a</b>	<b>924±184a</b>	<b>1409±166a</b>	<b>87.0±6.0a</b>	<b>4832±333a</b>
P <sub>90</sub>	NA	<b>7.9±1.17a</b>	<b>11.1±1.81a</b>	6.7±0.35a	15.1±0.73a	<b>7.9±1.48a</b>	<b>979±271a</b>	<b>1308±234a</b>	<b>91.4±5.6a</b>	<b>5077±309a</b>
<i>Sub-treatments (cropping systems)</i>										
Mono (cowpea or maize)	166666	<b>11.1±1.22a</b>	<b>14.9±1.89a</b>	7.3±0.43a	14.4±0.26b	<b>12.2±1.20a</b>	<b>2024±207a</b>	<b>2024±207a</b>	83.6±5.2a	4646±289a
Maize/cowpea inter-row	111111	<b>7.3±0.49b</b>	<b>9.8±0.86b</b>	6.6±0.39a	15.0±0.13a	<b>7.3±0.67b</b>	<b>816±75b</b>	<b>1223±112b</b>	70.6±7.7a	3920±430a
Maize/cowpea intra- row	55555	<b>5.4±0.57c</b>	<b>6.5±0.81c</b>	6.4±0.33a	15.3±0.15a	<b>5.3±1.05bc</b>	<b>292±58c</b>	<b>877±175bc</b>	78.9±9.8a	4385±542a
Maize/cowpea intra - hole	55555	<b>4.0±0.41c</b>	<b>7.7±1.01bc</b>	6.4±0.31a	15.4±0.18a	<b>4.3±0.71c</b>	<b>240±40c</b>	<b>720±119c</b>	83.0±6.3a	4614±352a

Table 2.3. Estimated yield advantage measured as Land Equivalent Ratio under different cropping systems and plant densities.

Treatment	Plant Density		Land equivalent ratio	
	Cowpea	Maize	2003	2004
Sole Cowpea + 0P	166666	-	-	-
Sole Maize + 0P	-	55555	-	-
Cowpea/maize inter-row cropping + 0P	111111	55555	1.59±0.05	1.24±0.10
Cowpea/maize hole intra-row cropping + 0P	55555	55555	1.15±0.08	1.04±0.15
Cowpea/maize hole intra-hole cropping + 0P	55555	55555	1.10±0.05	1.18±0.11
Sole Cowpea + 40P	166666	-	-	-
Sole Maize + 40P	-	55555	-	-
Cowpea/maize inter-row cropping + 40P	111111	55555	1.29±0.09	1.58±0.20
Cowpea/maize hole intra-row cropping + 40P	55555	55555	1.24±0.06	1.14±0.22
Cowpea/maize hole intra-hole cropping + 40P	55555	55555	1.20±0.14	1.06±0.08
Sole Cowpea + 80P	166666	-	-	-
Sole Maize + 80P	-	55555	-	-
Cowpea/maize inter-row cropping + 80P	111111	55555	1.35±0.10	1.04±0.07
Cowpea/maize hole intra-row cropping + 80P	55555	55555	1.18±0.14	1.05±0.12
Cowpea/maize hole intra-hole cropping + 80P	55555	55555	1.19±0.08	1.12±0.10

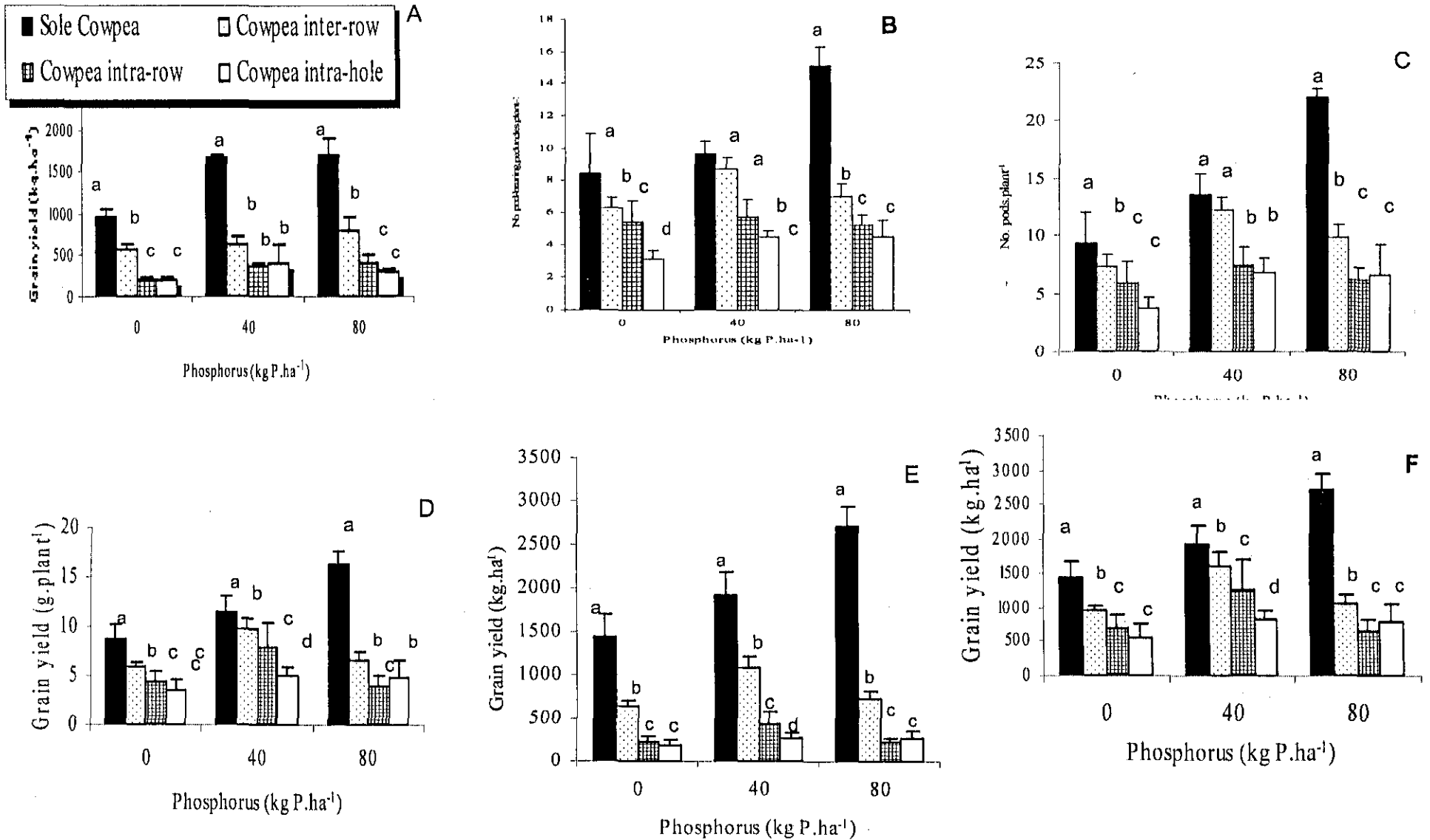


Figure 2.1. Interactive effects of P and cropping system on cowpea grain yield in 2003 (A), interactive effects of P and cropping system on number of pod-bearing peduncles per plant in 2004 (B), interactive effects of P and cropping system on number of pods per plant in 2004 (C), interactive effects of P and cropping system on yield per cowpea plant in 2004 (D), interactive effects of P and cropping system on cowpea grain yield 2004 (E), interactive effects of P and cropping system on cowpea grain yield in 2004, based on equal plant density basis (F).

### CHAPTER 3

ALTERATION IN PLANT-AVAILABLE NUTRIENT CONCENTRATIONS IN THE  
RHIZOSPHERE OF NODULATED COWPEA (*VIGNA UNGUICULATA* L. WALP.) AND  
MAIZE (*ZEA MAYS* L.) PLANTS SUPPLIED WITH EXOGENOUS P IN DIFFERENT MIXED  
CULTURE.

### 3.1 Introduction

Several studies on mineral nutrition of symbiotic legumes have concluded that there is a requirement of mineral nutrients for plant growth and another for symbiotic N<sub>2</sub> fixation (Anderson and Spencer, 1950; Ahmed and Evans, 1960; Gates and Wilson, 1974; Robson *et al.*, 1981; Jacobsen, 1985; Singleton *et al.*, 1985; Israel, 1987). Supplying exogenous P to purely symbiotic versus NO<sub>3</sub>-fed (nodulated and un-nodulated) legumes have, for example, shown that symbiotic N<sub>2</sub> fixation has a higher P requirement than NO<sub>3</sub> assimilation or host plant growth *per se* (Gates and Wilson, 1974; Robson *et al.*, 1981; Jacobsen, 1985; Singleton *et al.*, 1985; Israel, 1987). In symbiotic legumes supplied with P, this difference was manifested as an increase in whole-plant N concentration and in the ratio of nodule weight to whole-plant biomass, while there was a decrease in whole-plant N concentration in NO<sub>3</sub>- fed plants provided with P (Israel, 1987). Broadly speaking, the findings of these studies seem to suggest that purely symbiotic legumes have a higher internal requirement for P than species solely dependent on mineral N for their N nutrition. So far, however, few studies have been conducted to provide comparative data on nutrient uptake by legumes and cereals, including changes in rhizosphere nutrient concentrations. It is, therefore, still unclear whether nodulated legumes make a greater demand on the ecosystem for P and other mineral nutrients compared to non-N<sub>2</sub>-fixing species.

Several workers have shown that, in mixed plant cultures involving legumes and cereals, the growth and nutrient uptake of the cereal is greatly improved by the legume (Gardner and Boundy, 1983; Kahm *et al.*, 1999; Cu *et al.*, 2005). The mechanism underlying this growth stimulation of cereals by legumes includes transfer of fixed-N from legume to cereal (Eaglesham *et al.*, 1981), mobilisation of insoluble nutrients from soil (e.g. Fe-P, Ca-P and Al-P) by plant root exudates (Dakora and Phillips, 2002), and the chelation of P-bound cations to release P by organic acid



anions exuded from cluster roots (Neumann *et al.*, 2000; Roelofs *et al.*, 2001; Cu *et al.*, 2005). In a sorghum/pigeon pea mixed culture, P uptake by cereal was also enhanced by the legume through release of piscidic acid in pigeon pea root exudates, which mobilised and increased P availability (Ae *et al.*, 1990). A few studies have, however, shown that the reverse situation exists whereby cereal plants promote legume growth through facilitated uptake of nutrients (Römheld, 1991; Bryan and Hopking, 1992; Hopkins *et al.*, 1992) solubilised by the release of phytosiderophores in their root exudates. For example, Fe uptake by groundnut in a maize/groundnut intercrop was markedly improved by the cereal, especially when their root systems were intermingled (Zhang *et al.*, 2004).

Root activity, therefore, plays a major role in the nutrient economy of soils. For example, protons released into the soil as a by-product of nodule function in N<sub>2</sub>-fixing legumes, can alter soil pH and affect nutrient availability to plants (Dakora and Phillips, 2002). Recently, Cheng *et al.* (2004) showed that, in low pH soils, *Medicago sativa* released lots of protons into the rhizosphere which increased acidity, and decreased nodulation by *Sinorhizobium medicae* possibly as a result of reduced rhizobial growth and survival. In contrast, *Medicago murex*, which did not acidify its rhizosphere, was better nodulated than *Medicago sativa*. It is also generally accepted that, compared to cereals and other crops, symbiotic legumes take up an excess of cations over anions from soil solution during N<sub>2</sub> fixation, and release protons, which decrease rhizosphere pH (Jarvis and Robson, 1983; McLay *et al.*, 1997; Tang *et al.*, 1998, 2001) as well as affect nutrient availability in the rhizosphere (Brady, 1990). However, Rao *et al.* (2000) have shown that under illumination, but not darkness, NO<sub>3</sub>-fed non-fixing legumes, can still acidify their rhizosphere, indicating that rhizosphere acidification in the Leguminosae is regulated by photosynthetic activity and not excess uptake of cations (Rao *et al.*, 2002). Whatever the case, these changes of rhizosphere pH can affect nutrient availability such as enhancing P mobilisation and uptake from

Ca-P, Fe-P and Al-P present in soil but unavailable to plants (Dakora and Phillips 2002). Besides the effects of plant root activity and metabolism on the nutrient economy of soils, very few studies have assessed the impact of applying exogenous mineral nutrients to plants on the availability of other nutrient elements in the rhizosphere.

This study examines the effects of i) exogenous P supply, ii) planting system, and iii) plant species on the concentrations and uptake rates of plant-available nutrients in the rhizosphere of cowpea and maize.

## **3.2 Materials and Methods**

### **3.2.1 Experimental site**

Field experiments were conducted under irrigation at the Agricultural Research Council Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa, during the 2003 and 2004 summer seasons at two different locations within Nietvoorbij. The site characteristics are described in detail in chapter 2 section 2.2.1.

### **3.2.2 Experimental design**

The experimental treatments consisted of three levels (0, 40, and 80 kg P.ha<sup>-1</sup>) of triple superphosphate [46% P, 13.5% Ca, 1.5% S, 0.0007% Cu, 0.008% Zn, 0.021% Mn, 0.053% B and 0.0009% Mo (Cherney, 2003; FAS, 2005)] and four cropping systems (namely, monoculture, maize/cowpea inter-row, maize/cowpea intra-row, and maize/cowpea intra-hole cropping), which were used in both 2003 and 2004. The experimental layout, the crop varieties used and other cultural practices used are described in chapter 2 section 2.2.2.

### **3.2.3 Collection and preparation of bulk soil**

Prior to P application and planting, soil samples were collected with auger (0-20 cm depth) from several locations within each replicate plot and mixed for determination of the initial nutrient concentrations in the soil. The soil samples were air-dried in the laboratory, and sieved (2 mm) for analysis of nutrients and determination of pH and organic matter.

### **3.2.4 Collection and preparation of rhizosphere soil**

At 60 d after planting, “rhizosphere soil”, defined as soil rich in roots and/or adhering to the roots and influenced by root activity, was collected from around maize and cowpea plants for nutrient analysis. To achieve this, soil was carefully excavated from around single plants or their pairs down to 30 cm or more (depending on root depth), and “island of soil” around the plant dug up and removed, with the plant and its roots intact inside the lump of soil. Using one’s hands, the volume of soil containing intact plant(s) was removed from the exterior down to a root-rich “rhizosphere soil” material of about 30-50 g. This sample was shaken into a labelled plastic bag and the process repeated for up to 16 cowpea plants per plot or 8 maize plants per plot. Sometimes the lump or “island of soil” with intact plant roots broke and disintegrated in the course of removal, in which case only the bit of soil still adhering to plant roots was shaken into the labelled plastic bag. These rhizosphere soil samples were air-dried in the laboratory, and sieved (2 mm) for analysis of nutrients and the determination of pH and organic matter. Where maize and cowpea were sown in one hole (“intra-hole plants”), the collected rhizosphere soil was common to both species and the data obtained was, therefore, the same for both plant species.

### **3.2.5 Measurement of soil pH and organic matter**

The pH of soil was measured in 0.01 M CaCl<sub>2</sub> solution using a 1:2.5 soil-to-solution ratio. Organic carbon in soil was determined using the wet digestion method of Walkley and Black (Jackson, 1967). Soil organic matter (SOM) was determined in air-dried soil samples as loss on ignition at 450°C for 24 h after drying at 105°C for 12 h.

### **3.2.6 Determination of plant-available nutrients in rhizosphere soil**

The determination of S in soil was done by adding 20 g of soil in 0.01 M Ca(H<sub>2</sub>PO<sub>4</sub>)<sub>2</sub>·H<sub>2</sub>O extracting solution (FSSA, 1974), followed by filtering, and S determined by direct aspiration on a calibrated simultaneous inductively coupled plasma (ICP) spectrophotometer (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusetts, USA).

The extractable P, K, Na, Ca and Mg were determined by citric acid method as developed by Dyer (1894) and modified by the Division of Chemical Services (DCS, 1956) and Du Plessis and Burger (1964). A 20 g air-dried soil sample was extracted in 200 mL of 1% (w/v) citric acid, heated to 80 °C, shaken for 2 min at 10-min intervals over a total period of 1 h and filtered. A 50 mL aliquot was heated to dryness on a water bath, digested with 5 mL of concentrated HCl and HNO<sub>3</sub>, evaporated to dryness on a water bath, and 5 mL of concentrated HNO<sub>3</sub> and 20 mL of de-ionised water added. The mixture was heated to dissolve the dry residue, and the sample filtered. Measurements of P, K, Na, Ca and Mg were then done directly by direct aspiration on the calibrated simultaneous ICP.

The trace elements Cu, Zn, Mn, Fe, and Al were extracted from soil using di-ammonium ethylenediaminetetraacetic (EDTA) acid solution [Trierweiler and Lindsay (1969), as modified by Beyers and Coetzer (1971)]. The extractants were analysed for Cu, Zn, Mn, Fe, and Al using the calibrated simultaneous ICP spectrophotometer. Boron in the soil was determined following the method of FSSA (1974) and values measured using the ICP spectrophotometer.

### **3.2.7 Measurement of nutrient uptake rates**

To understand any changes in the nutrient concentrations in the rhizosphere of test species, nutrient uptake rates were measured. The plants dug up for collection of rhizosphere soil were separated into nodulated roots and shoots, oven-dried, weighed, and ground to fine powder for analysis of the major and minor nutrient elements. Measurements of macronutrients (P, K, Ca, Mg, and Na) and micronutrients (Cu, Zn, Mn, Fe, Al, B) were determined by ashing 1 g ground sample in a porcelain crucible at 500 °C overnight. This was followed by dissolving the ash in 5 mL of 6 M HCl and placing it in an oven at 50 °C for 30 min; 35 mL of deionised water were added and extract filtered through Whatman no. 1 filter paper. Nutrient concentrations in plant extracts were determined (Giron, 1973) using the ICP. Sulphur was determined by wet digestion procedure using 65% nitric acid. In each case, 1 g of milled plant material was digested overnight with 20 mL of 65% nitric acid in a 250 mL glass beaker. The beaker containing the extract was then placed on a sand bath and gently boiled until approximately 1 mL of the extract was left. After that, 10 mL of 4 M nitric acid was added and boiled for 10 min. The beaker was then removed from the sand bath, cooled, and the extract washed completely in a 100 mL volumetric flask and the extract filtered through Whatman no. 2 filter paper. The S in the sample was then determined (FSSA, 1974) by direct aspiration on the calibrated simultaneous ICP.

For each plant part, the measured concentrations of nutrients were multiplied by plant dry matter to obtain the organ's content of the mineral. The total content of each nutrient per plant was calculated from values of shoots and roots, and divided by plant age to obtain averageuptake rates ( $\text{mg.plant}^{-1}.\text{d}^{-1}$ ).

### **3.2.8 Statistical analysis**

A 2-factorial design (2-way ANOVA) involving P and cropping system was used to analyse soil pH, SOM and nutrient concentrations in the rhizosphere. However, a one-way ANOVA was used to compare nutrient concentrations associated with the rhizosphere of the two plant species, cowpea and maize. The analysis was done using the software of STATISTICA program 1997. Fisher's least significant difference was used to compare treatment means at  $P \leq 0.05$  level of significance (Steel and Torrie, 1980).

## **3.3 Results**

### **3.3.1 Soil properties at planting**

The chemical properties of the field soil at planting in 2003 and 2004 are shown in Table 3.1. Although the pH, soil organic matter (SOM), Mg and S levels were similar at the two sites used in 2003 and 2004, the concentrations of other nutrients were considerably different.

### **3.3.2 Effects of P supply, planting system and plant species on pH and organic matter content of the rhizosphere soil**

Rhizosphere soil pH was significantly decreased by P application in both 2003 and 2004 (Table 3.2). Except for intra-hole planted cowpea where pH was markedly higher in 2003 but not 2004, with cowpea there was no effect of cropping system on pH (Table 3.2). There was also no effect

of cropping system on the pH of maize rhizosphere in both 2003 and 2004, except for intra-hole planted maize where the pH was lowered relative to the other cropping systems (Table 3.2).

Although SOM was significantly increased in the rhizosphere by the application of 40 and 80 kg P.ha<sup>-1</sup> in 2003 and 2004 respectively (Table 3.2), cropping system had no effect on SOM levels (Table 3.2). The effects of plant species on pH and SOM was marked in both 2003 and 2004. The rhizosphere soil pH was significantly decreased by nodulated cowpea in both 2003 and 2004 relative to maize. With SOM, however, the opposite effect was obtained in that maize produced more organic matter in the rhizosphere compared to the legume (Table 3.2).

### **3.3.3 Effects of P supply, planting system and plant species on macronutrient concentrations in the rhizosphere**

The rhizosphere concentration of P, Ca, Mg, S, and Na were generally increased by exogenous supply of P in 2003 (Table 3.3). A similar pattern was obtained in 2004 with P, Ca, S, and Na being markedly increased with P supply (Table 3.4). Monocultured crop and inter-row planted cowpea showed lower concentrations of P, K, Mg, S, and Na in the rhizosphere compared with intra-hole and, to some extent, intra-row planted cowpea in 2003 (Table 3.3). In contrast, data for 2003 maize showed decreased levels of P, K and S in intra-hole relative to sole-planted and the other mixed cultures (Table 3.3). The rhizosphere nutrient concentrations obtained in 2004 were similar in pattern to those of 2003. The level of C, Mg, S and Na were generally decreased in the rhizosphere of sole, inter-row and intra-row planted cowpea relative to their intra-hole counterparts (Table 3.3). At the species level, cowpea showed significantly decreased concentrations of P, K, Ca, Mg, and S in its rhizosphere relative to maize in 2003 (Table 3.3). In 2004, however, only P, K, Mg, and Na showed decreased concentrations in cowpea rhizosphere compared to maize (Table 3.3). Furthermore, the P x cropping system interaction was significant

for C, Mg, Na, Zn, and Al in cowpea rhizosphere, as well as C, Na and Zn in maize rhizosphere (Figure 3.1).

### **3.3.4 Effects of P supply, planting system and plant species on micronutrient concentrations in the rhizosphere**

Relative to zero-P control, the application of exogenous P in 2003 significantly increased the concentrations of Zn and Fe in the rhizosphere of cowpea plants (Table 3.4). Supplying P at 40 kg P.ha<sup>-1</sup> also significantly increased the level of B in the rhizosphere of cowpea when compared to zero-P control or the other P treatment (Table 3.4). The data for 2004 also showed increased concentrations of Fe in the rhizosphere of P-treated cowpea (Table 3.4). With maize, only B and Fe showed changes in rhizosphere concentration with P application. Applying 40 kg.P.ha<sup>-1</sup> in 2003 increased the concentrations of B and Fe in maize rhizosphere over control (Table 3.4). Except for Al, which showed decreased concentration in mixed culture relative to sole-planted counterparts, planting system had no effect on nutrient concentration in the rhizosphere of cowpea (Table 3.4). However, with maize, Cu, Zn, Mn and B all showed decreased rhizosphere concentrations in 2003 in intra-hole plants relative to mono and mixed cultured counterparts (Table 3.4).

Species effect on micronutrient concentrations in the rhizosphere was highly pronounced. The levels of Cu, Zn, Mn, B and Fe were all significantly reduced in the rhizosphere of cowpea compared with maize in 2003 (Table 4). However, in 2004, only Zn, Mn and Fe showed decreased concentrations in the rhizosphere of cowpea relative to maize (Table 3.4).



### **3.3.5 Effects of P supply and planting system on nutrient uptake rates at whole-plant level in cowpea and maize**

Except P, the uptake rates of nutrients by cowpea were not affected significantly by P supply in Year 1 (data not shown). However, applying P to maize significantly increased the uptake rates of P, K, Mg, Cu and Zn relative to control in Year 1 (Table 3.5). Phosphorus application to cowpea in Year 2 also significantly increased the uptake rates of P, K, Mg, Ca, S, Na, Zn, Mn, B, Fe and Al with the exception of Cu (Table 3.5). However, only P, S and Mn exhibited significantly higher uptake rates with P application to maize in Year 2 (Table 3.5).

The effects of planting system on rates of nutrient uptake by test species was assessed and found to be markedly different. As shown in Table 5, the uptake rates of P, Zn, and Mn by cowpea in Year 1 were higher in monoculture relative to the mixed cultures, especially the intra-hole plants. In Year 2, the rates of uptake of P, K, Mg, Ca, S, Na, Cu, Zn, Mn, B, Fe and Al by cowpea were again significantly higher in monoculture compared to the mixed cultures, with intra-hole plants showing the lowest rates of nutrient uptake (Table 3.5). With maize, however, there was no effect of planting system on nutrient uptake rates in both seasons, except for S which showed reduced uptake rate in mixed culture (Table 3.5). In all instances, where planting system had an effect on nutrient absorption, the uptake rates were markedly higher in the monoculture, and much lower in the mixed cultures, especially where cowpea and maize were co-planted in one hole.

### **3.3.6 Interactive effects of applied P and planting system on the concentrations of nutrients in the rhizosphere of cowpea and maize planted in Year 2**

At zero-P, the co-planting of cowpea and maize in one hole produced significantly more organic C in the rhizosphere of cowpea compared with the other cropping systems (Figure 3.1A).

Although with maize the intra-hole C was similarly greater in the rhizosphere, it was significant over only intra-row material (Figure 3.1 F). As with organic C, at zero-P or 40 kg.ha<sup>-1</sup>, the concentrations of Mg, Na and Zn were significantly greater in the rhizosphere of intra-hole cowpea plants relative to monoculture (Figure 3.1B, C and D). At 80 kg.ha<sup>-1</sup>, however, Mg, and Na levels in the rhizosphere of sole-planted cowpea were either significantly greater than the mixed culture, or similar in magnitude (Figure 3.1 B, and C). The Al concentrations in the rhizosphere of monocultured cowpea plants supplied with 40 kg.ha<sup>-1</sup> was also markedly elevated relative to mixed cultures, especially the intra-hole plants (Figure 3.1E), results similar to that of Zn in the rhizosphere of maize receiving 80 kg.ha<sup>-1</sup> (Figure 3.1H). At zero-P, however, Zn and Na concentrations were significantly reduced in the rhizosphere of intra-row planted maize compared to the other planting systems (Figure 3.1G and H).

### 3.4 Discussion

In this study, an increase in the concentrations of C, P, Ca, Mg, S, Na, Zn, B, and Fe was observed in the rhizosphere of cowpea plants supplied with 40 or 80 kg P.ha<sup>-1</sup> as triple superphosphate in both Year 1 and Year 2 (Tables 3.3 and 3.4). Similarly, the rhizosphere concentrations of P, Mg, Ca, S, B, and Fe were also increased with external P supply to maize (Tables 3.3 and 3.4). While it is still unknown how P makes these mineral nutrients more available in the rhizosphere (Li *et al.*, 2004), there is evidence that exogenous P supply can decrease uptake of micronutrients such as Zn and Fe (Barrow, 1987), an event that would reflect as an increase in concentration relative to zero-P control. The change in pH caused by P supply (Table 3.2) can also increase nutrient availability, including that of P (Cline *et al.*, 1986).

Interestingly, planting system also had an effect on nutrient concentrations in the rhizosphere. Unlike Al, macronutrients such as P, K, Mg, S, and Na showed significantly decreased

concentrations in the rhizosphere of sole and inter-row cowpea relative to those of intra-row and intra-hole plants. Significantly lower concentrations of P, K, S, Cu, Zn, Mn, and B were also obtained in the rhizosphere of intra-hole maize relative to sole-planted or the other mix-cultured maize plants (Tables 3.3 and 3.4). These decreases in rhizosphere nutrient levels were, in part, due to increased uptake rates by plant roots, thus leading to depletion in rhizosphere soil solution (Table 3.5). This effect was, no doubt, enhanced by the higher plant density associated with those planting systems. That the higher uptake rates were responsible for their decreased concentrations in the rhizosphere were also confirmed by the significantly higher nutrient concentrations in organs of cowpea and maize plants from the respective planting systems (data not shown). Clearly, the decreased nutrient concentrations observed in maize and cowpea rhizospheres with planting pattern directly mirrored nutrient depletion by root uptake and accumulated nutrient concentrations of tissues.

When the plant species were compared against each other, cowpea and maize differed markedly in the concentrations of plant-available nutrients present in their rhizospheres. For the two years of experimentation, the concentrations of P, K, Mg, Zn, Mn, and Fe were always significantly lower in the rhizosphere of cowpea relative to maize, with Ca, S, Cu, and B being affected in only first year or the other (Tables 3.3 and 3.4). While the species differences obtained here could be attributed to the fact that, relative to non-legumes, N<sub>2</sub>-fixing plants have a higher nutrient demand to meet symbiotic requirements (Sa and Israel, 1999), it could be argued that legumes also mobilise soil minerals better than cereals (Koide and Mooney, 1987; Ae *et al.*, 1990; Marschner, 1995). Additionally, monocots and dicots differ in the nature and profile of root transport proteins (e.g. K, Fe and phosphate transporters), used to facilitate nutrient uptake from soil solution (Grusak *et al.*, 1999; Kochian *et al.*, 2002; Mäser *et al.*, 2001; Smith, 2002), just as they differ in the density of these ion transporters found in the root plasmalemma membrane. So,

the decreased mineral concentrations observed in the rhizosphere of cowpea relative to maize could be attributed to the presence of species-specific ion transporters or physiological variation that influenced greater nutrient uptake by the nodulated cowpea.

It is worth noting that the provision of exogenous P to soil also resulted in increased acidity (or low pH) of the rhizosphere environment in both 2003 and 2004 (Table 3.2), a result consistent with the findings of Huffman (1962) and Ownby *et al.* (2005), which showed decreased pH with super-phosphate supply to plants. But, as reported by Dakora and Phillips (2002), a change in the cation/anion ratio of soil as a consequence of differential root uptake activity can alter rhizosphere pH. In general, the release of protons by plants (whether legume or non-legume) to counter-balance an excess cation over anion uptake is the major cause of root-induced changes in rhizosphere pH of most plant species (Dakora and Phillips, 2002; Hinsinger *et al.*, 2003). It is, therefore, likely that, in this study, the exogenous supply of P to plants altered the pattern of nutrient uptake by roots, and thus affected the rhizospheric cation/anion balance, leading to increased proton extrusion and low pH.

The chemical changes observed in the rhizosphere appeared to have resulted from biological activity associated with roots. For example, consistent with the data of Kanchikerimath and Singh (2001), the level of organic matter in the rhizosphere soil of maize and cowpea plants supplied with P in 2003 and 2004 was significantly increased (Table 3.2), possibly as a result of enhanced root exudation and/or root-residue decomposition. Consequently, organic C in the rhizosphere was also significantly increased in 2004 (Table 3.3; Figure 3.1A and F). However, this increase varied with species and planting system. With both cowpea and maize from zero-P plots, the highest C, Mg, Na and Zn concentrations were associated with intra-hole plants relative to sole or other mixed cultures (Figure 3.1A and F).

In the two years of experimentation, the rhizosphere pH of cowpea was always significantly lower than that of maize (Table 3.2). This observation is consistent with the common view that, relative to cereals, symbiotic legumes acidify their rhizosphere environment from excess uptake of cations over anions (Jarvis and Robson, 1983; McLay *et al.*, 1997; Tang *et al.*, 1998; Gregory and Hinsinger, 1999; Dakora and Phillips, 2002; George *et al.*, 2002; Cheng *et al.*, 2004), a process that causes proton extrusion and low pH (Jarvis and Robson, 1983; McLay *et al.*, 1997; Tang *et al.*, 2001; Cheng *et al.*, 2004). Rao *et al.* (2000, 2002) have, however, recently shown that, under illumination but not darkness, NO<sub>3</sub>-fed non-symbiotic cowpea plants significantly increased proton concentration in the rhizosphere, an observation which led them to conclude that rhizosphere acidification is a function of photosynthetic activity and not excess uptake of cations. Furthermore, it is a well-known fact that rhizobia and other N<sub>2</sub>-fixing diazotrophs, especially when supplied with host-plant photosynthate, actively release protons as by-product of N<sub>2</sub> fixation and, thus, lower rhizosphere pH. Whatever the mechanisms underlying rhizosphere acidification may be, any change of pH as obtained in this study (Table 3.2) is likely to affect nutrient mobilisation and mineral uptake in the root environment (Dakora and Phillips 2002; Cheng *et al.*, 2004; Li *et al.*, 2004). So, the observed decrease in rhizosphere pH caused by P supply, planting system and/or legume species (Table 3.2) probably affected nutrient availability or uptake by plants in the mixed culture, and resulted in reducing rhizosphere nutrient concentrations.

In conclusion, we have shown that an exogenous supply of P at 40 or 80 kg P.ha<sup>-1</sup> to maize and cowpea significantly increased the concentrations of plant-available P, Ca, Mg, S, Na, Zn, and Fe in the rhizosphere. In 2003, the concentrations of P, Ca, Mg, S, Na, Cu, Zn, Mn, B, and Fe were all significantly lower in the rhizosphere of cowpea relative to maize. However, in 2004, only P,

K, Mg, Na, Zn, Mn, and Fe were decreased in the legume's rhizosphere relative to the cereal. While the mechanism remains unknown for the observed increase in nutrient concentrations with the provision of P, the decreased levels of nutrients in the rhizosphere of cowpea was likely due to increased uptake as a result of higher plant density and possibly greater nutrient availability from change of rhizosphere pH.

*Table 3. 1.* Concentration of extractable mineral elements in bulk soil sampled prior to P application and planting. Each value represents an average of 15 soil samples collected from different points within each replicate plot.

Year	Concentration of mineral elements (mg.kg <sup>-1</sup> )														
	pH (CaCl <sub>2</sub> )	SOM	C	P	K	Ca	Mg	S	Na	Cu	Zn	Mn	B	Fe	Al
2003	6.3 ± 0.1	3200 ± 100	900 ± 100	40.0 ± 3.5	103.5 ± 10.3	842.1 ± 80.2	121.5 ± 12.2	3.1 ± 0.2	0.10 ± 0.001	8.4 ± 0.3	2.47 ± 0.3	20.5 ± 1.9	0.7 ± 0.04	122.8 ± 11.5	
2004	6.3 ± 0.1	3100 ± 200	1900 ± 100	8.8 ± 0.8	141.8 ± 5.9	521.3 ± 40.1	121.5 ± 3.7	3.7 ± 0.2	0.067 ± 0.002	0.8 ± 0.04	1.79 ± 0.2	9.7 ± 0.1	0.3 ± 0.02	131.5 ± 5.02	0.7 ± 0.04

Table 3. 2. pH and soil organic matter (SOM) in the rhizosphere of nodulated cowpea and maize Plants supplied with exogenous P in different cropping systems in 2003 and 2004. Values followed by dissimilar letters in the same column (bold type) differ significantly at  $P \leq 0.05$ . A 2-way ANOVA was used to analyze P and cropping system effects while a one-way ANOVA was used to compare pH and SOM changes associated with the rhizosphere of both cowpea and maize.

Treatment	pH (CaCl <sub>2</sub> )		SOM (mg kg <sup>-1</sup> )	
	2003	2004	2003	2004
<b>Cowpea</b>				
<b>P level (kg P.ha<sup>-1</sup>)</b>				
P0	5.9±0.07b	6.0±0.02b	3200±90b	4220±181b
P40	5.9±0.05b	6.0±0.02a	3600±140a	4380±156b
P80	5.8±0.05a	5.9±0.03a	3400±90a	4881±197a
<b>Cropping system</b>				
Sole cowpea	5.8±0.06b	6.0±0.03a	3300±100a	4243±185b
Inter-row cowpea	5.8±0.06b	6.0±0.02a	3500±100a	4450±235b
Intra- row cowpea	5.8±0.06b	6.0±0.02a	3300±200a	4384±215b
Intra – hole cowpea	6.1±0.08a	6.0±0.03a	3400±100a	4897±214a
<b>Maize</b>				
P0	6.2±0.07b	6.2±0.01a	3543±92a	4718±189a
P40	6.2±0.04b	6.2±0.01a	3934±260a	4585±139a
P80	6.0±0.05a	6.1±0.01a	3549±84a	5029±168a
<b>Cropping system</b>				
Sole maize	6.2±0.07a	6.2±0.07a	3737±106a	4700±200a
Inter-row maize	6.2±0.05a	6.2±0.04a	3665±113a	4900±200a
Intra- row maize	6.2±0.05a	6.2±0.03a	3912±328a	4700±200a
Intra – hole maize	6.1±0.08a	6.0±0.03b	3387±139a	4900±200a
<b>Species</b>				
Cowpea	5.9±0.034b	6.0±0.01b	3400±100b	4500±100b
Maize	6.2±0.032a	6.2±0.03a	3700±100a	4800±100a



*Table 3. 3.* Concentration of extractable mineral elements in the rhizosphere soil of nodulated cowpea and maize plants supplied with exogenous P in different cropping systems in 2003 and 2004. Values followed by dissimilar letters in the same column (**bold type**) for P level, cropping system, or species are significant at  $P \leq 0.05$ . A 2-way ANOVA was used to analyze P and cropping system effects while a one-way ANOVA was used to compare mineral elements concentrations associated with the rhizosphere of both cowpea and maize

Treatment	Concentration of macroelements (mg.kg <sup>-1</sup> )													
	C		P		K		Mg		Ca		S		Na	
	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004
<b>Cowpea</b>														
<b>P level (kg P.ha<sup>-1</sup>)</b>														
P0	1000±40b	1900±110b	44.6±2.8b	9.5±1.3c	79.6±4.3a	102.9±4.6a	106.4±5.1b	168.4±9.3a	711.9±42.7b	578.1±35.8b	3.4±0.37b	4.5±0.20c	70.8±2.5a	86.5±3.1b
P40	1100±50a	2100±40a	106.6±8.8a	69.9±5.7b	77.1±4.2a	103.1±3.4a	123.6±5.9a	152.1±5.9a	877.6±43.0a	735.7±63.1a	4.7±0.25ab	6.2±0.36b	76.1±2.5a	87.5±3.0b
P80	1100±40a	2200±110a	129.6±11.1a	103.5±8.7a	86.2±4.2a	96.0±3.3a	110.9±4.6a	166.4±9.7a	760.9±35.6ab	711.0±41.4a	5.0±0.29a	7.0±0.38a	68.5±2.9a	94.3±3.9a
<b>Cropping systems</b>														
Sole cowpea	1000±60a	1900±120b	84.2±12.3c	57.2±12.7a	82.8±5.2b	98.8±3.9a	104.7±4.9c	159.0±12.1b	735.0±38.6a	736.2±82.7a	3.8±0.41c	5.5±0.46b	69.5±2.7bc	85.0±4.1b
Inter-row cowpea	1100±50a	1900±110b	79.7±10.3d	53.4±13.3a	81.7±3.3b	97.8±4.3a	115.5±7.0b	149.1±8.3b	758.7±48.3a	597.2±37.2a	4.6±0.32b	5.4±0.44b	70.8±1.9b	84.3±2.6b
Intra-row cowpea	1100±50a	2100±80ab	109.2±19.8a	68.9±14.7a	66.8±3.0c	99.4±3.7a	108.2±6.1b	163.8±8.0a	811.9±60.1a	685.7±61.9a	5.2±0.44ab	6.6±0.48a	81.1±2.7a	96.1±4.5a
Intra-hole cowpea	1100±50a	2300±90a	101.3±12.0b	64.4±13.5a	92.6±5.0a	106.3±5.5a	126.1±5.8a	177.3±9.7a	828.2±52.7a	680.7±38.4a	4.1±0.37b	6.2±0.47a	65.8±3.2c	92.3±3.6a
<b>Maize</b>														
P0	1100±53a	2160±102a	51.9±2.4c	11.1±1.2c	108.7±5.8a	116.6±5.1a	125.3±4.5b	186.8±8.3a	783.2±39.3b	676.8±40.6a	4.0±0.3b	5.3±0.3b	64.7±2.3ab	95.0±4.0a
P40	1200±63a	2190±79a	127.4±8.6b	70.3±5.3b	108.8±5.8a	112.7±4.8a	139.9±4.4a	178.2±8.1a	948.4±41.0a	727.1±39.1a	5.4±0.3a	6.3±0.4a	69.4±2.3a	93.8±3.3a
P80	1100±39a	2265±108a	166.9±11.8a	116.8±9.6a	106.9±7.1a	103.7±6.3a	129.7±5.3ab	173.1±8.7a	840.8±30.8ab	711.5±30.4a	5.7±0.3a	6.7±0.3a	61.1±2.2b	98.0±4.8a
<b>Cropping systems</b>														
Sole maize	1200±60a	2200±130a	127.1±22.4ab	71.7±16.5a	115.8±6.2a	110.8±5.8a	134.7±7.2a	182.6±9.8a	864.7±54.6a	709.1±33.7a	4.9±0.36ab	5.7±0.42b	63.0±2.4a	97.8±5.3a
Inter-row maize	1100±60a	2100±90a	104.1±13.5ab	65.5±17.2a	109.4±8.3a	104.3±6.8a	129.8±4.9a	182.5±11.5a	863.8±35.6a	708.3±56.9a	5.3±0.33a	5.9±0.31b	65.4±2.2a	96.9±4.7a
Intra-row maize	1100±70a	2300±130a	129.3±18.4a	60.9±12.3a	114.7±7.2a	112.7±5.3a	136.0±4.5a	175.3±8.2a	873.2±46.4a	722.5±40.9a	5.7±0.40a	6.6±0.33a	66.0±3.1a	95.3±5.2a
Intra-hole maize	1100±50a	2300±90a	101.3±12.0b	66.2±13.4a	92.6±5.0b	106.3±7.7a	126.1±5.8a	177.3±9.7a	828.2±52.7a	680.7±38.4a	4.1±0.37b	6.2±0.47a	65.8±3.3a	92.3±3.6a
<b>Species</b>														
Cowpea	1100±30a	2100±50a	93.6±7.0b	61.0±6.6b	80.9±2.5b	100.7±2.2b	113.6±3.1b	162.3±4.9b	783.5±25.1b	674.9±29.1a	4.4±0.2b	5.9±0.2a	71.8±1.55a	89.4±2.0b
Maize	1100±30a	2200±60a	115.4±8.5a	66.1±7.3a	108.1±3.6a	111.0±3.2a	131.6±2.8a	179.4±4.8a	857.5±23.3a	705.1±21.1a	5.0±0.2a	6.1±0.2a	65.1±1.36b	95.6±2.3a

*Table 3.4.* Concentration of extractable mineral elements in the rhizosphere soil of nodulated cowpea and maize plants supplied with exogenous P in different cropping systems in 2003 and 2004. Values followed by dissimilar letters in the same column (bold type) for P level, cropping system, or species are significant at  $P \leq 0.05$ . A 2-way ANOVA was used to analyze P and cropping system effects while a one-way ANOVA was used to compare mineral elements concentrations associated with the rhizosphere of cowpea and maize. nd = not determined.

Treatment	Concentration of microelements (mg.kg <sup>-1</sup> )											
	Cu		Zn		Mn		B		Fe		Al	
	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004	2003	2004
<b>Cowpea</b>												
<b>P level (kg P.ha<sup>-1</sup>)</b>												
P0	7.0±0.37a	0.84±0.12a	<b>3.1±0.25b</b>	1.9±0.13a	15.4±0.71a	11.1±0.78a	<b>0.51±0.023b</b>	0.56±0.20a	<b>125±7.1b</b>	<b>106±3.9b</b>	nd	1.1±0.1a
P40	6.9±0.37a	0.96±0.17a	<b>3.7±0.32ab</b>	2.2±0.22a	17.3±0.99a	10.7±0.74a	<b>0.58±0.022a</b>	0.34±0.02a	<b>142±6.7a</b>	<b>146±11.2a</b>	-	1.1±0.1a
P80	7.6±0.25a	0.86±0.14a	<b>4.3±0.56a</b>	2.1±0.13a	16.4±0.72a	11.4±0.81a	<b>0.51±0.024b</b>	0.36±0.04a	<b>141±6.1a</b>	<b>148±11.6a</b>	-	1.1±0.1a
<b>Cropping systems</b>												
Sole cowpea	6.9±0.45a	0.87±0.13a	3.6±0.46a	1.8±0.17a	15.3±0.72a	10.4±0.7a	0.52±0.029a	0.33±0.031a	128±6a	135±4a	-	<b>1.3±0.12a</b>
Inter-row cowpea	7.3±0.33a	0.91±0.18a	3.5±0.37a	2.0±0.17a	17.3±1.03a	11.3±1.0a	0.53±0.027a	0.35±0.042a	127±8a	130±12a	-	<b>1.1±0.10b</b>
Intra-row cowpea	7.2±0.44a	1.01±0.24a	3.9±0.70a	2.3±0.22a	16.6±1.16a	11.6±0.9a	0.55±0.031a	0.60±0.269a	145±8a	132±10a	-	<b>1.0±0.09b</b>
Intra-hole cowpea	7.4±0.36a	0.76±0.09a	3.8±0.33a	2.1±0.19a	16.3±0.86a	11.0±1.0a	0.54±0.027a	0.39±0.043a	144±8a	137±13a	-	<b>0.9±0.10b</b>
<b>Maize</b>												
P0	8.2±0.29a	0.91±0.10a	4.4±0.25a	2.09±0.16a	18.3±0.68a	13.1±1.24a	0.59±0.018b	0.36±0.02a	<b>138±3.8b</b>	<b>141±11b</b>	-	0.82±0.06a
P40	7.8±0.28a	0.96±0.11a	4.9±0.31a	2.24±0.17a	19.0±0.88a	11.7±0.86a	0.66±0.033a	0.36±0.02a	<b>161±9.6a</b>	<b>143±8b</b>	-	0.97±0.14a
P80	8.0±0.27a	0.73±0.07a	4.8±0.42a	2.32±0.20a	18.3±0.90a	11.6±0.81a	0.56±0.024b	0.35±0.03a	<b>150±7.5ab</b>	<b>180±11a</b>	-	1.03±0.16a
<b>Cropping systems</b>												
Sole maize	<b>8.2±0.4ab</b>	0.94±0.12a	<b>5.0±0.4a</b>	2.4±0.25a	<b>19.0±1.0ab</b>	11.8±1.0a	<b>0.63±0.03a</b>	0.34±0.019a	151±11a	159±11a	-	1.0±0.20a
Inter-row maize	<b>8.3±0.2a</b>	0.87±0.14a	<b>5.0±0.3a</b>	2.4±0.20a	<b>19.2±0.9a</b>	13.1±1.7a	<b>0.62±0.03a</b>	0.35±0.019a	146±8a	169±16a	-	0.9±0.07a
Intra-row maize	<b>8.2±0.2ab</b>	0.91±0.09a	<b>5.1±0.4a</b>	2.0±0.14a	<b>19.7±0.7a</b>	12.6±0.7a	<b>0.63±0.03a</b>	0.35±0.019a	158±9a	152±11a	-	1.0±0.19a
Intra-hole maize	<b>7.4±0.4b</b>	0.76±0.09a	<b>3.8±0.3b</b>	2.1±0.19a	<b>16.3±0.9b</b>	11.0±1.0a	<b>0.54±0.03b</b>	0.39±0.043a	144±8a	137±13a	-	0.9±0.10a
<b>Species</b>												
Cowpea	<b>7.2±0.19b</b>	0.89±0.08a	<b>3.7±0.2b</b>	<b>2.0±0.10b</b>	<b>16.4±0.5b</b>	<b>11.1±0.4b</b>	<b>0.54±0.01b</b>	0.42±0.07a	<b>136±4b</b>	<b>133±6b</b>	-	1.08±0.05a
Maize	<b>8.0±0.16a</b>	0.87±0.06a	<b>4.7±0.2a</b>	<b>2.2±0.10a</b>	<b>18.6±0.57a</b>	<b>12.1±0.6a</b>	<b>0.61±0.02a</b>	0.36±0.01a	<b>150±4a</b>	<b>155±7a</b>	-	0.94±0.07a

Table 3.5. Effects of P supply and cropping system on the uptake rate of mineral elements in whole of cowpea and maize planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (bold type) for are significant at  $P \leq 0.05$ . nd = not determined. The effects of P supply and cropping system on some mineral elements in whole plant uptake rate in cowpea and maize were not significant in Year 1.

Treatment	mg plant <sup>-1</sup> d <sup>-1</sup>						µg plant <sup>-1</sup> d <sup>-1</sup>					
	P	K	Mg	Ca	S	Na	Cu	Zn	Mn	B	Fe	Al
<b>Year 1: Effects of cropping system on uptake rate in cowpea</b>												
Mono cowpea	2.0±0.2a	8.1±0.7a	1.4±0.1a	6.7±0.8a	1.1±0.1a	0.3±0.02a	5.3±0.5a	25.9±3.2a	16.8±2.3a	19.2±1.9a	300.9±30.6a	-
Inter-row cowpea	1.7±0.1ab	7.4±0.5a	1.3±0.1a	6.2±0.4a	1.0±0.1a	0.3±0.02a	4.6±0.3a	21.6±1.8ab	13.5±1.0ab	17.8±1.3a	259.5±26.7a	-
Intra- row cowpea	1.6±0.1ab	7.2±0.6a	1.3±0.1a	5.5±0.5a	1.1±0.1a	0.3±0.02a	4.5±0.3a	19.8±2.1ab	12.1±1.2ab	17.4±1.5a	286.4±35.8a	-
Intra – hole cowpea	1.5±0.2b	6.3±0.6a	1.1±0.1a	5.1±0.7a	0.9±0.1a	0.2±0.03a	4.1±0.4a	18.4±2.6b	11.5±1.6b	16.2±2.2a	293.1±42.3a	-
<b>Year 1: Effects of P on uptake rate in maize</b>												
P0	3.7±0.46b	28.9±2.5b	3.2±0.30b	4.6±0.34a	0.56±0.05a	0.88±0.08a	11.0±0.8b	45.3±6.1b	33.5±2.9a	8.3±0.64a	896.7±63.5a	nd
P40	3.7±0.32b	29.2±2.1b	3.1±0.24b	4.9±0.33a	0.59±0.04a	0.87±0.10a	13.2±2.4b	40.0±5.1b	30.9±2.7a	8.8±0.56a	901.9±42.7a	-
P80	5.3±0.40a	36.8±2.9a	4.0±0.27a	5.4±0.30a	0.67±0.04a	0.90±0.05a	17.3±2.7a	58.4±5.9a	39.6±3.0a	9.9±0.55a	973.4±111.4a	-
<b>Year 2: Effects of P on uptake rate in cowpea</b>												
P0	1.0±0.21b	8.3±1.7b	1.3±0.19b	5.7±0.8b	1.1±0.13b	0.36±0.05b	4.4±0.8b	21.7±2.6b	22.4±2.8b	19.6±3.0b	189.0±24.0b	275.3±24.9b
P40	2.0±0.22a	12.9±1.8a	2.0±0.19a	9.9±1.1a	1.6±0.13a	0.58±0.07a	6.2±0.9a	26.7±3.5a	35.4±4.9a	30.8±3.6a	258.9±27.2a	394.8±40.9a
P80	2.2±0.39a	12.9±2.5a	2.1±0.32a	9.0±1.6a	1.7±0.31a	0.56±0.08a	5.8±1.2a	27.6±6.0a	37.1±5.8a	28.3±4.6a	232.6±33.9a	337.7±36.5a
<b>Year 2: Effects of cropping system on uptake rate in cowpea</b>												
Mono cowpea	3.1±0.45a	20.2±2.8a	2.9±0.34c	13.7±1.8a	2.5±0.32a	0.77±0.09a	10.1±1.3a	47.1±5.5a	44.9±6.3a	42.4±5.1a	360.3±26.6a	478.5±31.4a
Inter-row cowpea	1.6±0.20b	11.3±1.3b	1.8±0.15b	8.0±0.8b	1.4±0.12b	0.53±0.05b	5.2±0.5b	23.7±2.0b	37.5±5.5b	26.2±2.5b	238.0±27.4b	356.6±43.4b
Intra- row cowpea	1.2±0.17bc	7.6±1.5bc	1.3±0.18bc	5.9±0.9bc	1.1±0.11bc	0.39±0.07bc	3.6±0.7bc	16.4±2.2bc	24.7±3.6bc	19.3±3.5bc	167.1±18.9bc	266.7 <sup>c</sup> ±23.9b
Intra – hole cowpea	1.0±0.13c	6.4±1.1c	1.1±0.15c	5.2±0.7c	0.9±0.12c	0.32±0.05c	3.0±0.4c	14.1±1.7c	19.6±3.8c	17.0±2.5c	142.1±19.2c	242.0±29.8c
<b>Year 2: Effects of P on uptake rate in maize</b>												
P0	1.7±0.29b	17.2±2.3	2.3±0.28	2.4±0.36	0.65±0.07b	0.76±0.11	3.3±0.47	38.7±4.0	30.7±3.6b	6.0±0.80	411.9±93.9	571.8±137.8
P40	3.1±0.35a	23.2±2.3	3.1±0.34	3.1±0.30	1.03±0.10a	0.88±0.09	4.1±0.38	53.9±6.0	44.0±5.3a	8.1±0.83	401.6±49.3	518.8±62.1
P80	2.8±0.35a	22.2±2.2	3.1±0.37	3.0±0.36	1.21±0.24a	0.78±0.07	4.0±0.39	53.5±6.9	53.7±6.6a	8.0±0.86	482.8±71.2	699.1±130.5
<b>Year 2: Effects of cropping system on uptake rate in maize</b>												
Mono cowpea	2.7±0.43a	22.7±2.5a	3.3±0.43a	3.2±0.48a	1.37±0.32a	0.96±0.10a	4.4±0.51a	53.5±7.8a	46.1±7.3a	8.6±1.10a	478.4±106.3a	664.8±185.5a
Inter-row cowpea	2.5±0.46a	19.2±3.3a	2.7±0.47a	2.7±0.43a	0.79±0.12b	0.79±0.11a	3.1±0.43a	47.1±8.9a	40.2±7.3a	6.5±1.07a	357.3±43.7a	475.9±53.3a
Intra- row cowpea	2.6±0.44a	21.3±2.7a	2.7±0.35a	2.6±0.34a	0.84±0.10b	0.76±0.10a	3.8±0.50a	49.0±5.0a	38.5±5.3a	7.0±0.93a	464.3±68.5a	626.0±97.9a
Intra – hole cowpea	2.4±0.36a	20.1±2.4a	2.6±0.29a	2.7±0.33a	0.84±0.08b	0.71±0.11a	3.7±0.46a	45.2±5.5a	46.4±6.9a	7.4±0.81a	428.4±107.0a	619.7±160.8a

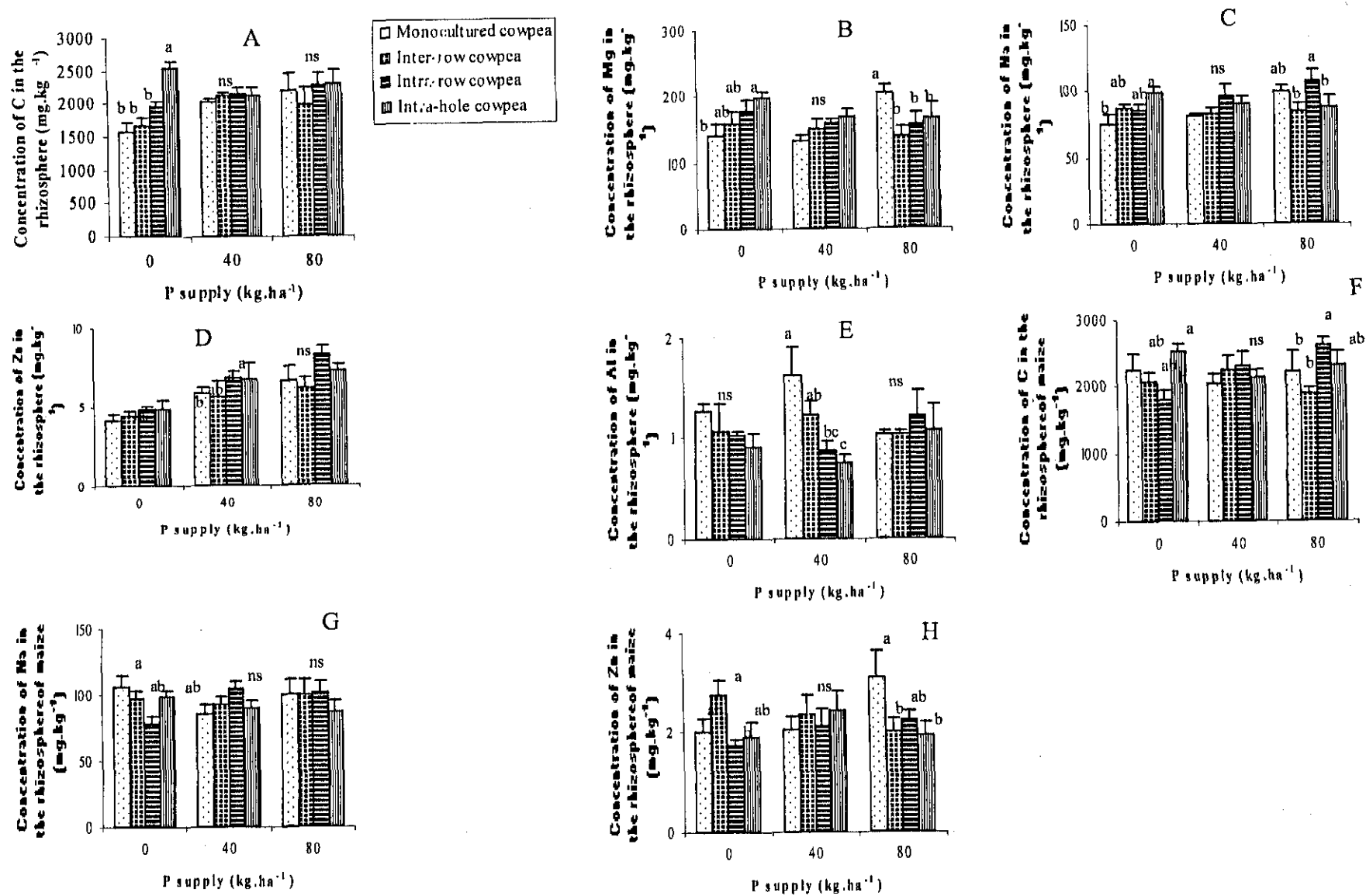


Figure 3.1 . Interactive effects of P and cropping system on the concentration of mineral elements in rhizosphere soil of cowpea planted in 2004 (Year 2). A-E = concentration of mineral elements in cowpea rhizosphere plants; F-H = concentration of mineral elements in maize rhizosphere plants. Vertical line on bars represent the standard error of mean. For each P level, different letters on bars indicate significantly different means at P = 0.05. ns = not significant

## CHAPTER 4

TISSUE CONCENTRATION OF MINERAL NUTRIENTS IN NODULATED COWPEA (*VIGNA UNGUICULATA* L. WALP.) AND MAIZE (*ZEA MAYS* L.) SUPPLIED WITH EXOGENOUS P IN MIXED PLANT CULTURE.

#### 4.1 Introduction

Studies on mineral nutrition of plants have been well documented (Marschner, 1995). However, many of those studies have either been limited to a few single mineral nutrients, or conducted in monoculture. Yet plant ecologists and agronomists tend to work with all the nutrients in mixed plant stands in natural ecosystems or intercropped material in agricultural systems. This approach has resulted in limited knowledge of plant-plant, plant-nutrient, and nutrient-nutrient interactions in the rhizosphere of field plants. Detailed studies are currently lacking on the uptake profile and tissue accumulation of the major mineral nutrients in relation to planting patterns (i.e. monoculture vs. mixed cultures), and/or when supplemented with exogenous nutrient elements.

Nutrient uptake and accumulation in plants can be influenced by the level of other nutrients in the soil solution and inside plant tissues. For example, studies by Baligar *et al.* (2001) have shown that, Al supply and its accumulation in alfalfa can significantly increase tissue concentrations of N, P, S, K, Mg, Zn, and Mn from decreased growth of shoots and roots. Low tissue P is also known to induce the accumulation of carbohydrates, which indirectly affect the uptake of other nutrient elements (Reinbott and Blevins, 1997). This is because an increase in the plant's internal P status can decrease soluble carbohydrates in roots and root exudates (Graham *et al.*, 1981; Same *et al.*, 1983) and, thus, affect nutrient uptake. However, adequate P nutrition is also known to enhance the uptake of other nutrients through improved overall plant growth, especially root development (Reinbott and Blevins, 1997). It has, however, been shown that increasing P availability and uptake can lower the level of available Zn in soil (Lindsay *et al.*, 1962). Carroll and Loneragan (1998) measured root and shoot concentrations of Zn in the presence of excess P and showed that as much as 35% of total absorbed Zn remained in roots, resulting in Zn deficiency symptoms in shoots. Thus, excess uptake of P by legumes and cereals can increase Zn accumulation in roots at the expense of its translocation to shoots (Takkar *et al.*, 1976). Applying

P to common bean and maize was found to also decrease the concentration of Zn in tissues (Christensen and Jackson, 1981; Singh *et al.*, 1988; Gianquinto *et al.*, 2000).

Root exudates also play a major role in the mineral nutrition of plants (Marschner, 1995; Dakora and Phillips, 2002). Of the root exudates released by some tree species, 71% were found to be cations ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{NH}_4^+$ ,  $\text{Mg}^{2+}$ ), 12% anions ( $\text{SO}_4^{2-}$ ,  $\text{Cl}^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{NO}_3^-$ ), 11% organic acids, 5% carbohydrates and 1% amino acids (Smith, 1976). The presence of minerals in root exudates can, therefore, significantly alter the composition of nutrients in soil solution, and hence increase or decrease their uptake, as well as modify their concentrations in plant organs. Furthermore, protons released into the rhizosphere of symbiotic legumes from  $\text{N}_2$ -fixing activity in nodules can also alter soil pH and affect nutrient mobility, availability and uptake by plants (Schubert *et al.*, 1990; McLay *et al.*, 1997; Dakora and Phillips, 2002; Cheng *et al.*, 2004). Such a pH change could enhance or inhibit mineral availability (e.g. P) in the soil solution, as well as affect the uptake of Ca, Fe, and Al by plant roots (Hinsinger and Gilkes, 1996; Dakora and Phillips, 2002).

Differences in organ concentrations of minerals have been reported for several plant species in monoculture (Hart *et al.*, 1981; Brink and Fairbrother, 1992; Ciarelli *et al.*, 1998; Pederson *et al.*, 2002), with some data showing that forage and food legumes accumulate greater concentrations of nutrients in tissue relative to grasses and cereals (Fleming, 1963; Kubota, 1983; Minson, 1990; Pederson *et al.*, 2002; Fageria, 2004). While such findings could be attributed to species differences in nutrient acquisition, they could also be ontogenic, stemming from dilution of tissue mineral concentrations by added biomass during plant development (Loneragan *et al.*, 1979; Jarrell and Beverly, 1981; Sing *et al.*, 1988; Fageria, 2004).

So far, however, most studies of mineral nutrition in legumes and cereals have focused on monoculture and the analysis of single or few nutrient elements. To our knowledge, no study has yet examined mineral uptake and tissue accumulation in complex mixed cultures, even though the findings of such studies could benefit plant ecologists, plant nutritionists, and field agronomists. This study assesses the effect of exogenous P supply on tissue concentrations of minerals in nodulated cowpea and maize in mixed plant cultures.

## **4.2 Materials and Methods**

### **4.2.1 Plant growth and growth conditions**

Field experiments were conducted under irrigation at the Agricultural Research Council Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa, during the 2003 and 2004 summer seasons at two different locations within Nietvoorbij. The site characteristics were described in detail in chapter 2 section 2.2.1.

### **4.2.2 Experimental design**

The experimental treatments consisted of three levels (0, 40, and 80 kg P.ha<sup>-1</sup>) of triple superphosphate [46% P, 13.5% Ca, 1.5% S, 0.0007% Cu, 0.008% Zn, 0.021% Mn, 0.053% B and 0.0009% Mo (Cherney, 2003; FAS, 2005)] and 4 planting patterns (namely, monoculture, maize/cowpea inter-row, maize/cowpea intra-row, and maize/cowpea intra-hole planting). The experimental design and layout, and other cultural practices are well described in chapter 2 section 2.2.2

### **4.2.3 Plant harvest and sample preparation**

At 60 d after planting, cowpea and maize plants were sampled for nutrient analysis. About 16 and 8 plants were sampled for cowpea and maize respectively from the middle rows of each plot. The border plants within each row were excluded. The plants were carefully dug out with



their entire root system, washed, and cowpea plants separated into nodules, roots, shoot and pods, while maize plants were divided into roots and shoots. The plant organs were oven-dried at 60 °C for 48 h and ground into a fine powder for the analysis of mineral nutrients.

#### **4.2.4 Characterisation of soil chemical properties**

The determination of soil pH, soil organic carbon, soil organic matter (SOM), S, P, K, Na, Ca, Mg, Cu, Zn, Mn, Fe, and Al were extracted from soil following the procedures outlined in chapter 3 sections 3.2.5 and 3.2.6.

#### **4.2.5 Measurement of mineral nutrients in organs**

Measurements of macronutrients (P, K, Ca, Mg, and Na) and micronutrients (Cu, Zn, Mn, Fe, Al, B) were determined by ashing 1 g ground sample in a porcelain crucible at 500 °C overnight. This was followed by dissolving the ash in 5 mL of 6 M HCl and placing it in an oven at 50 °C for 30 min; 35 mL of deionised water were added and extract filtered through Whatman no. 1 filter paper. Nutrient concentrations in plant extracts were determined using the ICP spectrophotometer (Giron, 1973). Sulphur was determined by wet digestion procedure using 65% (v/v) nitric acid. In each case, 1 g of milled plant material was digested overnight with 20 mL of 65% (v/v) nitric acid in a 250 mL glass beaker. The beaker containing the extract was then placed on a sand bath and gently boiled until approximately 1 mL of the extract was left. After that, 10 mL of 4 M nitric acid was added and boiled for 10 min. The beaker was then removed from the sand bath, cooled, and the extract washed completely in a 100 mL volumetric flask and the extract filtered through Whatman no. 2 filter paper. The S in the sample was then determined by (FSSA, 1974) direct aspiration on the calibrated simultaneous ICP spectrophotometer.

#### **4.2.6 Statistical analysis**

A 2-factorial design (2-way ANOVA) was used to analyse for mineral in plant organs. However, a one-way ANOVA was used to compare nutrient concentrations in the legume and cereal. The analysis was done using the software of STATISTICA program 1997. Fisher's least significant difference was used to compare treatment means at  $P \leq 0.05$  (Steel and Torrie, 1980).

### **4.3 Results**

#### **4.3.1 Soil properties at planting**

The soil chemical properties from the experimental plots were measured before establishing the experiment in 2003 (Year 1) and 2004 (Year 2). In Year 1, the soil pH was 6.3; SOM: 3.2 g.kg<sup>-1</sup>; C: 0.9 g.kg<sup>-1</sup>; P: 40 mg.kg<sup>-1</sup>; K: 104 mg.kg<sup>-1</sup>; Ca: 8.4 mg.kg<sup>-1</sup>; Mg: 122 mg.kg<sup>-1</sup>; S: 3.1 mg.kg<sup>-1</sup>; Na: 0.10 mg.kg<sup>-1</sup>; Cu: 8.4 mg.kg<sup>-1</sup>; Zn: 2.5 mg.kg<sup>-1</sup>; Mn: 20 mg.kg<sup>-1</sup>; B: 0.7 mg.kg<sup>-1</sup>; and Fe was 123 mg.kg<sup>-1</sup>.

In Year 2, soil pH was 6.3; SOM: 3.1 g.kg<sup>-1</sup>; C: 1.9 g.kg<sup>-1</sup>; P: 8.8 mg.kg<sup>-1</sup>; K: 142 mg.kg<sup>-1</sup>; Ca: 521 mg.kg<sup>-1</sup>; Mg: 122 mg.kg<sup>-1</sup>; S: 3.7 mg.kg<sup>-1</sup>; Na: 0.07 mg.kg<sup>-1</sup>; Cu: 0.80 mg.kg<sup>-1</sup>; Zn: 1.8 mg.kg<sup>-1</sup>; Mn: 9.7 mg.kg<sup>-1</sup>; B: 0.34 mg.kg<sup>-1</sup>; Fe: 131 and Al was 0.68 mg.kg<sup>-1</sup>.

#### **4.3.2 Effects of P supply on mineral concentrations in shoots, roots and whole plants of cowpea and maize**

Applying P to cowpea plants significantly increased shoot concentration of P in both Year 1 and 2, and Cu and Zn in Year 2 only (Table 4.1). Exogenous P supply also increased shoot levels of Ca and P in maize plants in Year 1, and only P in year 2 (Table 4.1).

With roots, however, there were major changes in the concentrations of nutrients with P application to both cowpea and maize. Provision of P significantly increased root concentrations of K, S and P in cowpea plants in Year 1, but decreased those of Cu, Zn and Mn (Table 4.1). In Year 2, the levels of S, Na, and P were elevated with mineral P supply to cowpea, while those of Cu, Zn and B were again decreased relative to control (Table 4.1). With maize, the effect of P application also showed increased Ca and P, and decreased Zn in roots of Year 1 plants (Table 4.1). In year 2, only P concentration was increased in maize roots with exogenous P supply (Table 4.1).

At whole-plant level, nutrient concentrations in cowpea were more altered with P application than in maize. As shown in Table 4.1, supplying cowpea with mineral P in Year 1 increased tissue levels of Ca, S, and P, but decreased the concentration of Cu relative to zero-P control. In Year 2, tissue levels of S, Na and P were also elevated with P supply, whereas those of Cu, Zn and B were reduced compared with control (Table 4.1). As with shoots, only Ca and P showed increased concentrations at whole-plant level with external P supply in Year 1 and 2 (Table 4.1).

#### **4.3.3 Effects of exogenous P supply and mixed plant culture on mineral concentration in pods and nodules of cowpea**

Applying P to cowpea plants significantly increased nodule concentration of Mg, S and P, but not Ca or K in Year 1 (Table 4.2). In contrast, the concentration of micronutrients such as Cu, Zn, Mn and Al were significantly reduced with P supply in Year 1 (Table 4.2). Nodule concentration of P and S were increased in Year 2 with P supply, whereas that of Zn decreased relative to zero-P control (Table 4.1).

The concentrations of mineral nutrients in cowpea pods were also altered with P supply. For example, in Year 1, the levels of P, Mg and Ca were increased in pods with P application, whereas those of Cu and Zn decreased (Table 4.2). In Year 2, the pod concentrations of P, K, and Mg also increased with P fertilisation, whereas those of Cu, Zn and B again decreased relative to zero-P control (Table 4.2).

The effects of each separate planting system on nodule concentration of nutrients were assessed and found to be more clearly defined in Year 1 than in Year 2. As shown in Table 4.2, the nodule concentrations of P, K and Al were significantly more reduced in intra-hole planted cowpea relative to monoculture and the other mixed cultures. However, intra-hole cowpea nodules showed greater Ca concentration, followed by the other mixed cultures, and least the monoculture. With Mn, the nodule concentration was greater in mono-cowpea, followed by intra-hole, and least in inter-row and intra-row cowpea plants in Year 1 (Table 4.2). In Year 2, the levels of Cu and Fe were decreased in mix-cultured cowpea relative to monoculture, Al was significantly increased in nodules of intra-hole cowpea compared with mono- and mix-cultured cowpea (Table 4.2).

As with nodules, cowpea pods also showed changes nutrient concentrations with planting system in Year 1, but not Year 2. The levels of Cu and Zn in cowpea pods were, for example, decreased by mixed culture compared with monoculture (Table 4.2).

#### **4.3.4 Effects of planting pattern on nutrient concentrations in shoots, roots and whole plants of cowpea and maize**

Separating the effects of the individual components of the planting system for each species revealed significant differences in nutrient concentrations of plant organs. In cowpea, the shoot levels of P, Cu and Zn were decreased in mixed culture relative to monoculture in Year 1, with Fe the concentration was significantly greater in intra-hole cowpea compared with mono-cowpea or the other mixed cultures (Table 4.3). In Year 2, shoot concentrations of K and Cu were lowest in intra-hole cowpea, but greater in monocultured material (Table 4.3). Shoot Ca and S were also greater in intra-hole and intra-row cowpea compared to the other planting patterns. In Year 2, there was also a significant increase in shoot B levels in intra-hole and intra-row cowpea plants compared with mono-cowpea (Table 4.3).

Except for Fe, which showed significantly increased concentration in intra-hole planted maize relative to monoculture, there was little effect of planting pattern on nutrient concentrations in maize shoots in Year 1 (Table 4.3). However, shoot levels of K and Zn in Year 2 were much greater in intra-hole maize compared with the monoculture, just as shoot B of intra-hole maize was also significantly greater than monoculture (Table 4.3). Shoot concentration of Mg in inter-row maize was similarly increased relative to monoculture and intra-hole plants in Year 2 (Table 4.3).

The concentrations of minerals in cowpea roots were altered by planting pattern in Year 1. As shown in Table 4.3, root levels of P were significantly decreased by mixed culture relative to monoculture in Year 1. The concentration of K in roots was lowered in only intra-hole cowpea, but not in the other planting systems. Root levels of Na in Year 1 were significantly increased in intra-hole and intra-row cowpea plants compared with monoculture, while with Mn the root

concentration in intra-hole cowpea was significantly greater than that of the other mixed cultures (Table 4.3). In Year 2, root concentrations of P were significantly lower in inter-row and intra-hole cowpea plants, while with B it was lower in only inter-row cowpea (Table 4.3).

Although the planting system had an effect on root concentrations of K, Mg and Na in maize in Year 1, there was no effect in Year 2 (Table 4.3). The concentrations of K, Mg and Na in roots of intra-row maize were significantly increased relative to only intra-hole plants in Year 1 (Table 4.3).

At whole-plant level, the tissue concentrations of P and, to some extent, K, Zn and Mn in cowpea were decreased in mixed culture relative to monoculture in Year 1 (Table 4.4). But the concentrations of Na and Fe in Year 1 were significantly increased in intra-hole cowpea when compared with monoculture or other mixed cultures (Table 4.4). However, the levels of K and Cu were relatively decreased in mixed cultures compared with monoculture (Table 4.4). In contrast, the concentrations of Ca and B were significantly increased in intra-hole cowpea compared with monoculture or inter-row plants (Table 4.4).

Except for Na and K, there was no effect of planting pattern on mineral concentration in maize. Relative to the other planting systems, Na levels were lower in intra-hole cowpea in Year 1 and 2, and in monoculture in Year 1 (Table 4.4). Compared with monoculture, mix-cultured maize showed greater K concentration in tissues, especially in intra-row plants (Table 4.4).

Comparing the effects of planting system across species for the two years showed significantly greater nutrient concentrations in cowpea organs relative to maize (data not shown)

#### **4.3.5 Species effect on nutrient concentrations in shoots, roots and whole plants of cowpea and maize**

A one-way ANOVA analysis was done to compare nutrient concentrations in organs of the two test species (cowpea and maize). In all instances, the concentrations of P, K, Mg, Ca, S, Na, Cu, Zn, Mn, B, Fe and Al were significantly greater in shoots, roots and whole plants of the legume (cowpea) relative to the cereal (maize) in both Year 1 and 2 (Table 4.5).

#### **4.3.6 Interactive effects of applied P and mixed plant culture on the concentration of Cu in cowpea and maize shoots, and in root nodules of cowpea**

There was a significant interactive effect between applied P and shoot concentrations of Cu. At 40 kg P.ha<sup>-1</sup> or 80 kg P.ha<sup>-1</sup>, shoot concentration of Cu in monocultured cowpea was significantly greater than that of mixed cultures in Year 2 (Figure 4.2).

The nodule concentration of Cu in cowpea was also significantly affected by the interactive effects of P supply and planting system in Year 1. At zero-P, monocultured cowpea showed increased concentration of Cu in root nodules, followed by intra-hole cowpea (Figure 4.1). At 80 kg P.ha<sup>-1</sup>, nodules from monoculture and intra-hole cowpea showed significantly elevated concentrations of Cu relative to the other planting patterns (Figure 4.1). However, at 40 kg P.ha<sup>-1</sup>, root nodules of inter-row cowpea exhibited the greatest concentration of Cu (Figure 4.1).

### **Discussion**

#### **Effects of exogenous P on plant growth and tissue nutrient concentrations**

In this study, the exogenous supply of P to cowpea and maize in two consecutive field experiments altered the mineral concentration in all organs analysed (Tables 4.1 and 4.2).

However, P application significantly increased the concentrations of P, Mg, Ca and K in cowpea shoots and pods in Year 2 (Tables 4.1 and 4.2), and led to increased organ and plant growth (Table 6.1) even though micronutrient levels (Cu, Zn and B) were significantly decreased (Tables 4.1 and 4.2). Similar changes in nutrient concentrations were observed in belowground organs. As shown in Tables 4.1 and 4.2, applying P to cowpea plants in Year 1 and 2 increased root and nodule concentrations of P, K, Mg, S and Na, but decreased those of the trace elements Cu, Zn, Mn, B and Al. The decreased level of Zn in root nodules in Year 2 (Table 4.2) was likely due to dilution by the significantly increased nodule dry matter (Table 6.1; Loneragan *et al.*, 1979; Jarrell and Beverly, 1981; Sing *et al.*, 1988; Fageria, 2004), which was, in turn, caused by increased accumulation of P and S in those organs with external P supply (Table 4.2).

Integrating the data at whole-plant level showed that tissue concentrations of P, Ca, S and Na were significantly increased in both Year 1 and 2 in cowpea, but those of Zn, Cu and B were decreased in Year 2 from dilution by accumulated biomass (Table 6.1; Loneragan *et al.*, 1979; Jarrell and Beverly, 1981; Sing *et al.*, 1988; Fageria, 2004). While the data for maize showed a limited effect of external P supply, the levels of Ca and P were significantly increased in shoots, roots and whole plants in Year 1 and 2. Considered across all organs, the concentrations of P, Mg, Ca, K, S and Na in cowpea were found to increase with external P supply, while those of Zn, Cu, Mn, B and Al decreased either in the first or second year of experimentation, or both. In Africa, where cowpea leaves and green pods are eaten as vegetables, P supplementation to this legume would, therefore, have the added advantage of increased concentrations of dietary Ca, Mg, K and P, but at the expense of decreased micronutrients such as Zn, Cu and B (Tables 4.1 and 4.2).



While the mechanisms underlying P-induced changes in tissue mineral concentration are still unknown, data do exist which show that this phenomenon is widespread among plant species. For example, as found with cowpea in this study, increasing P supply to monocultured winter wheat significantly increased shoot concentrations of P, Ca and Mg (Reinbott and Blevins, 1991; 1994). P-induced Zn deficiency has also been detected in common bean and cotton, especially under low Zn conditions in soil (Cakmak and Marschner, 1986; Gianquinto *et al.*, 2000), a finding consistent with the results obtained here. The data from this study seem to suggest that P supply in Year 2 promoted plant growth and biomass accumulation, leading to dilution of some trace elements in tissues (Tables 4.1 and 4.2; Loneragan *et al.*, 1979; Jarrell and Beverly, 1981; Sing *et al.*, 1988; Fageria, 2004). Additionally, however, other mechanisms exist which can also alter mineral concentrations in plant tissues. For example, shoot level of nutrients such as Cu, Mn, Mg, Ca are reported to increase in pea and *Arabidopsis* plants when exposed to low Fe regime (Welch *et al.*, 1993; Rodecap *et al.*, 1994). *Commelina communis* has also been shown to increase its tissue accumulation of Cu when Fe is deficient, an event that was linked to elevated activity of Fe (III) chelate reductase (Chen *et al.*, 2004). However, whether the decreased concentrations of micronutrients (e.g. Zn, Cu, B, Mn and Al) obtained in this study for organs whose growth was unaltered by P supply in Year 1, were due to low Fe or external P supply, still remain to be properly assessed.

#### **Effects of mixed culture and species differences on mineral concentration in cowpea and maize**

Apart from the effects of applied P, tissue concentration of nutrients in maize and cowpea were also significantly altered by the components of the planting system used in this study. With cowpea, shoot levels of P, Cu and Zn in Year 1 and K and Cu in Year 2 were, for example,

significantly greater in monoculture compared with mixed culture (Table 4.3), possibly due to better mobilisation of mineral nutrients by the legume's root exudates in the monoculture (Ae *et al.*, 1990; Dakora and Phillips 2002). Relative to the other mixed cultures, the co-planting of cowpea and maize in one hole appeared to have also generally increased tissue concentration of many minerals, especially micronutrients, in the legume partner, with little change in the cereal. For example, shoot concentrations of Fe, Ca and B in Year 1 and 2, whole plant Na, Fe, Ca and B in Year 1 and 2, as well as nodule Ca and Al in Year 1, were all significantly increased by co-planting of cowpea with maize (in one hole) when compared with monoculture, or inter-row and intra-row grown plants. These findings are consistent with those of a recent report which showed that tissue concentration of Fe was significantly increased in groundnut plants grown with their roots closely intermingled with those of maize in a mixed culture (Zhang *et al.*, 2004).

Whether in this study the improved mineral nutrition of cowpea co-planted in one hole with maize was due to increased nutrient availability from mobilisation by phytosiderophores, phenolics and/or organic acid anions (Römheld, 1991; Hopkins *et al.*, 1992; Dakora and Phillips, 2002), was not assessed. However, the 22 instances of numerical increase in mineral concentration of cowpea organs and some 10 cases of significant elevation in tissue nutrient levels in cowpea co-planted with maize in one hole (but not in monoculture or other mixed cultures) clearly suggests the need for further experimentation. It is, however, interesting to note that Cu levels can be managed for optimal tissue concentrations using planting systems as monocultured cowpea and maize were found to increase their tissue levels of this mineral nutrient relative to mixed cultures (Figures 4.1 and 4.2).

A one-way ANOVA analysis was done to compare nutrient levels in cowpea (a legume) and maize (a cereal). The results revealed significantly large differences between these two species in

nutrient concentrations of shoots, roots and whole plants. In both Year 1 and 2, there was a significantly greater concentration of P, K, Mg, Ca, S, Na, Cu, Zn, Mn, B, Fe and Al in shoots, roots and whole plants of cowpea relative to maize (Table 4.5). These results are consistent with the findings of a few studies that compared single nutrients in legumes and cereals (Gladstones and Loneragan, 1967; Minson, 1990; Pederson *et al.*, 2002; Fageria, 2004). Analysis of nutrients in food grains from legumes and cereals also showed that the concentrations of N, P, K, Ca, Mg, Zn, Cu, Fe and B were greater in soybean and common bean relative to rice and corn (Fageria, 2004). This clearly indicates that species-specific differences exist between legumes and cereals (or grasses) in root uptake and tissue accumulation of minerals. Even among legumes, cereals, or within species and cultivars, strong differences exist in terms of tissue concentration of nutrients (Gross and Jung 1978; Baligar *et al.*, 2002; McCrimmon, 2002; Pederson *et al.*, 2002; McLaughlin *et al.*, 2004; Pomper and Grusak, 2004; Gahoonia *et al.*, 2005).

In conclusion, external P application significantly increased the concentrations of P, Mg, Ca and K in shoots and pods, but decreased the levels of Zn, Cu and B in the two years of experimentation. Exogenous P supply also increased root concentrations of P, K, S and Na, but decreased those of Zn, Cu, Mn and B. The concentrations of P, Mg and S were increased in nodules, while those of Zn, Cu, Mn and Al were decreased with P supply in Year 1. Similarly, nodule concentrations of P and S were increased in Year 2 in contrast to Zn which was decreased. At whole-plant level, tissue levels of P, Ca and S were increased in the two-year study, while the levels of Zn, Cu and B were decreased in only Year 2. At the species level, the concentrations of P, K, Ca, Mg, S, Na, Cu, Zn, Mn, B, Fe and Al were significantly greater in shoots, roots, and whole plants of cowpea relative to maize in the two years of study. With cowpea, shoot levels of P, K, Cu and B were greater in monoculture compared with mixed cultures in both Year 1 and Year 2, probably as a consequence of better mobilisation of mineral nutrients by legume root

exudates. The co-planting of cowpea with maize in one hole increased shoot Fe (Year 1), Mg, Ca and B (Year 2), as well as whole-plant Fe and B (Year 1) compared with monoculture, or the other mixed cultures. This suggests that organic molecules in root exudates of the cereal partner probably increased the availability and uptake of Fe and B by the legume.

Table 4.1. Effects of P application on the concentration of mineral elements in shoots, roots and whole plants of nodulated cowpea and maize planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (bold type) are significant at  $P \leq 0.05$ . Mg, Na, B and Fe were not statistically significant in Year 1; Ca, Mg, K, Mn, Fe and Al were also not significant in Year 2.

Treatment	mg g DM <sup>-1</sup>				µg g DM <sup>-1</sup>			mg g DM <sup>-1</sup>			µg g DM <sup>-1</sup>		
	P	K	Ca	S	Cu	Zn	Mn	P	S	Na	Cu	Zn	B
<b>SHOOTS</b>													
Year 1							Year 2						
<b>Cowpea (Shoot + Pods)</b>													
P0	<b>6.6±0.17b</b>	28.5±0.75a	24.8±0.85a	3.7±0.07a	17.4±17.4a	92.5±4.1a	57.9±4.1a	<b>4.3±0.23c</b>	4.9±0.22a	1.2±0.04a	<b>18.6±0.42a</b>	100.6±7.2a	87.9±2.4a
P40	<b>7.2±0.20a</b>	28.6±0.94a	26.4±0.72a	3.8±0.15a	16.8±16.8a	82.8±4.7a	51.8±3.2a	<b>5.6±0.26b</b>	4.6±0.28a	1.3±0.05a	<b>16.4±0.53b</b>	<b>73.4±4.5b</b>	86.1±1.6a
P80	<b>7.3±0.22a</b>	29.3±0.97a	24.9±0.80a	4.0±0.31a	16.5±16.5a	87.42±4.8a	59.3±3.4a	<b>6.3±0.34a</b>	4.8±0.22a	1.2±0.06a	<b>15.3±0.50c</b>	<b>69.9±4.3b</b>	83.0±2.1a
<b>Maize</b>													
P0	<b>3.2±0.23b</b>	24.8±0.59a	<b>3.7±0.13b</b>	0.42±0.02a	6.7±0.36a	30.0±3.6a	26.2±1.7a	<b>1.7±0.14b</b>	0.64±0.03a	0.43±0.02a	3.4±0.20a	46.8±3.8a	6.5±0.16a
P40	<b>3.2±0.19b</b>	25.0±0.56a	<b>4.0±0.08a</b>	0.43±0.02a	8.1±1.26a	37.2±3.2a	23.5±1.4a	<b>2.4±0.25a</b>	0.74±0.02a	0.42±0.01a	3.2±0.12a	45.7±3.1a	6.7±0.19a
P80	<b>3.6±0.17a</b>	25.2±0.81a	<b>4.2±0.13a</b>	0.44±0.01a	9.4±1.45a	33.7±3.5a	25.5±1.8a	<b>2.3±0.15a</b>	1.07±0.36a	0.39±0.01a	3.3±0.07a	44.9±1.5a	6.8±0.16a
<b>ROOTS</b>													
<b>Cowpea (Nodule + Root)</b>													
P0	<b>3.6±0.14b</b>	<b>31.0±1.16b</b>	8.3±0.23a	<b>5.4±0.27b</b>	<b>34.6±2.7a</b>	<b>75.0±6.1a</b>	<b>34.4±1.9a</b>	<b>2.5±0.13b</b>	<b>4.8±0.19b</b>	<b>5.4±0.20b</b>	<b>12.3±0.49a</b>	<b>87.6±6.4a</b>	<b>42.9±2.4a</b>
P40	<b>4.1±0.20a</b>	<b>32.1±1.21b</b>	8.8±0.32a	<b>6.6±0.37a</b>	<b>27.4±2.0b</b>	<b>71.4±4.8b</b>	<b>32.7±2.0a</b>	<b>3.3±0.15a</b>	<b>5.4±0.18a</b>	<b>5.6±0.24ab</b>	<b>11.5±0.37ab</b>	<b>56.6±5.1b</b>	<b>39.4±1.2b</b>
P80	<b>4.3±0.12a</b>	<b>34.2±1.15a</b>	9.2±0.26a	7.0±0.18a	<b>26.4±2.1b</b>	<b>65.7±4.4b</b>	27.8±1.6b	<b>3.6±0.21a</b>	<b>5.8±0.25a</b>	6.1±0.27a	<b>11.2±0.46b</b>	<b>60.8±7.4b</b>	<b>37.5±1.2b</b>
<b>Maize</b>													
P0	<b>0.75±0.05c</b>	7.5±0.37a	<b>1.2±0.04b</b>	0.35±0.02a	13.9±0.49a	<b>15.0±1.3a</b>	18.6±0.63a	<b>0.71±0.05c</b>	0.59±0.03a	2.3±0.79a	0.95±0.30a	2.2±0.41a	0.68±0.21a
P40	<b>0.91±0.07b</b>	7.3±0.46a	<b>1.3±0.05a</b>	0.38±0.02a	12.4±0.48a	<b>13.6±1.3b</b>	17.4±0.60a	1.11±0.10a	0.64±0.03a	1.6±0.09a	1.17±0.24a	4.2±1.34a	0.73±0.14a
P80	<b>1.04±0.05a</b>	7.2±0.38a	<b>1.4±0.06a</b>	0.37±0.02a	13.6±0.74a	<b>11.9±1.1b</b>	17.7±0.61a	<b>0.93±0.07b</b>	0.63±0.03a	1.4±0.13a	1.03±0.12a	3.5±0.44a	0.69±0.09a
<b>WHOLE PLANT</b>													
<b>Cowpea</b>													
P0	<b>5.1±0.12b</b>	29.6±0.72a	<b>16.6±0.46b</b>	<b>4.6±0.15b</b>	<b>26.0±1.3a</b>	83.8±4.4a	46.1±2.3a	<b>3.4±0.17b</b>	<b>4.8±0.15b</b>	<b>3.3±0.10b</b>	<b>15.5±0.35a</b>	<b>94.2±10.2a</b>	<b>130.8±3.1a</b>
P40	<b>5.7±0.16a</b>	30.4±0.85a	<b>17.6±0.36a</b>	<b>5.2±0.19a</b>	<b>22.1±0.9b</b>	77.2±3.6a	42.3±2.1a	<b>4.5±0.19a</b>	<b>5.0±0.19a</b>	<b>3.5±0.12ab</b>	<b>14.0±0.35b</b>	<b>60.0±2.8b</b>	<b>125.5±2.0b</b>
P80	<b>5.8±0.14a</b>	31.8±0.86a	<b>17.1±0.47ab</b>	<b>5.5±0.19a</b>	<b>21.4±1.1b</b>	76.6±3.7a	43.6±2.1a	<b>10.0±0.26a</b>	<b>5.3±0.19a</b>	<b>3.7±0.13a</b>	<b>13.6±0.37c</b>	<b>65.4±4.4b</b>	<b>120.5±2.9b</b>
<b>Maize</b>													
P0	<b>2.0±0.13b</b>	16.1±0.32a	<b>2.5±0.07b</b>	0.31±0.02a	10.3±0.3a	22.5±2.2a	22.4±0.7a	<b>1.2±0.10b</b>	0.62±0.03a	1.37±0.41a	2.2±0.25a	24.5±2.1a	3.6±0.19a
P40	<b>2.1±0.12b</b>	16.2±0.41a	<b>2.7±0.05a</b>	0.41±0.01a	10.3±0.7a	25.4±2.1a	20.5±0.8a	<b>1.8±0.17a</b>	0.69±0.03a	1.01±0.05a	2.2±0.15a	25.0±2.2a	3.7±0.17a
P80	<b>2.4±0.10a</b>	16.2±0.41a	<b>2.8±0.08a</b>	0.40±0.02a	11.5±0.7a	22.8±2.2a	43.2±1.1a	<b>1.6±0.10a</b>	0.85±0.07a	0.90±0.07a	2.2±0.10a	24.2±0.97a	3.8±0.13a

Table 4.2. Effects of P supply and mixed plant culture on the concentration of mineral elements in nodules and pods of cowpea planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (bold type) for are significant at  $P \leq 0.05$ . ns = not significant. The effects of planting system on pod mineral elements concentrations were not significant in Year 2.

Treatment	mg g DM <sup>-1</sup>						µg g DM <sup>-1</sup>					
	P	K	Mg	Ca	S		Cu	Zn	Mn	B	Fe	Al
<b>Year 1: Effects of P on nodules</b>												
P0	<b>2.5±0.11b</b>	18.4±0.67a	<b>4.7±0.17b</b>	3.3±0.12a	<b>2.0±0.09b</b>		15.4±1.99a	<b>53.0±4.6a</b>	18.0±1.47a	ns	4454±297a	<b>6031±404a</b>
P40	<b>2.8±0.11a</b>	19.4±0.70a	<b>5.0±0.25ab</b>	3.3±0.09a	<b>2.4±0.19ab</b>		13.7±1.58b	<b>48.9±3.6b</b>	18.3±1.61a	-	3902±221a	<b>5400±273b</b>
P80	<b>2.8±0.09a</b>	20.1±0.68a	<b>5.5±0.27a</b>	3.2±0.11a	<b>2.5±0.11a</b>		<b>10.5±0.96c</b>	<b>41.4±3.8c</b>	12.9±1.21b	-	3964±368a	<b>5222±310b</b>
<b>Year 2: Effects of P on nodules</b>												
P0	<b>1.6±0.10b</b>	11.3±0.68a	3.6±0.19a	2.8±0.25a	<b>2.0±0.11b</b>		6.1±0.40	<b>63.9±15.7a</b>	15.8±0.90a	-	4149±394a	7006±344a
P40	<b>1.9±0.14ab</b>	10.1±0.61a	3.4±0.23a	3.2±0.22a	<b>2.4±0.13ab</b>		5.7±0.28	<b>36.6±5.6b</b>	14.9±0.76a	-	3765±197a	6899±312a
P80	<b>2.1±0.14a</b>	10.7±0.69a	3.4±0.21a	2.9±0.12a	<b>2.7±0.19a</b>		5.6±0.35	<b>40.4±6.6b</b>	15.1±0.74a	-	3638±228a	6663±247a
<b>Year 1: Effects of P on pods</b>												
P0	<b>3.8±0.16b</b>	12.2±0.17a	<b>2.4±0.06b</b>	<b>3.7±0.18b</b>	ns		<b>8.1±0.18a</b>	<b>41.9±1.2a</b>	ns	22.0±0.44a	ns	ns
P40	<b>4.1±0.16a</b>	12.7±0.38a	<b>2.6±0.10a</b>	<b>4.4±0.30a</b>	-		<b>7.9±0.15ab</b>	<b>38.7±1.6ab</b>	-	22.1±0.46a	-	-
P80	<b>3.9±0.17a</b>	12.7±0.24a	<b>2.6±0.08ab</b>	<b>4.1±0.24a</b>	-		<b>7.6±0.15b</b>	<b>36.7±0.9b</b>	-	22.1±0.48a	-	-
<b>Year 2: Effects of P on pods</b>												
P0	<b>3.0±0.14b</b>	11.0±0.15b	<b>2.3±0.05b</b>	3.3±0.18a	-		<b>8.9±0.15a</b>	<b>41.0±2.4a</b>	-	<b>23.0±0.67a</b>	-	-
P40	<b>3.6±0.13a</b>	11.0±0.14b	<b>2.4±0.07ab</b>	3.7±0.25a	-		<b>8.5±0.16b</b>	<b>35.0±2.0ab</b>	-	<b>21.2±0.50b</b>	-	-
P80	<b>4.0±0.15a</b>	11.6±0.20a	<b>2.6±0.07a</b>	3.5±0.19a	-		<b>8.3±0.19c</b>	<b>34.3±1.7b</b>	-	<b>20.6±0.41b</b>	-	-
<b>Year 1: Effects of planting system on nodules</b>												
Mono cowpea	<b>3.0±0.13a</b>	<b>20.3±0.83a</b>	4.7±0.26a	<b>3.1±0.07c</b>	2.4±0.18a		14.7±2.41a	47.5±6.0a	20.4±2.40a	-	4436±457a	<b>5438±442b</b>
Inter-row cowpea	<b>2.8±0.11a</b>	<b>19.3±0.89a</b>	5.0±0.32a	<b>3.2±0.14b</b>	2.1±0.14a		13.8±1.98a	45.4±3.3a	14.2±1.41c	-	3704±276a	<b>5256±386b</b>
Intra-row cowpea	<b>2.8±0.09a</b>	<b>20.3±0.49a</b>	5.4±0.26a	<b>3.2±0.10b</b>	2.4±0.21a		10.9±1.13a	43.7±2.5a	13.2±0.93c	-	3989±357a	<b>5113±260b</b>
Intra-hole cowpea	<b>2.4±0.10b</b>	<b>17.3±0.68b</b>	5.14±0.29a	<b>3.5±0.13a</b>	2.2±0.11a		13.3±1.79a	54.5±7.6a	17.8±1.68b	-	4297±276a	<b>6397±385a</b>
<b>Year 2: Effects of planting system on nodules</b>												
Mono cowpea	2.0±0.20a	11.5±0.78a	3.7±0.23a	3.1±0.27a	2.4±0.15a		6.7±0.55a	41.7±10.5a	15.0±0.80a	-	4651±395a	<b>6685±416b</b>
Inter-row cowpea	1.7±0.15a	10.3±0.79a	3.3±0.29a	2.9±0.35a	2.5±0.21a		5.3±0.32b	36.6±8.6a	14.8±1.12a	-	3770±223b	<b>6355±282b</b>
Intra-row cowpea	2.0±0.17a	11.0±0.72a	3.6±0.24a	3.0±0.15a	2.5±0.23a		5.6±0.22b	42.3±11.5a	15.2±0.76a	-	3522±292b	<b>6767±240b</b>
Intra-hole cowpea	1.8±0.12a	10.1±0.78a	3.1±0.20a	2.8±0.14a	2.1±0.14a		5.6±0.35b	67.2±16.4a	16.1±1.02a	-	3461±300b	7617±349a
<b>Year 1: Effects of planting system on pods</b>												
Mono cowpea	3.9±0.18a	12.8±0.33a	2.5±0.09a	4.1±0.26a	-		<b>8.45±0.18a</b>	<b>41.6±1.4a</b>	-	21.6±0.56a	-	-
Inter-row cowpea	4.0±0.20a	12.6±0.44a	2.6±0.13a	4.3±0.42a	-		<b>7.83±0.18b</b>	<b>39.0±1.8b</b>	-	22.1±0.65a	-	-
Intra-row cowpea	3.8±0.18a	12.4±0.16a	2.5±0.06a	3.8±0.20a	-		<b>7.56±0.10b</b>	<b>38.7±1.4b</b>	-	22.1±0.35a	-	-
Intra-hole cowpea	4.0±0.20a	12.4±0.32a	2.5±0.09a	4.0±0.27a	-		<b>7.63±0.19b</b>	<b>37.0±1.4b</b>	-	22.4±0.53a	-	-

Table 4.3. Effects of planting pattern on the concentration of mineral elements in shoots and roots of nodulated cowpea and maize sown in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (bold type) are significant at  $P \leq 0.05$ . nd = not determined. For cowpea, shoots include pods, and roots include nodules. The effects of planting pattern on root mineral elements concentration of maize were not significant in Year 2.

Treatment	mg g DM <sup>-1</sup>						µg g DM <sup>-1</sup>				
	P	K	Mg	Ca	S	Na	Cu	Zn	Mn	B	Fe
<b>Year 1: Effects of planting pattern on cowpea shoots (Shoot + Pods)</b>											
Mono cowpea	<b>3.9±0.09a</b>	14.8±0.61a	2.6±0.05a	13.0±0.44a	1.9±0.05a	0.38±0.04a	<b>9.3±0.18a</b>	<b>50.2±1.85a</b>	31.7±3.00a	35.3±0.33a	<b>244±12b</b>
Inter-row cowpea	<b>3.4±0.12b</b>	14.2±0.47a	2.6±0.09a	13.1±0.54a	1.9±0.07a	0.38±0.07a	<b>8.2±0.25b</b>	<b>42.7±1.95b</b>	28.1±1.75a	35.1±0.70a	<b>247±16b</b>
Intra- row cowpea	<b>3.3±0.13b</b>	14.25±0.50a	2.6±0.09a	12.3±0.55a	2.1±0.21a	0.41±0.06a	<b>8.0±0.15b</b>	<b>41.0±2.45b</b>	26.2±1.45a	34.4±0.37a	<b>228±17b</b>
Intra – hole cowpea	<b>3.5±0.08b</b>	14.4±0.48a	2.5±0.08a	12.4±0.30a	1.8±0.07a	0.39±0.06a	<b>8.3±0.28b</b>	<b>41.4±3.35b</b>	26.7±1.60a	35.4±0.42a	<b>306±32a</b>
<b>Year 2: Effects of planting pattern on cowpea shoots (Shoot + Pods)</b>											
Mono cowpea	2.8±0.20a	<b>18.2±0.51a</b>	2.7±0.14a	<b>13.1±0.50b</b>	<b>2.4±0.17ab</b>	0.60±0.02a	9.4±0.17a	46.2±3.7a	49.2±7.7a	<b>40.0±0.92c</b>	202±33a
Inter-row cowpea	2.5±0.20a	<b>17.6±0.72ab</b>	2.8±0.14a	<b>12.9±0.48b</b>	<b>2.2±0.08b</b>	0.65±0.03a	<b>8.2±0.33b</b>	38.1±3.3a	63.7±9.0a	<b>41.6±0.90bc</b>	186±17a
Intra- row cowpea	2.8±0.23a	<b>16.5±0.57ab</b>	3.0±0.11a	<b>14.2±0.50ab</b>	<b>2.6±0.12a</b>	0.65±0.03a	<b>8.0±0.37b</b>	39.4±3.7a	62.9±6.6a	<b>43.7±1.06ab</b>	204±22a
Intra – hole cowpea	2.8±0.21a	<b>16.4±0.56b</b>	3.0±0.12a	<b>14.7±0.45a</b>	<b>2.5±0.14ab</b>	0.60±0.04a	<b>8.1±0.34b</b>	39.1±4.1a	54.5±4.5a	<b>46.2±1.14a</b>	187±17a
<b>Year 1: Effects of planting pattern on maize shoots</b>											
Mono maize	1.6±0.13a	12.3±0.35a	1.3±0.05a	1.9±0.08a	0.22±0.02a	0.22±0.04a	3.6±0.38a	16.9±1.95a	12.0±1.10a	3.1±0.12a	<b>61±3b</b>
Inter-row maize	1.7±0.11a	12.4±0.35a	1.4±0.07a	1.9±0.08a	0.22±0.01a	0.23±0.06a	3.3±0.21a	18.2±1.95a	11.4±0.75a	3.2±0.10a	<b>65±3ab</b>
Intra- row maize	1.7±0.11a	12.6±0.39a	1.4±0.06a	2.1±0.08a	0.23±0.01a	0.21±0.04a	4.6±0.86a	17.7±1.70a	13.4±0.75a	3.3±0.13a	<b>63±3ab</b>
Intra – hole maize	1.8±0.12a	12.8±0.45a	1.4±0.04a	2.0±0.06a	0.22±0.01a	0.20±0.04a	4.8±0.88a	21.2±2.20a	13.5±1.05a	3.2±0.08a	<b>72±6a</b>
<b>Year 2: Effects of planting pattern on maize shoots</b>											
Mono maize	1.0±0.07a	<b>7.8±0.52b</b>	<b>1.1±0.04b</b>	1.1±0.07a	0.58±0.25a	0.22±0.01a	1.6±0.08a	<b>20.3±1.50b</b>	17.3±1.90a	<b>3.2±0.14b</b>	111±17a
Inter-row maize	1.2±0.16a	<b>8.8±0.35ab</b>	<b>1.3±0.05a</b>	1.3±0.08a	0.36±0.02a	0.21±0.01a	1.6±0.07a	<b>24.1±1.25ab</b>	22.1±2.40a	<b>3.3±0.07ab</b>	165±34a
Intra- row maize	1.1±0.12a	<b>9.2±0.27a</b>	<b>1.2±0.04ab</b>	1.2±0.04a	0.35±0.02a	0.20±0.01a	1.8±0.10a	<b>25.2±1.45a</b>	19.7±1.55a	<b>3.4±0.08ab</b>	191±37a
Intra – hole maize	1.1±0.10a	<b>8.5±0.38ab</b>	<b>1.1±0.04b</b>	1.2±0.05a	0.35±0.02a	0.20±0.01a	1.7±0.07a	<b>22.1±2.20ab</b>	22.1±2.45a	<b>3.5±0.10a</b>	133±17a
<b>Year 1: Effects of planting pattern on nodulated cowpea roots (Roots + Nodule)</b>											
Mono cowpea	<b>2.35±0.11a</b>	<b>17.5±0.66a</b>	2.9±0.06	4.4±0.19	3.4±0.20	<b>1.4±0.07b</b>	13.2±1.18	37.9±3.40	<b>17.2±1.31ab</b>	31.7±1.69	2754±256
Inter-row cowpea	<b>1.95±0.09b</b>	<b>16.4±0.85a</b>	2.8±0.09	4.4±0.19	3.0±0.17	<b>1.4±0.06b</b>	14.8±1.16	33.1±2.25	<b>14.3±1.04c</b>	30.1±2.11	2486±180
Intra- row cowpea	<b>1.95±0.07b</b>	<b>16.6±0.51a</b>	2.7±0.09	4.5±0.14	3.2±0.22	<b>1.6±0.06a</b>	14.3±1.44	32.2±1.45	<b>14.4±0.91bc</b>	37.1±3.17	2725±219
Intra – hole cowpea	<b>1.75±0.08c</b>	<b>14.4±0.44b</b>	2.85±0.10	4.3±0.15	2.9±0.145	<b>1.6±0.05a</b>	16.7±1.75	38.3±3.95	<b>17.4±1.03a</b>	37.2±4.84	2972±159
<b>Year 2: Effects of planting pattern on nodulated cowpea roots (Roots + Nodule)</b>											
Mono cowpea	<b>1.8±0.15a</b>	11.1±0.43	3.6±0.16	2.9±0.13	2.8±0.15	2.8±0.15	61±0.29	33.4±3.02	14.6±0.80	<b>21.8±0.73a</b>	3171±203
Inter-row cowpea	<b>1.5±0.09b</b>	10.2±0.45	3.6±0.30	2.8±0.19	2.7±0.14	2.8±0.18	5.6±0.19	29.1±2.91	16.0±1.24	<b>18.9±1.05b</b>	2614±167
Intra- row cowpea	<b>1.7±0.13ab</b>	10.7±0.51	3.6±0.19	2.9±0.11	2.7±0.16	2.9±0.15	5.7±0.22	30.8±3.41	14.9±0.64	<b>19.1±0.72ab</b>	2413±170
Intra – hole cowpea	<b>1.5±0.08b</b>	10.0±0.52	3.2±0.16	2.6±0.11	2.5±0.08	2.9±0.09	6.1±0.32	43.5±4.18	15.3±0.78	<b>20.2±1.37ab</b>	245±163
<b>Year 1: Effects of planting pattern on maize roots</b>											
Mono maize	0.47±0.04	<b>3.9±0.22a</b>	<b>0.39±0.02ab</b>	0.65±0.03	0.18±0.01	<b>0.65±0.13b</b>	6.6±0.42	7.15±0.85	8.8±0.42	2.7±0.08	1407±41
Inter-row maize	0.44±0.04	<b>3.7±0.21ab</b>	<b>0.39±0.02a</b>	0.70±0.03	0.19±0.01	<b>0.75±0.11ab</b>	6.9±0.31	6.6±0.60	9.4±0.33	2.8±0.05	1430±77
Intra- row maize	0.49±0.04	<b>4.1±0.22a</b>	<b>0.41±0.02a</b>	0.65±0.03	0.19±0.02	<b>1.05±0.18a</b>	6.8±0.24	6.6±0.60	8.7±0.29	2.8±0.07	1362±41
Intra – hole maize	0.42±0.04	<b>3.1±0.19b</b>	<b>0.35±0.02b</b>	0.65±0.05	0.18±0.01	<b>0.55±0.13b</b>	6.4±0.39	6.8±0.90	8.9±0.38	2.8±0.08	1390±45

Table 4.4. Effects of cropping system on the concentration of mineral elements in whole plants of nodulated cowpea and maize planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (bold type) are significant at  $P \leq 0.05$ . nd = not determined

Treatment	P	K	Ca	Na	Cu	Zn	Mn	B	Fe
-----mg g DM <sup>-1</sup> ----- -----µg g DM <sup>-1</sup> -----									
<b>Year 1: Effects of planting pattern on whole cowpea plant</b>									
mono cowpea	3.1 ± 0.07a	16.1 ± 0.58a	8.7 ± 0.23a	<b>0.90 ± 0.04b</b>	11.3 ± 0.63a	44.1 ± 2.1a	24.4 ± 1.6a	33.5 ± 0.9a	1499 ± 126b
inter-row cowpea	2.7 ± 0.09b	15.3 ± 0.33ab	8.8 ± 0.30a	<b>0.90 ± 0.03b</b>	11.5 ± 0.53a	37.9 ± 2.0ab	21.2 ± 1.0ab	32.6 ± 1.2a	1366 ± 89b
intra-row cowpea	2.7 ± 0.08b	15.5 ± 0.40ab	8.4 ± 0.29a	<b>0.98 ± 0.02ab</b>	11.2 ± 0.73a	36.6 ± 1.7b	20.3 ± 1.1b	35.8 ± 1.7a	1476 ± 111b
intra-hole cowpea	2.7 ± 0.07b	14.4 ± 0.35b	8.4 ± 0.18a	<b>1.00 ± 0.03a</b>	12.5 ± 0.88a	39.8 ± 3.1ab	22.0 ± 0.9ab	36.3 ± 2.5a	1639 ± 74a
<b>Year 2: Effects of planting pattern on whole cowpea plant</b>									
mono cowpea	2.3 ± 0.17a	14.6 ± 0.38a	<b>8.0 ± 0.27b</b>	1.7 ± 0.08a	7.7 ± 0.18a	39.8 ± 4.0a	31.8 ± 4.1a	30.8 ± 0.7b	1687 ± 107a
inter-row cowpea	2.0 ± 0.14a	13.9 ± 0.48ab	<b>7.8 ± 0.27b</b>	1.8 ± 0.08a	6.9 ± 0.22b	33.6 ± 3.4a	39.8 ± 4.8a	30.2 ± 0.8b	1400 ± 86a
intra-row cowpea	2.2 ± 0.17a	13.5 ± 0.45ab	<b>8.5 ± 0.26ab</b>	1.8 ± 0.07a	6.9 ± 0.22b	35.0 ± 4.2a	38.9 ± 3.5a	31.4 ± 0.8ab	1309 ± 85a
intra-hole cowpea	2.1 ± 0.14a	13.2 ± 0.28b	<b>8.6 ± 0.24a</b>	1.8 ± 0.04a	7.1 ± 0.27b	41.2 ± 5.3a	34.9 ± 2.5a	33.2 ± 0.7a	1305 ± 85a
<b>Year 1: Effects of planting pattern on whole maize plant</b>									
mono maize	1.0 ± 0.07a	8.1 ± 0.21a	1.3 ± 0.04a	<b>0.43 ± 0.07b</b>	5.1 ± 0.23a	12.0 ± 1.4a	10.4 ± 0.7a	2.9 ± 0.08a	734 ± 21a
inter-row maize	1.1 ± 0.07a	8.0 ± 0.15a	1.3 ± 0.05a	<b>0.48 ± 0.06ab</b>	5.1 ± 0.16a	12.4 ± 1.1a	10.3 ± 0.4a	3.0 ± 0.07a	748 ± 40a
intra-row maize	1.1 ± 0.07a	8.3 ± 0.25a	1.4 ± 0.04a	<b>0.63 ± 0.09a</b>	5.7 ± 0.44a	12.2 ± 1.0a	11.1 ± 0.4a	3.1 ± 0.09a	713 ± 21a
intra-hole maize	1.1 ± 0.07a	7.9 ± 0.24a	1.3 ± 0.04a	<b>0.38 ± 0.07b</b>	5.6 ± 0.47a	13.9 ± 1.5a	11.2 ± 0.5a	3.0 ± 0.06a	731 ± 22a
<b>Year 2: Effects of planting pattern on whole maize plant</b>									
mono maize	0.7 ± 0.05a	6.6 ± 0.32b	0.8 ± 0.03a	<b>0.50 ± 0.03ab</b>	2.2 ± 0.14a	14.1 ± 1.2a	12.2 ± 0.9a	2.5 ± 0.09a	660 ± 56a
inter-row maize	0.8 ± 0.11a	7.1 ± 0.25ab	1.0 ± 0.05a	<b>0.73 ± 0.26a</b>	2.2 ± 0.06a	17.0 ± 0.8a	14.9 ± 1.3a	2.6 ± 0.12a	705 ± 80a
intra-row maize	0.8 ± 0.09a	7.5 ± 0.20a	0.9 ± 0.06a	<b>0.50 ± 0.03ab</b>	2.3 ± 0.13a	18.6 ± 1.9a	13.3 ± 0.9a	2.4 ± 0.06a	615 ± 62a
intra-hole maize	0.8 ± 0.07a	7.1 ± 0.27ab	0.9 ± 0.03a	<b>0.45 ± 0.04b</b>	2.2 ± 0.07a	32.7 ± 1.7a	15.0 ± 1.3a	2.7 ± 0.11a	711 ± 106a



Table 4.5. Species differences in the concentration of mineral elements in shoots, roots and whole plants of nodulated cowpea and maize planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (**bold type**) are significant at  $P \leq 0.05$ . nd = not determined

Treatment	mg g DM <sup>-1</sup>						µg g DM <sup>-1</sup>					
	P	K	Mg	Ca	S	Na	Cu	Zn	Mn	B	Fe	Al
<b>Year 1</b>												
<b>A: Shoots</b>												
Cowpea	<b>7.0±0.12a</b>	<b>28.8±0.51a</b>	<b>5.1±0.07a</b>	<b>25.4±0.46a</b>	<b>3.84±0.12a</b>	<b>0.77±0.02a</b>	<b>16.9±0.26a</b>	<b>87.6±2.6a</b>	<b>56.3±2.09a</b>	<b>70.1±0.48a</b>	<b>512±27a</b>	nd
Maize	<b>3.4±0.11b</b>	<b>25.0±0.38b</b>	<b>2.7±0.05b</b>	<b>4.0±0.07b</b>	<b>0.43±0.01b</b>	<b>0.43±0.01b</b>	<b>8.1±0.66b</b>	<b>37.0±2.0b</b>	<b>25.1±0.94b</b>	<b>6.3±0.11b</b>	<b>130±4b</b>	-
<b>B: Roots</b>												
Cowpea	<b>3.98±0.10a</b>	<b>32.4±0.69a</b>	<b>5.60±0.08a</b>	<b>8.8±0.16a</b>	<b>6.27±0.19a</b>	<b>3.0±0.06a</b>	<b>29.4±1.41a</b>	<b>70.7±2.95a</b>	<b>31.6±1.1a</b>	<b>68.0±3.22a</b>	<b>5468±206a</b>	-
Maize	<b>0.90±0.04b</b>	<b>7.3±0.23b</b>	<b>0.76±0.02</b>	<b>1.3±0.03b</b>	<b>0.36±0.01b</b>	<b>1.5±0.15b</b>	<b>13.3±0.34b</b>	<b>13.5±0.72b</b>	<b>17.9±0.35b</b>	<b>5.5±0.07b</b>	<b>2794±52b</b>	-
<b>C: Whole plant</b>												
Cowpea	<b>5.5±0.09a</b>	<b>30.6±0.48a</b>	<b>5.4±0.12a</b>	<b>17.1±0.25a</b>	<b>5.1±0.12a</b>	<b>1.9±0.03a</b>	<b>23.2±0.69a</b>	<b>79.2±2.3a</b>	<b>44.0±1.3a</b>	<b>69.1±1.7a</b>	<b>2990±103a</b>	-
Maize	<b>2.2±0.07b</b>	<b>16.2±0.22b</b>	<b>1.8±0.06b</b>	<b>2.7±0.05b</b>	<b>0.40±0.01b</b>	<b>1.0±0.08b</b>	<b>10.7±0.35b</b>	<b>25.3±1.3b</b>	<b>21.5±0.5b</b>	<b>6.0±0.08b</b>	<b>1463±26b</b>	-
<b>Year 2</b>												
<b>A: Shoots</b>												
Cowpea	<b>5.4±0.20a</b>	<b>34.3±0.62a</b>	<b>5.7±0.13a</b>	<b>27.4±0.52a</b>	<b>4.78±0.14a</b>	<b>1.24±0.03a</b>	<b>16.8±0.34a</b>	<b>81.3±3.7a</b>	<b>115.1±7.1a</b>	<b>85.7±1.19a</b>	<b>390.23±22a</b>	<b>515±37a</b>
Maize	<b>2.1±0.12b</b>	<b>17.1±0.40b</b>	<b>2.4±0.04b</b>	<b>2.4±0.06b</b>	<b>0.81±0.12b</b>	<b>0.41±0.01b</b>	<b>3.3±0.08b</b>	<b>45.8±1.7b</b>	<b>40.6±2.1b</b>	<b>6.6±0.10b</b>	<b>299.67±28b</b>	<b>414±40b</b>
<b>B: Roots</b>												
Cowpea	<b>3.2±0.12a</b>	<b>20.9±0.48a</b>	<b>6.9±0.21a</b>	<b>5.53±0.13a</b>	<b>5.31±0.13a</b>	<b>5.7±0.14a</b>	<b>11.7±0.26a</b>	<b>68.3±6.42a</b>	<b>30.4±0.88a</b>	<b>39.93±1.02</b>	<b>5311±193a</b>	<b>9227±227a</b>
Maize	<b>0.91±0.05b</b>	<b>11.3±0.25b</b>	<b>1.0±0.03b</b>	<b>0.97±0.02b</b>	<b>0.62±0.02b</b>	<b>1.8±0.27b</b>	<b>1.1±0.13b</b>	<b>3.3±0.49b</b>	<b>2.74±0.29b</b>	<b>0.70±0.09</b>	<b>499±723b</b>	<b>662±94b</b>
<b>C: Whole plant</b>												
Cowpea	<b>4.3±0.14a</b>	<b>27.6±0.42a</b>	<b>6.4±0.11a</b>	<b>16.5±0.28a</b>	<b>5.1±0.11a</b>	<b>3.5±0.07a</b>	<b>14.3±0.24a</b>	<b>74.9±4.3a</b>	<b>72.7±3.8a</b>	<b>62.8±0.82a</b>	<b>2851±99a</b>	<b>4871±114a</b>
Maize	<b>1.6±0.56b</b>	<b>14.2±0.27b</b>	<b>1.7±0.03b</b>	<b>1.8±0.04b</b>	<b>0.7±0.07b</b>	<b>1.1±0.14b</b>	<b>4.4±0.11b</b>	<b>33.0±1.5b</b>	<b>27.7±1.2b</b>	<b>5.1±0.10b</b>	<b>1345±72.5b</b>	<b>1799±77b</b>

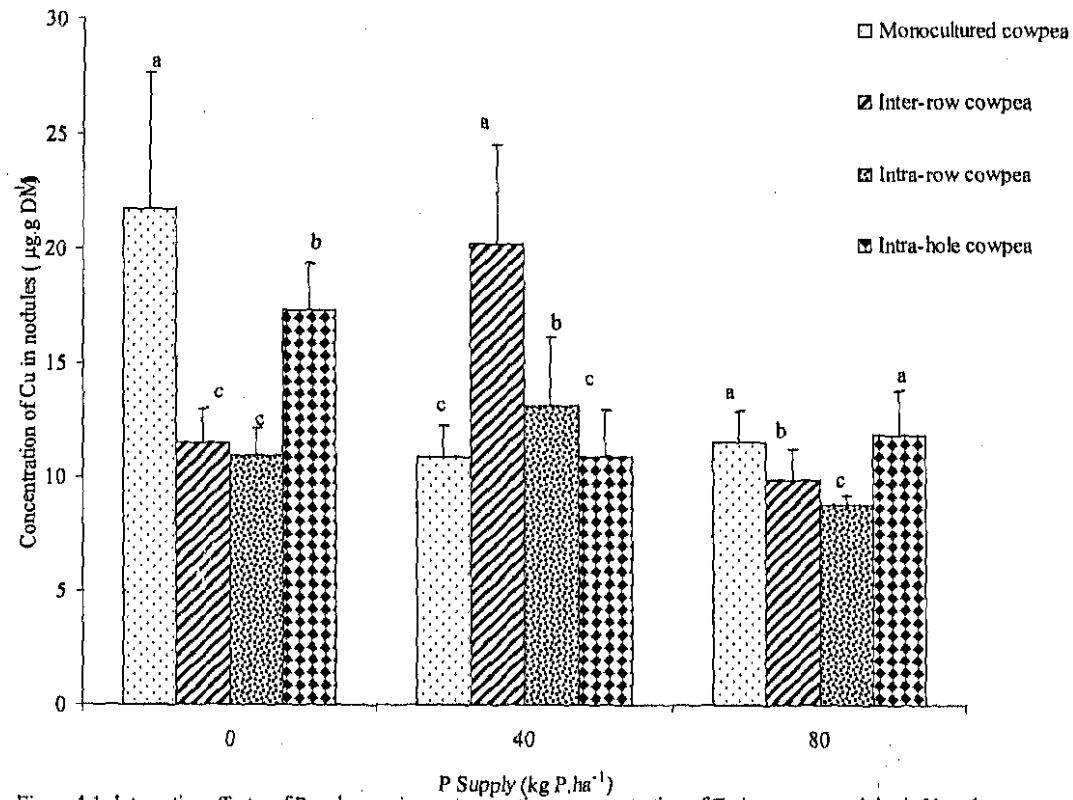


Figure 4.1 Interactive effects of P and cropping system on tissue concentration of Cu in cowpea nodules in Year 1.

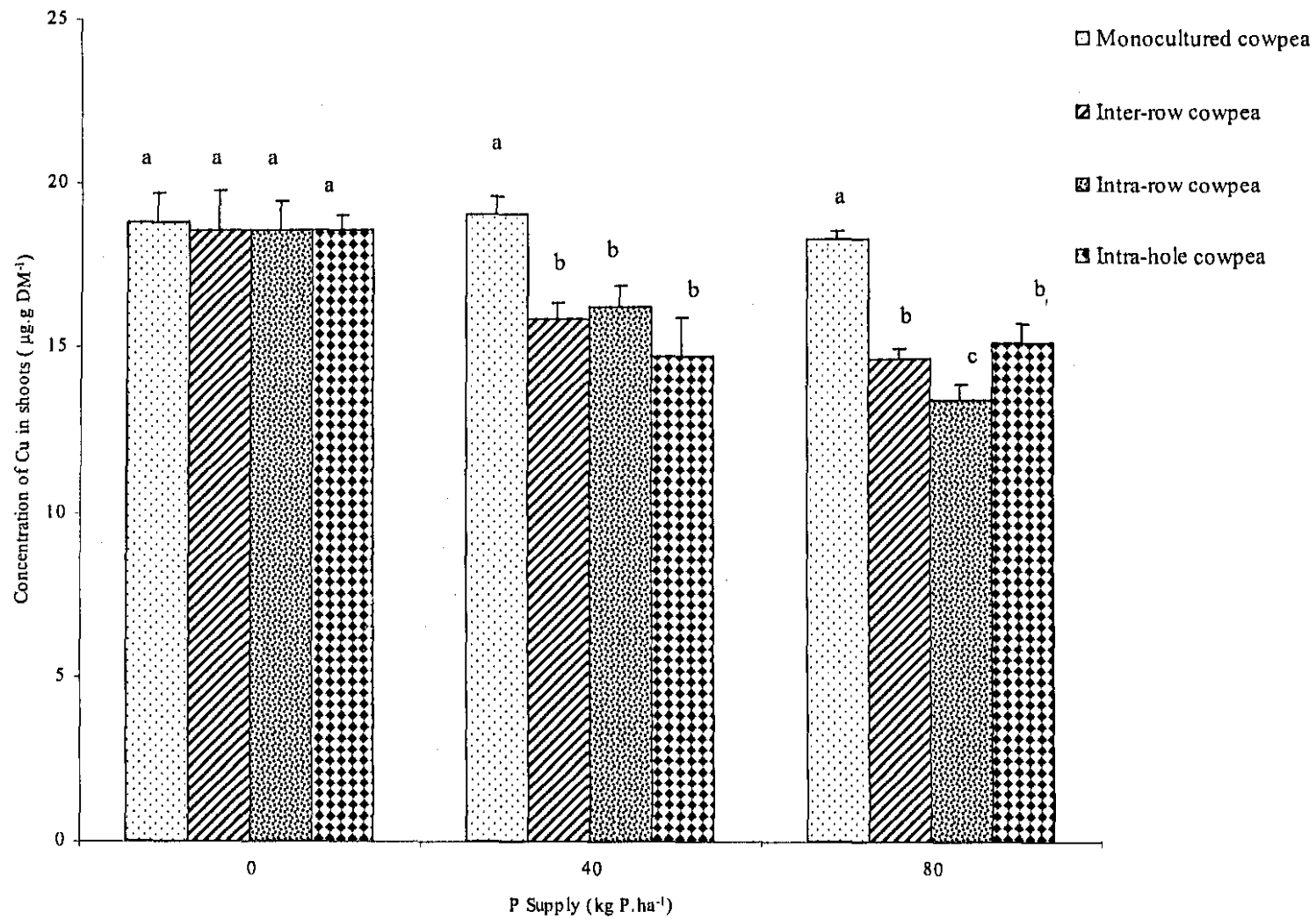


Figure 4.2. Interactive effects of P and cropping system on tissue concentration of Cu in cowpea shoots in Year 2.

## CHAPTER 5

EFFECT OF EXOGENOUS P SUPPLY AND CROPPING SYSTEM ON MINERAL PARTITIONING IN NODULATED COWPEA (*VIGNA UNGUICULATA* L. WALP.) AND MAIZE (*ZEA MAYS* L.).

## 5.1 Introduction

Mixed culture (or intercropping) of legumes and cereals is an old practice in tropical agriculture that dates back to ancient civilization. As a result, considerable research has been conducted on this system. Many of these past studies have, however, centred on various aspects, including inter-specific competition for water and nutrients, pest control, N contribution by nodulated legumes, plant growth, grain yield, and yield advantage of the component crop species involved, usually measured as land equivalent ratios (Wahua 1983; Chang and Shibles 1985a&b; Ofori and Stern, 1986&1987; Buerkert *et al.* 2001; Carsky 2003). Later studies of intercropping have compared the uptake of single or few selected mineral nutrients in mixed culture with monoculture (Craig *et al.*, 1981; Burton *et al.*, 1983; Gardner and Boundy, 1983). Recent studies have, however, started to focus on belowground interaction between component plant species. It has, for example, been shown that in mixed cultures involving legumes and cereals, the growth of the latter is greatly enhanced by the former (Kahm *et al.* 1999; Cu *et al.*, 2005) either from transfer of fixed-N to the non-legume (Eaglesham *et al.* 1981), or mobilisation of insoluble minerals by organic acids secreted by legume (Ae *et al.*, 1990; Dakora and Phillips 2002). Conversely, the uptake of Fe by the legume in a maize/groundnut intercrop was enhanced by the cereal partner, especially when their roots were closely intermingled (Zhang *et al.*, 2004).

Although studies on mineral nutrition of plants have been well documented (Marschner, 1995), these have either been limited to a few single minerals or conducted in monoculture. No study has so far evaluated and compared plant uptake and partitioning of all the major and minor nutrient elements in mixed cultures involving nodulated legumes and cereals. Yet plant ecologists and agronomists tend to work with these nutrients in mixed plant stands in natural ecosystems, or as intercropped material in agricultural systems. Detailed studies are currently lacking on the uptake profile, tissue accumulation, and the nutrient allocation to plant organs in relation to

cropping systems (i.e. monoculture vs. mixed culture), and/or exogenous nutrient supply. As a result, we do not have a proper understanding of how nutrient uptake rates are affected by planting pattern and/or the exogenous supply of minerals. More information is, therefore, needed on nutrient uptake and partitioning to organs of plants growing in complex mixed cultures and supplied with external mineral nutrients.

This study assesses the effect of exogenous P supply and cropping system on the accumulation and partitioning of both major and minor nutrients to organs of nodulated cowpea and maize in sole and mixed cultures.

## **5.2 Materials and Methods**

### **5.2.1 Experimental site**

Field experiments were conducted under irrigation at the Agricultural Research Council Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa, during the 2003 (Year 1) and 2004 (Year 2) summer seasons at two different locations within Nietvoorbij. The site description is outlined in chapter 2 section 2.2.1.

### **2.2 Experimental design and treatments**

The experimental treatments consisted of three levels (0, 40, and 80 kg P.ha<sup>-1</sup>) of triple superphosphate [46% P, 13.5% Ca, 1.5% S, 0.0007% Cu, 0.008% Zn, 0.021% Mn, 0.053% B and 0.0009% Mo (Cherney, 2003; FAS, 2005)] and 4 cropping systems (namely, monoculture, maize/cowpea inter-row, maize/cowpea intra-row, and maize/cowpea intra-hole planting). The experimental layout, crop varieties used and other cultural practices are described in chapter 2 section 2.2.2.

### **5.2.3 Plant harvest and sample preparation**

At 60 d after planting, cowpea and maize plants were sampled for nutrient analysis. The procedure followed is described in chapter 4 section 4.3.2.

### **5.2.4 Determination of soil chemical properties**

The determination of soil pH, soil organic carbon, soil organic matter (SOM), S, P, K, Na, Ca, Mg, Cu, Zn, Mn, Fe, and Al were extracted from soil following the procedures outlined in chapter 3 in sections 3.2.5 and 3.2.6.

### **5.2.5 Measurement of mineral nutrients in plant organs**

The amount of macronutrients (P, K, Ca, Mg, and Na) and micronutrients (Cu, Zn, Mn, Fe, Al, B) in plant samples were determined as described in chapter 4 section 4.2.5. The amount of nutrients in each plant part was determined by multiplying the dry weight by the corresponding elemental concentration and expressed as g (macronutrient) or  $\mu\text{g}\cdot\text{plant}^{-1}$  (micronutrient). The total amount of each nutrient per plant was obtained by adding the values of all organs or plant parts.

### **5.2.6 Statistical analysis**

A 2-factorial design (2-way ANOVA) was used to statistically analyse for mineral uptake in plant organs. However, a one-way ANOVA was used to compare nutrient uptake in the legume and cereal. The analysis was done using the software of STATISTICA program 1997. Fisher's least

significant difference was used to compare significant treatment means at  $P \leq 0.05$  (Steel and Torrie, 1980).

### **5.3 Results**

#### **5.3.1 Effects of P supply on nutrient content of shoots, roots and whole plants of cowpea and maize**

Relative to control, exogenous P supply to cowpea significantly increased its shoot content of P only in Year 1 (Table 5.1), whereas in Year 2 the amounts of P, K, Mg, Ca, S, Na, Zn, Mn, B, Fe and Al were all significantly increased in cowpea shoots (Table 5.2). Maize shoots also showed increased amounts of P, K, Mg, Na, Cu, Zn, Mn and Al with external P supply in Year 1 (Table 5.1), while in Year 2 only P, S, Zn and Mn were significantly increased (Table 5.2).

The application of P to plants also increased the content of P, Ca and S in cowpea roots in Year 1, while with maize, only P was significantly increased in roots (Table 5.1). Supplying P in Year 2 also increased root content of P, K, Mg, Ca, S, Na and Mn in cowpea and only P in maize relative to zero-P control (Table 5.2).

At whole-plant level, the contents of P, K, Mg, Cu and Zn were significantly increased by P application to maize in Year 1, while only P and K were increased in cowpea (Table 5.1). In Year 2, however, the contents of P, K, Mg, Ca, S, Na, Zn, Mn, B, Fe and Al all rose in cowpea, while only P, S and Mn were increased in maize with P application (Table 5.2).



### **5.3.2 Effects of exogenous P supply and cropping system on the nutrient content of pods and nodules in cowpea**

Applying P to cowpea plants significantly increased nodule content of only P and S in Year 1 (data not shown), while in Year 2 the amounts of P, K, Mg, Ca, S, Na, Cu and Mn in nodules were all increased (Table 5.3).

The nutrient content of cowpea pods were more altered with P supply in Year 2 compared to Year 1. For example, while only Ca was increased in pods in Year 1 (data not shown), P, K, Mg, Ca, S, Na, Cu, Zn, Mn and B were all significantly increased with P supply (Table 5.3).

The effect of each separate cropping system on nutrient content of nodules were assessed and found to be more clearly defined in Year 2 than in Year 1. As shown in Table 5.3, the nodule content of P, K, Mg, Ca, S, Na, Cu, Mn, B and Fe were significantly decreased in intercropped cowpea relative to those in monoculture. However, the amounts of these nutrients in nodules of intra-hole cowpea was also significantly lower compared to the other intercropping systems (Table 5.3).

With pods, only Cu was significantly decreased by cropping system in Year 1 (data not shown). In Year 2, however, pod content of P, K, Mg, Ca, S, Na, Cu, Zn, Mn, B and Fe were all significantly decreased in mixed culture relative to monoculture. As with nodules, the amounts of nutrients in pods were significantly lower in the intra-hole cowpea compared with the other intercropping systems (Table 5.3).

### **5.3.3 Effect of cropping system on the nutrient content of shoots, roots and whole plants of cowpea and maize**

Separating the effects of the individual components of the cropping system for each species revealed significant differences in the nutrient content of plant organs. The shoot levels of P, Zn and Mn in Year 1 were significantly decreased in cowpea from mixed culture relative to monoculture (data not shown). In Year 2, the shoot content of P, K, Mg, Ca, S, Na, Cu, Zn, Mn, B, Fe and Al were all reduced in mixed culture compared with monocultured cowpea (Table 5.4). But again, the nutrient levels were also significantly lower in intra-hole cowpea, relative to the other intercrop treatments (Table 5.4).

Except for S, which showed a significantly decreased content in maize shoots with intercropping, the other nutrients were not affected in both Year 1 (data not shown) and Year 2 (Table 5.4).

Cowpea roots showed no changes in nutrient content with intercropping in Year 1 (data not shown). However, in Year 2 the amounts of P, K, Mg, Ca, S, Na, Zn and B in roots were significantly decreased when grown in mixed culture relative to monoculture (Table 5.4). As observed previously the intra-hole cowpea showed the lowest content of all nutrient elements relative to the other cropping systems.

With maize, however, there was no effect of cropping system on root content of nutrients in both planting seasons, except for Year 2, where root Na was significantly lower in intra-hole plants compared with the other planting systems (Table 5.4).

Whole-plant content of P, Zn and Mn in cowpea were significantly decreased when intercropped than monocropped in Year 1 (Table 4). In Year 2, whole-plant content of P, K, Mg, Ca, S, Na,

Cu, Zn, Mn, B, Fe and Al were significantly decreased in mixed culture relative to the monoculture (Table 5.4), with intra-hole cowpea exhibiting the lowest nutrient content (Table 5.4). At whole-plant level, monocultured maize plants showed significantly greater S content relative to those in mixed culture (data not shown).

#### **5.3.4 Species effect on nutrient content of shoots, roots and whole plants of cowpea and maize**

A one-way ANOVA analysis was done to compare nutrient content of organs as well as whole plants of the two test species (cowpea and maize). Except for Ca, S, and B which showed greater levels in cowpea shoots than maize shoots, the amounts of the remaining minerals (i.e. P, K, Mg, Na, Cu, Zn, Mn, Fe and Al) were all consistently greater in maize shoots than in cowpea shoots (Table 5.5). With roots, however, all the nutrients studied were significantly greater in cereal relative to legume (Table 5.5). Whole-plant contents of Ca, S, B (in Year 1 and 2) and Cu (in only Year 2) were again greater in cowpea than maize, while the contents of P, K, Mg, Na, Zn, Mn, Fe and Al were all lower in the legume relative to the cereal (Table 5.5).

#### **5.3.5 Interactive effects of applied P and cropping system on nutrient content of pods, shoots, and whole cowpea plants**

There was a significant interactive effect between applied P and cropping system on the nutrient content of pods, shoots and whole cowpea plants. Applying exogenous P at 0, 40 or 80 kg.ha<sup>-1</sup> altered the pod content of P, K, Mg, Na, Cu, Zn, shoot levels of P, Ca, S, and Zn, as well as whole-plant content of P, Ca, S, and Zn (Figures 5.1 and 5.2). Whether at 0, 40 or 80 P.ha<sup>-1</sup>, the monocultured cowpea plants were always significantly greater in nutrient content relative to those in mixed cultures (Figure 5.1 and 5.2). In all instances, where 40 kg P.ha<sup>-1</sup> was applied, inter-row cowpea plants also consistently showed significantly greater nutrient content compared

to their intra-hole counterparts (Figure 5.1 and 5.2). Furthermore, in all cases involving the application of 80 P.ha<sup>-1</sup>, to plants, the nutrient contents were not significantly different for the mixed cultures (i.e. inter-row, intra-row and intra-hole systems).

#### 5.4 Discussion

Applying exogenous P to cowpea and maize altered organ content of various mineral nutrients. Irrespective of the plant part concerned, nutrient accumulation in cowpea was more affected than maize, just as plant response to P in Year 2 was also more pronounced than in Year 1. The differences in response between Year 1 and Year 2 is site-related. As shown in Table 3.1 (Chapter 3), the concentration of endogenous P in the bulk soil of the field used in Year 1 was 4.5-fold greater than that of the soil used in Year 2. As a result, alteration in rhizosphere nutrient concentrations as well as tissue accumulation with P supply was reduced in Year 1 relative to Year 2 (Tables 5.1; 5.2; and 5.3). Thus, the significantly greater levels of P, K, Mg, Ca, S, Na, Zn, Cu, Mn, B, Fe and Al in shoots, pods, nodules and roots of cowpea with exogenous P supply in Year 2 stemmed from the low endogenous soil P of the sites used, an effect which manifested in significantly increased mineral content at whole-plant level (Reinbott and Blevins, 1991; 1994 & 1997; Li *et al.*, 2004b; Tables 5.1; 5.2 and 5.3). This was in contrast to maize which showed limited response to the provision of external P.

Perhaps the most intriguing aspect of this study was the observation that cropping system altered nutrient partitioning to organs of cowpea. Although less marked in Year 1 relative to Year 2, intercropping was found to decrease the tissue content of both major and minor elements in all organs of cowpea when compared to sole cropping. The decrease in mineral content of plant parts was more dramatic with intra-row and intra-hole planting. Even at whole-plant level, the level of minerals in tissues was significantly decreased by the intercropping of maize with cowpea,

especially where the legume and cereal were co-planted in one hole (Table 5.4). The data in Fig 5.1 and 5.2 shows that, in all instances, the amounts of P, K, Mg, Na, Cu and Zn as well as P, Ca, S and Zn in shoots and whole plants were significantly reduced with intercropping relative to monoculture at all rates of P application. Although the intercropping treatments were not significantly different between themselves at zero and 80 kg P.ha<sup>-1</sup> in respect of the specific nutrients, at 40 kg P.ha<sup>-1</sup> the levels of these nutrients in pods, shoots and whole plants were consistently lower in cowpea from intra-hole planting relative to inter-row or sole culture (Figs. 5.1 and 5.2).

These changes in mineral content of plant organs could have implications in the dietary use of cowpea leaves as vegetables in Africa. Farmers in the tropics, including Africa, grow their crops as mixtures. So the finding that intercropping can reduce the level of mineral nutrients in leaves and shoots of vegetable crops such as cowpea calls for detailed studies on the effect of mixed cropping on the food quality of crop plants. However, the decrease in mineral content of cowpea with intercropping could also have positive effects on ecosystem functioning, especially where farmers remove crop residues from the field. The decreased content of mineral nutrients in residues of intercropped cowpea implies that the complete removal of such a crop at harvest where intensive cultivation is practiced could potentially reduce nutrient supply to subsequent crops relative to monoculture where the legume accumulated greater amounts of soil nutrients.

A comparison of cowpea with maize revealed large species differences in nutrient accumulation by organs and whole plants (Table 5.5). Except for Ca, S and B, which showed consistently higher levels in shoots, roots and whole plants of cowpea, the tissue content of P, K, Mg, Na, Cu, Zn, Mn, Fe and Al were all greater in maize relative to cowpea (Table 5.5), suggesting that the cereal is more likely to exhaust the soil of mineral nutrients relative to the legume (Dalal, 1974).

However, when the plant densities in monocultures and nutrient content per plant (Table 5.6) were used to estimate the level of nutrient mining by the two species on a per-hectare basis, the cowpea was found to deplete more soil nutrients than the maize because of its greater plant density (Table 5.6). Thus, the production of legumes in monocultures (as practised by commercial farmers), is more likely to exhaust soil nutrients than sole cropped cereals. However, when cowpea is intercropped with maize as done in traditional systems, the nutrient depletion by this legume is significantly reduced (Table 5.7).

The provision of external nutrients as fertilisers to cropping systems as shown in this study also appears to promote greater nutrient uptake and accumulation in tissues, with the result that removal of crop residues at harvest could lead to nutrient mining and the creation of an unsustainable cultural system. However, the cropping system had no effect on nutrient accumulation in maize, except for S (Table 5.8). The findings obtained here clearly suggest the need for further experimentation on the effects of mixed cultures on the nutrient quality of food grain and leafy vegetables as well as on nutrient balance of the cropping system.

Table 5.1. Effect of P application on the amount of mineral elements in shoots, roots and whole plants of nodulated cowpea and maize planted in 2003 (Year 1). Values followed by dissimilar letters in the same column (bold type) are significant at  $P \leq 0.05$ .

Treatment	P	K	Mg	Ca	S	Na	Cu	Zn	Mn	B	Fe	Al
mg plant <sup>-1</sup>						µg plant <sup>-1</sup>						
<i>SHOOTS</i>												
<b>Cowpea (Shoot + Pods)</b>												
P0	<b>79.7±7.2b</b>	342.1±28.5a	60.5±5.2a	297.2±25.1a	44.5±3.7a	9.7±1.0a	209±18a	1055±104a	697±74a	855±69a	5843±605a	
P40	<b>97.2±10.2ab</b>	372.2±28.8a	70.7±7.2a	359.0±38.5a	51.3±5.3a	10.0±0.9a	226±23a	1159±156a	709±94a	945±94a	6726±753a	
P80	<b>103.2±9.3a</b>	412.3±36.2a	70.7±6.3a	348.3±29.2a	53.9±4.1a	10.8±1.0a	238±24a	1236±127a	841±88a	982±88a	8121±1469a	
<b>Maize</b>												
P0	<b>210.5±27.1b</b>	<b>1611±152b</b>	<b>179.6±18.1b</b>	256.8±20.4a	27.9±3.1a	<b>28.1±2.4b</b>	<b>427±38b</b>	<b>2500±356b</b>	<b>1698±174b</b>	405±39a	<b>8464±668b</b>	
P40	<b>209.6±19.0b</b>	<b>1629±125b</b>	<b>176.0±14.5b</b>	271.1±19.9a	29.2±2.5a	<b>27.4±2.1b</b>	<b>586±141ab</b>	<b>2208±298b</b>	<b>1558±164b</b>	431±33a	<b>8182±795b</b>	
P80	<b>298.4±24.4a</b>	<b>2085±171a</b>	<b>225.5±16.3a</b>	302.0±19.1a	34.3±2.6a	<b>32.8±1.5a</b>	<b>813±155a</b>	<b>3268±357a</b>	<b>2078±182a</b>	500±31a	<b>10766±836a</b>	
<i>ROOTS</i>												
<b>Cowpea (Nodule + Root)</b>												
P0	<b>6.2±0.5b</b>	54.2±5.1a	9.6±0.9a	<b>14.5±1.4b</b>	<b>9.2±0.8b</b>	5.0±0.4a	57.8±5.8a	127.7±13.7a	59.5±6.8a	115±12.1a	10255±1271a	
P40	<b>7.3±0.8ab</b>	56.3±5.9a	10.5±1.3a	<b>15.4±1.6ba</b>	<b>11.9±1.5ab</b>	5.4±0.7a	49.1±6.1a	132.2±20.6a	59.3±7.3a	134±24.9a	9125±995a	
P80	<b>8.8±1.2a</b>	70.6±9.6a	11.2±1.5a	<b>19.2±2.8a</b>	<b>14.4±2.0a</b>	6.3±0.8a	55.7±10.1a	141.1±24.2a	59.3±9.9a	147±29.7a	11222±1953a	
<b>Maize</b>												
P0	<b>12.0±1.1b</b>	120.9±11.3a	12.2±0.8a	21.7±2.1a	5.7±0.5a	25.0±3.9a	229.8±27.9a	217.6±25.1a	309±35a	93±11a	45335±3831a	
P40	<b>15.2±1.5a</b>	125.1±13.9a	12.6±0.9a	23.9±2.3a	6.3±0.6a	24.5±5.6a	207.8±15.5a	193.8±16.3a	298±28a	96±7a	45932±2719a	
P80	<b>17.0±1.9a</b>	120.7±17.0a	12.7±1.6a	19.9±2.9a	5.8±0.6a	20.9±3.4a	224.1±28.0a	233.6±23.3a	299±41a	91±13a	47636±6753a	
<i>WHOLE PLANT</i>												
<b>Cowpea</b>												
P0	<b>85.9±7.1b</b>	396.3±28.5a	70.1±5.2a	311.7±25.3a	<b>53.7±3.8b</b>	14.7±1.1a	267±17.6a	1183±103a	757±74a	970±71a	16098±1365a	
P40	<b>104.5±10.5ab</b>	428.5±29.3a	81.2±7.4a	374.5±38.7a	<b>63.2±6.0ab</b>	15.4±1.2a	275±23.0a	1291±161a	769±95a	1079±99a	15851±1297a	
P80	<b>112.1±9.3a</b>	482.9±35.9a	81.9±6.2a	367.5±29.6a	<b>68.4±4.7a</b>	17.2±1.4a	294±22.8a	1377±129a	900±89a	1130±99a	19343±2339a	
<b>Maize</b>												
P0	<b>222.5±27.3b</b>	<b>1732.1±151.0b</b>	<b>191.9±18.0b</b>	278.5±20.2a	33.5±3.0a	53.0±4.9a	<b>657±49.8b</b>	<b>2717±364.7b</b>	2008±172.0a	498±38.6a	53799±3807a	
P40	<b>224.7±19.0b</b>	<b>1753.8±124.0b</b>	<b>188.6±14.4b</b>	295.0±19.9a	35.5±2.5a	52.0±6.1a	<b>794±141.4b</b>	<b>2402±304.1b</b>	1855±164.6a	527±33.5a	54113±2561a	
P80	<b>315.3±23.7a</b>	<b>2205.3±172.8a</b>	<b>238.2±16.2a</b>	322.0±18.1a	40.1±2.4a	53.7±3.2a	<b>1037±160.6a</b>	<b>3501±353.7a</b>	2377±177.4a	592±33.0a	58402±6682a	

Table 5.2. Effect of P application on the amount of mineral elements in shoots, roots and whole plants of nodulated cowpea and maize planted in 2004 (Year 2). Values followed by dissimilar letters in the same column (**bold type**) are significant at  $P \leq 0.05$ .

Treatment	P	K	Mg	Ca	S	Na	Cu	Zn	Mn	B	Fe	Al
mg plant <sup>-1</sup>						µg plant <sup>-1</sup>						
<i>SHOOTS</i>												
<b>Cowpea (Shoot + Pod)</b>												
P0	<b>57.4±12.4b</b>	<b>473.0±97.8b</b>	<b>69.7±10.7b</b>	<b>339.3±46.2b</b>	<b>60.0±7.6b</b>	<b>16.3±2.6b</b>	250.7±45.2a	<b>1218±157b</b>	<b>1315±164b</b>	<b>1133±176b</b>	<b>5122±734b</b>	<b>6478±958b</b>
P40	<b>114.6±13.0a</b>	<b>746.8±103.2a</b>	<b>110.9±10.7a</b>	<b>585.6±67.3a</b>	<b>89.3±7.5a</b>	<b>26.9±3.5a</b>	354.3±50.5a	<b>1515±206a</b>	<b>2084±293a</b>	<b>1791±212a</b>	<b>7625±901a</b>	<b>9818±1282a</b>
P80	<b>125.7±22.7a</b>	<b>743.0±146.7a</b>	<b>117.1±18.4a</b>	<b>531.4±95.5a</b>	<b>96.2±18.2a</b>	<b>25.4±4.4a</b>	334.5±70.5a	<b>1566±353a</b>	<b>2184±343a</b>	<b>1646±270a</b>	<b>6662±1002a</b>	<b>8307±1228a</b>
<b>Maize</b>												
P0	<b>95±16b</b>	906±120a	126±15a	134±20a	<b>33±4b</b>	24±4a	178±23a	<b>2287±233b</b>	<b>1800±210b</b>	348±44a	14938±3098a	20773±4515a
P40	<b>172±20a</b>	1229±135a	174±20a	171±17a	<b>53±6a</b>	31±4a	223±20a	<b>3163±365a</b>	<b>2598±316a</b>	473±48a	15615±2191a	20204±3097a
P80	<b>157±21a</b>	1184±131a	170±23a	168±22a	<b>64±15a</b>	27±4a	223±24a	<b>3158±416a</b>	<b>3173±397a</b>	467±52a	21296±4720a	32018±8475a
<i>ROOTS</i>												
<b>Cowpea (Nodule + Root)</b>												
P0	<b>2.6±0.3b</b>	<b>22.3±2.7b</b>	<b>7.5±1.2b</b>	<b>5.5±0.7b</b>	<b>4.9±0.5b</b>	<b>5.4±0.5b</b>	12.9±1.6a	82.0±12.8a	<b>31.2±3.0b</b>	44.8±5.5a	6221±917a	10039±1143a
P40	<b>4.8±0.5a</b>	<b>29.2±2.9a</b>	<b>10.1±1.0a</b>	<b>8.4±0.8a</b>	<b>7.9±0.8a</b>	<b>7.9±0.6a</b>	16.6±1.6a	85.2±13.7a	<b>41.7±3.6a</b>	58.2±6.3a	7912±921a	13872±1648a
P80	<b>5.1±0.7a</b>	<b>30.2±3.7a</b>	<b>9.6±1.1a</b>	<b>7.6±0.8a</b>	<b>8.0±0.9a</b>	<b>8.2±0.7a</b>	15.6±1.8a	90.8±16.1a	<b>42.8±5.0a</b>	53.3±6.4a	7296±1146a	11957±1247a
<b>Maize</b>												
P0	<b>8.2±1.3b</b>	124.3±20.2a	11.6±1.9a	9.9±1.2b	6.0±0.8a	21.3±4.7a	18.0±7.7a	35.4±11.5a	43.0±18.2a	13.0±5.6a	9776±4754a	13534±6663a
P40	<b>15.1±1.7a</b>	160.2±17.5a	14.7±1.4a	13.1±1.1a	8.7±0.9a	21.5±2.6a	19.9±5.9a	69.7±27.5a	43.7±9.2a	12.1±3.3a	8479±2497a	10925±2944a
P80	<b>12.7±1.0a</b>	149.8±9.7a	14.4±1.5a	13.4±1.0a	8.7±0.7a	19.5±2.4a	15.7±2.5a	52.5±8.2a	47.9±8.3a	10.4±1.8a	7671±1539a	9930±1878a
<i>WHOLE PLANT</i>												
<b>Cowpea</b>												
P0	<b>60±13b</b>	<b>495±100b</b>	<b>77±11b</b>	<b>345±47b</b>	<b>65±8b</b>	<b>22±3b</b>	264±47a	<b>1300±158b</b>	<b>1346±165b</b>	<b>1178±180b</b>	<b>11343±1442b</b>	<b>16518±1496b</b>
P40	<b>119±13a</b>	<b>776±106a</b>	<b>121±11a</b>	<b>594±68a</b>	<b>97±8a</b>	<b>35±4a</b>	371±52a	<b>1600±211a</b>	<b>2125±294a</b>	<b>1849±217a</b>	<b>15536±1634a</b>	<b>23690±2454a</b>
P80	<b>131±23a</b>	<b>773±149a</b>	<b>127±19a</b>	<b>539±96a</b>	<b>104±19a</b>	<b>34±5a</b>	350±72a	<b>1657±363a</b>	<b>2227±347a</b>	<b>1699±275a</b>	<b>13958±2033a</b>	<b>20264±2190a</b>
<b>Maize</b>												
P0	<b>103±17b</b>	1031±137a	137±17a	143±21a	<b>39±4b</b>	45±7a	196±28a	2322±240a	<b>1843±219b</b>	361±48a	24714±5636a	34307±8266a
P40	<b>187±21a</b>	1390±140a	189±20a	184±18a	<b>62±6a</b>	53±5a	243±23a	3233±360a	<b>2642±315a</b>	485±50a	24094±2956a	31130±3727a
P80	<b>170±21a</b>	1333±132a	185±22a	181±22a	<b>72±15a</b>	47±4a	239±23a	3210±414a	<b>3221±395a</b>	477±52a	28967±4274a	41948±7832a



Table 5.3. Effect of P supply and cropping system on the amount of mineral elements in nodules and pods of cowpea planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (bold type) are significant at  $P \leq 0.05$ . ns = not significant. The effects of P supply and cropping systems on uptake of some mineral elements in nodules and pods were not significant in Year 1 and 2.

Treatment	P	K	Mg	Ca	S	Na	Cu	Zn	Mn	B	Fe
mg plant <sup>-1</sup>						µg plant <sup>-1</sup>					
<b>Year 2: Effects of P on nodules</b>											
P0	<b>0.38±0.07b</b>	<b>2.9±0.6b</b>	<b>0.9±0.2b</b>	<b>0.7±0.2b</b>	<b>0.50±0.09b</b>	<b>0.09±0.02b</b>	<b>1.6±0.3b</b>	12.2±3.2a	<b>4.0±0.8b</b>	4.1±0.9a	1133±229a
P40	<b>0.76±0.12a</b>	<b>4.3±0.7a</b>	<b>1.4±0.2a</b>	<b>1.3±0.2a</b>	<b>1.11±0.23a</b>	<b>0.16±0.02a</b>	<b>2.5±0.4a</b>	16.7±4.8a	<b>6.0±0.9a</b>	6.0±1.0a	1646±275a
P80	<b>0.89±0.23a</b>	<b>4.6±1.1a</b>	<b>1.4±0.3a</b>	<b>1.0±0.2a</b>	<b>1.04±0.21a</b>	<b>0.15±0.03a</b>	<b>2.3±0.6a</b>	11.8±3.0a	<b>5.2±1.0a</b>	4.9±1.0a	1470±387a
<b>Year 2: Effects of cropping system on nodules</b>											
Mono cowpea	<b>1.11±0.24a</b>	<b>6.1±1.0a</b>	<b>2.0±0.3a</b>	<b>1.6±0.3a</b>	<b>1.3±0.2a</b>	<b>0.21±0.03a</b>	<b>3.5±0.6a</b>	17.6±3.4a	<b>7.4±1.0a</b>	<b>7.7±1.1a</b>	<b>22877±336a</b>
Inter-row cowpea	<b>0.69±0.12b</b>	<b>4.6±0.9ab</b>	<b>1.5±0.3ab</b>	<b>1.2±0.2ab</b>	<b>1.1±0.3ab</b>	<b>0.15±0.02ab</b>	<b>2.4±0.5b</b>	17.7±6.9a	<b>6.1±1.1b</b>	<b>6.0±1.3b</b>	<b>1737±343ab</b>
Intra- row cowpea	<b>0.57±0.09b</b>	<b>3.3±0.6b</b>	<b>1.1±0.2b</b>	<b>0.9±0.1b</b>	<b>0.7±0.1bc</b>	<b>0.11±0.02b</b>	<b>1.6±0.3c</b>	11.7±3.6a	<b>4.5±0.8c</b>	<b>4.2±0.8c</b>	<b>1101±223b</b>
Intra – hole cowpea	<b>0.26±0.08c</b>	<b>1.4±0.4c</b>	<b>0.4±0.1c</b>	<b>0.4±0.1c</b>	<b>0.3±0.1c</b>	<b>0.06±0.01c</b>	<b>0.8±0.2d</b>	7.8±2.3a	<b>2.3±0.6d</b>	<b>2.2±0.6d</b>	<b>523±153c</b>
<b>Year 2: Effects of P on pods</b>											
P0	<b>23.8±3.8b</b>	<b>88.4±12.1b</b>	<b>18.2±2.3b</b>	<b>26.5±3.5b</b>	<b>12.2±1.6b</b>	<b>2.0±0.26b</b>	<b>71.0±8.7b</b>	<b>319.8±38.9b</b>	<b>177.9±22.0b</b>	<b>180.4±20.9b</b>	<b>653±127a</b>
P40	<b>45.2±5.2a</b>	<b>137.8±16.5a</b>	<b>29.8±3.5a</b>	<b>47.7±7.1a</b>	<b>17.4±2.2a</b>	<b>3.3±0.39a</b>	<b>106.5±13.1a</b>	<b>441.0±63.5a</b>	<b>252.4±31.4a</b>	<b>268.5±34.6a</b>	<b>842±111a</b>
P80	<b>45.8±8.4a</b>	<b>133.4±23.5a</b>	<b>29.1±5.0a</b>	<b>40.0±7.1a</b>	<b>15.5±2.6a</b>	<b>3.1±0.54a</b>	<b>100.5±19.7a</b>	<b>407.5±79.9a</b>	<b>247.0±39.4a</b>	<b>236.5±41.9a</b>	<b>730±130a</b>
<b>Year 2: Effects of cropping system on pods</b>											
Mono cowpea	<b>67.3±8.7a</b>	<b>202.6±22.4a</b>	<b>42.3±4.6a</b>	<b>64.0±6.8a</b>	<b>24.4±2.5a</b>	<b>4.6±0.50a</b>	<b>158.3±18.0a</b>	<b>711.2±74.6a</b>	<b>342.1±35.9a</b>	<b>378.4±39.5a</b>	<b>1318±147a</b>
Inter-row cowpea	<b>37.5±4.3b</b>	<b>120.1±11.3b</b>	<b>26.3±2.7b</b>	<b>37.4±5.0b</b>	<b>14.8±1.3b</b>	<b>2.8±0.31b</b>	<b>92.0±8.3b</b>	<b>370.9±27.1b</b>	<b>248.0±25.8b</b>	<b>228.4±21.3b</b>	<b>727±77b</b>
Intra- row cowpea	<b>27.3±4.7bc</b>	<b>87.3±16.3bc</b>	<b>19.2±3.7bc</b>	<b>29.2±7.7bc</b>	<b>11.6±2.3c</b>	<b>2.1±0.41bc</b>	<b>68.2±13.9bc</b>	<b>261.6±41.2bc</b>	<b>181.4±35.0bc</b>	<b>171.2±33.6bc</b>	<b>519±93bc</b>
Intra – hole cowpea	<b>20.9±3.1c</b>	<b>69.3±10.2c</b>	<b>15.1±2.4c</b>	<b>21.6±3.3c</b>	<b>9.3±1.4c</b>	<b>1.7±0.30c</b>	<b>52.1±7.6c</b>	<b>214.1±30.0c</b>	<b>131.6±21.7c</b>	<b>135.8±19.4c</b>	<b>403±56c</b>

Table 5.4. Effect of cropping system on the amounts of mineral elements in shoots roots and whole plant of nodulated cowpea and maize planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (bold type) are significant at  $P \leq 0.05$ . nd = not determined. The effects of cropping system on cowpea and maize shoots, and roots for some mineral elements uptake were not significant in Year 1. The effects of cropping system on whole plant uptake of mineral elements in maize were not significant in Year 1.

Treatment	P	K	Mg	Ca	S	Na	Cu	Zn	Mn	B	Fe	Al
	mg g DM <sup>-1</sup>											
<b>Year 2: Effects of cropping system on cowpea shoots (Shoot + Ped)</b>												
Mono cowpea	180.6±26.4a	1170.7±167.3a	163.5±19.4a	810.5±106.1a	139.2±18.6a	36.7±4.7a	585±75a	2712±325a	2641±376a	2471±303a	10472±698a	12450±1113a
Inter-row cowpea	93.3±11.4b	651.6±72.6b	99.6±8.3b	473.4±49.4b	77.0±6.1b	23.9±2.7b	296±27b	1334±104b	2208±331b	1521±145b	6822±966b	8890±1423b
Intra-row cowpea	67.0±9.9bc	429.7±89.0bc	71.9±9.9bc	347.8±55.0bc	60.6±6.4bc	16.9±3.5bc	204±40bc	914±129bc	1450±214bc	1116±205bc	4586±493bc	6186±753bc
Intra-hole cowpea	56.1±7.5c	365.0±65.3c	62.0±8.9c	310.1±43.5c	50.6±6.9c	13.9±2.9c	168±23c	773±102c	1145±223c	985±146c	3998±812c	5276±1191c
<b>Year 2: Effects of cropping system on maize shoots</b>												
Mono maize	152.8±25.4a	1214.1±145.7a	186.6±26.2a	182.8±29.1a	73.9±19.4a	36.5±6.0a	242±29a	3165±470a	2720±440a	502±65a	19202±6319a	27157±11328a
Inter-row maize	136.5±26.3a	1024.5±186.7a	148.1±27.4a	148.8±24.8a	40.4±6.93b	24.2±4.1a	172±23a	2783±532a	2373±430a	377±62a	13764±1827a	19067±3037a
Intra-row maize	144.1±25.6a	1123.1±153.0a	147.0±20.3a	146.1±20.2a	42.1±5.7b	24.6±3.3a	211±29a	2863±311a	2266±318a	409±55a	20493±4369a	27996±6291a
Intra-hole maize	131.6±20.3a	1064.0±129.5a	145.5±16.8a	152.5±18.8a	43.0±4.4b	24.9±3.7a	207±21a	2666±324a	2736±410a	429±44a	15674±2154a	23106±3763a
<b>Year 2: Effects of cropping system on cowpea roots (Nodules + Roots)</b>												
Mono cowpea	6.4±0.9a	38.9±4.0a	12.6±1.3a	10.1±1.0a	9.6±1.0a	9.6±0.8a	21.3±2.2a	111.3±14.8a	50.1±4.8a	75.26.5a	11145±1191a	16262±1795a
Inter-row cowpea	4.1±0.5b	28.4±3.3b	9.9±1.4b	7.6±0.9b	7.6±1.0b	7.6±0.8b	15.5±1.8a	87.1±20.2b	43.2±5.0a	53.77.1b	7458±990a	12508±1659a
Intra-row cowpea	3.5±0.4bc	23.4±2.8bc	7.9±0.9bc	6.1±0.6bc	5.9±0.6bc	6.2±0.6bc	12.2±1.2a	70.3±16.0b	32.2±3.2a	42.65.3bc	5439±771a	9814±1045a
Intra-hole cowpea	2.7±0.3c	18.2±1.5c	5.8±0.5c	4.8±0.4c	4.7±0.5c	5.2±0.5c	11.1±1.1a	75.4±11.7b	28.8±3.5a	37.03.6c	4529±542a	9241±1078a
<b>Year 2: Effects of cropping system on maize roots</b>												
Mono maize	12.0±1.9a	148.9±21.5a	13.9±2.0a	12.1±1.4a	7.9±1.2a	21.2±3.5a	20.4±7.6a	46.5±13.2a	46.6±12.5a	13.2±4.3a	9501±3337a	12728±4178a
Inter-row maize	11.7±1.8a	129.2±14.7a	14.3±2.3a	12.2±1.5a	7.3±0.9a	23.3±5.3a	14.2±3.9a	42.0±10.6a	40.0±11.7a	10.5±3.2a	7673±2369a	9485±2669a
Intra-row maize	13.4±2.0a	156.2±22.3a	13.4±1.8a	12.2±1.6a	8.6±1.2a	20.9±3.5ab	19.3±5.5a	78.2±35.6a	43.8±9.3a	11.0±2.8a	7365±1954a	9565±2249a
Intra-hole maize	10.9±1.2a	144.6±18.4a	12.7±1.5a	12.1±1.0a	7.4±0.7a	17.6±3.2b	17.4±8.9a	43.4±13.3a	49.0±22.4a	12.7±6.8a	10029±5997a	14074±8523a
<b>Year 1: Effects of cropping system on whole cowpea plant</b>												
Mono cowpea	121.1±12.3a	487.5±43.5a	86.0±8.2a	401.6±45.9a	67.8±6.0a	16.2±1.3a	320.9±30.2a	1554±194a	1006±138a	1151±114a	18053±1836a	nd
Inter-row cowpea	101.0±8.7ab	445.0±27.9a	80.6±5.6a	369.6±22.5a	62.0±4.8a	15.3±1.2a	278.8±17.4a	1294±106ab	812±59ab	1068±75a	15569±1604a	-
Intra-row cowpea	93.2±8.8ab	434.5±33.9a	76.7±7.0a	329.5±30.9a	65.3±6.5a	16.7±1.5a	268.2±18.6a	1185±125ab	729±71ab	1047±92a	17181±2150a	-
Intra-hole cowpea	88.0±11.5b	376.7±38.2a	67.7±8.2a	304.1±41.2a	52.0±5.5a	14.7±1.7a	246.8±26.4a	1102±155b	688±97b	972±134a	17587±2536a	-
<b>Year 2: Effects of cropping system on whole cowpea plant</b>												
Mono cowpea	187±27a	1210±170a	176±20a	821±107a	149±19a	46±5a	606±76a	2823±332a	2691±378a	2546±309a	21617±1594a	28712±1884a
Inter-row cowpea	97±12b	680±75b	109±9b	481±50b	85±7b	32±3b	311±28b	1421±118b	2251±333b	1574±150b	14280±1645b	21398±2604b
Intra-row cowpea	71±10bc	453±91bc	80±11bc	354±55bc	67±7bc	23±4bc	216±41bc	984±134bc	1482±216bc	1159±209bc	10026±1133bc	16001±1436bc
Intra-hole cowpea	59±8c	383±66c	68±9c	315±44c	55±7c	19±3c	180±24c	848±101c	1174±225c	1022±149c	8527±1150c	14517±1790c

Table 5.5. Species differences in the amount of mineral elements in shoots, roots and whole plants of nodulated cowpea and maize planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (**bold type**) are significant at  $P \leq 0.05$ . nd = not determined

Treatment	P	K	Mg	Ca	S	Na	Cu	Zn	Mn	B	Fe	Al
	mg plant <sup>-1</sup>						µg plant <sup>-1</sup>					
<b>Year 1</b>												
<b>A: Shoots</b>												
Cowpea (Shoot + Pod)	93.4 ± 5.3 <b>b</b>	375.6 ± 18.2 <b>b</b>	67.3 ± 3.6 <b>b</b>	334.9 ± 18.2 <b>a</b>	49.9 ± 2.6 <b>a</b>	10.2 ± 0.5 <b>b</b>	224.5 ± 12.5 <b>b</b>	1150.1 ± 74.7 <b>b</b>	749.3 ± 49.3 <b>b</b>	927 ± 48 <b>a</b>	6897.5 ± 90 <b>b</b>	nd
Maize	228.6 ± 10.7 <b>a</b>	1763.2 ± 85.9 <b>a</b>	189.4 ± 7.9 <b>a</b>	279.8 ± 13.9 <b>b</b>	30.3 ± 1.7 <b>b</b>	29.9 ± 1.6 <b>a</b>	608.9 ± 73.2 <b>a</b>	2658.4 ± 201.7 <b>a</b>	1778.1 ± 103.3 <b>a</b>	445 ± 20 <b>b</b>	9137 ± 466 <b>a</b>	-
<b>B: Roots</b>												
Cowpea (Nodule + Root)	7.4 ± 0.5 <b>b</b>	59.9 ± 4.0 <b>b</b>	10.1 ± 0.61 <b>b</b>	15.9 ± 0.9 <b>b</b>	11.4 ± 0.69 <b>a</b>	5.5 ± 0.3 <b>b</b>	54.2 ± 4.3 <b>b</b>	133.7 ± 11.3 <b>b</b>	59.4 ± 4.6 <b>b</b>	132 ± 13 <b>a</b>	10200 ± 836 <b>b</b>	-
Maize	14.7 ± 0.9 <b>a</b>	122.2 ± 8.1 <b>a</b>	12.5 ± 0.67 <b>a</b>	21.9 ± 1.4 <b>a</b>	5.9 ± 0.3 <b>b</b>	23.5 ± 2.5 <b>a</b>	220.6 ± 13.9 <b>a</b>	215.0 ± 12.6 <b>a</b>	301.8 ± 19.9 <b>a</b>	93 ± 6 <b>b</b>	46301 ± 2687 <b>a</b>	-
<b>C: Whole plant</b>												
Cowpea	100.8 ± 5.5 <b>b</b>	435.4 ± 19.9 <b>b</b>	77.5 ± 3.9 <b>b</b>	350.8 ± 18.5 <b>a</b>	61.3 ± 3.1 <b>a</b>	15.6 ± 0.71 <b>b</b>	278.7 ± 12.1 <b>b</b>	1283.7 ± 76.3 <b>b</b>	808.6 ± 49.9 <b>b</b>	1060 ± 52 <b>a</b>	17098 ± 1006 <b>b</b>	-
Maize	243.3 ± 11.0 <b>a</b>	1885.4 ± 89.7 <b>a</b>	201.3 ± 8.1 <b>a</b>	301.6 ± 14.7 <b>b</b>	36.2 ± 1.8 <b>b</b>	53.4 ± 3.4 <b>a</b>	829.5 ± 75.2 <b>a</b>	2873.4 ± 204.6 <b>a</b>	2079.9 ± 102.0 <b>a</b>	539 ± 21 <b>b</b>	55438 ± 2662 <b>a</b>	-
<b>Year 2</b>												
<b>A: Shoots</b>												
Cowpea (Shoot + Pod)	99.2 ± 10.4 <b>b</b>	654.3 ± 69.2 <b>b</b>	99.3 ± 8.4 <b>b</b>	485.5 ± 43.8 <b>a</b>	81.8 ± 7.3 <b>a</b>	22.9 ± 2.1 <b>b</b>	313.2 ± 32.6 <b>a</b>	1433.1 ± 144.6 <b>b</b>	1860.9 ± 166.6 <b>b</b>	1523 ± 132 <b>a</b>	6469 ± 523 <b>b</b>	8201 ± 687 <b>b</b>
Maize	141.2 ± 11.9 <b>a</b>	1106.4 ± 75.8 <b>a</b>	156.8 ± 11.4 <b>a</b>	157.5 ± 11.6 <b>b</b>	49.9 ± 5.6 <b>b</b>	27.6 ± 2.3 <b>a</b>	208.0 ± 13.1 <b>b</b>	2869.2 ± 204.8 <b>a</b>	2523.8 ± 197.0 <b>a</b>	429 ± 29 <b>b</b>	17283 ± 2019 <b>a</b>	24332 ± 3385 <b>a</b>
<b>B: Roots</b>												
Cowpea (Nodule + Root)	4.2 ± 0.4 <b>b</b>	27.2 ± 1.9 <b>b</b>	9.1 ± 0.64 <b>b</b>	7.2 ± 0.5 <b>b</b>	6.9 ± 0.48 <b>a</b>	7.2 ± 0.41 <b>b</b>	15.1 ± 1.0 <b>a</b>	86.0 ± 8.1 <b>a</b>	38.6 ± 2.4 <b>a</b>	52 ± 4 <b>a</b>	7143 ± 574 <b>a</b>	11956 ± 803 <b>a</b>
Maize	11.9 ± 0.9 <b>a</b>	144.8 ± 9.5 <b>a</b>	13.6 ± 0.94 <b>a</b>	12.1 ± 0.7 <b>a</b>	7.8 ± 0.49 <b>a</b>	20.7 ± 1.9 <b>a</b>	17.8 ± 3.3 <b>a</b>	52.5 ± 10.3 <b>b</b>	44.9 ± 7.2 <b>a</b>	11 ± 2 <b>b</b>	8642 ± 1826 <b>a</b>	11463 ± 2464 <b>a</b>
<b>C: Whole plant</b>												
Cowpea	103.4 ± 10.7 <b>b</b>	681.5 ± 70.7 <b>b</b>	108.3 ± 8.8 <b>b</b>	492.6 ± 44.2 <b>a</b>	88.8 ± 7.6 <b>a</b>	30.0 ± 2.5 <b>b</b>	328.2 ± 33.3 <b>a</b>	1519.1 ± 148.1 <b>b</b>	1899.4 ± 168.1 <b>b</b>	1575 ± 937 <b>a</b>	13612 ± 1004 <b>b</b>	20157 ± 1253 <b>b</b>
Maize	153.2 ± 12.4 <b>a</b>	1251.2 ± 80.6 <b>a</b>	170.4 ± 11.7 <b>a</b>	169.7 ± 11.8 <b>b</b>	57.7 ± 5.7 <b>b</b>	48.3 ± 3.2 <b>a</b>	225.8 ± 14.4 <b>b</b>	2921.8 ± 204.7 <b>a</b>	2568.7 ± 197.5 <b>a</b>	441 ± 29 <b>b</b>	25925 ± 2520 <b>a</b>	35795 ± 3964 <b>a</b>

Table 5.6. Effect of cropping system on the amount of mineral elements mined by sole cowpea and maize planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (**bold type**) are significant at  $P \leq 0.05$ . nd = not determined.

Treatment	P	K	Mg	Ca	S	Na	Cu	Zn	Mn	B	Fe	Al
<b>Year 1: Effects of cropping system on amounts of mineral elements mined by the two species (mg.plant<sup>-1</sup>) for macroelements and (µg.plant<sup>-1</sup>) for microelements</b>												
Mono cowpea	<b>121.1±12.3b</b>	<b>487.5±43.5b</b>	<b>86.0±8.2b</b>	<b>401.6±45.9a</b>	<b>67.8±6.0a</b>	<b>16.2±1.3b</b>	<b>320.9±30.2b</b>	<b>1554±194b</b>	<b>1006±138b</b>	<b>1151±114a</b>	<b>18053±1836b</b>	nd
Mono maize	<b>231.2±20.3a</b>	<b>1867.0±139.0a</b>	<b>193.9±12.5a</b>	<b>293.8±15.5b</b>	<b>36.0±2.6b</b>	<b>51.3±5.4a</b>	<b>752.9±103.9a</b>	<b>2576±300.2a</b>	<b>1943±129.9a</b>	<b>525.9±32.1b</b>	<b>60081±7265a</b>	
<b>Year 1: Effects of cropping system on amounts of mineral elements taken up by the two species (kg/ha)</b>												
Mono cowpea	<b>20.2±2.0a</b>	<b>81.2±7.3b</b>	<b>14.3±1.4a</b>	<b>66.9±7.6a</b>	<b>11.3±1.0a</b>	<b>2.70±0.22a</b>	<b>0.053±0.005a</b>	<b>0.26±0.032a</b>	<b>0.17±0.023a</b>	<b>0.19±0.019a</b>	<b>3.01±0.31a</b>	-
Mono maize	<b>12.8±1.1b</b>	<b>103.7±7.7a</b>	<b>10.8±0.7b</b>	<b>16.3±0.9b</b>	<b>2.0±0.15b</b>	<b>2.8±0.30a</b>	<b>0.042±0.006b</b>	<b>0.14±0.017b</b>	<b>0.11±0.007b</b>	<b>0.029±0.0018b</b>	<b>3.3±0.40a</b>	-
<b>Year 2: Effects of cropping system on amounts of mineral elements taken up by the two species (mg.plant<sup>-1</sup>) for macroelements and (µg.plant<sup>-1</sup>) for microelements</b>												
Mono cowpea	<b>187±27a</b>	<b>1210±170b</b>	<b>176±20b</b>	<b>821±107a</b>	<b>149±19a</b>	<b>46±5b</b>	<b>606±76a</b>	<b>2823±332b</b>	<b>2691±378a</b>	<b>2546±309a</b>	<b>21617±1594b</b>	<b>28712±1884b</b>
Mono maize	<b>165±26a</b>	<b>1363±150a</b>	<b>200±26a</b>	<b>195±29b</b>	<b>82±19b</b>	<b>58±6a</b>	<b>262±31b</b>	<b>3211466a</b>	<b>2767437a</b>	<b>515±66b</b>	<b>287026380a</b>	<b>3988611131a</b>
<b>Year 2: Effects of cropping system on amounts of mineral elements taken up by the two species (kg/ha)</b>												
Mono cowpea	<b>31.2±4.5a</b>	<b>201.6±28.3a</b>	<b>29.4±3.4a</b>	<b>136.8±17.8a</b>	<b>24.8±3.2a</b>	<b>7.7±0.87a</b>	<b>0.101±0.013a</b>	<b>0.47±0.055a</b>	<b>0.45±0.063a</b>	<b>0.42±0.051a</b>	<b>3.60±0.27a</b>	<b>4.79±0.31a</b>
Mono maize	<b>9.2±1.4b</b>	<b>75.7±8.3b</b>	<b>11.1±1.4b</b>	<b>10.8±1.6b</b>	<b>4.6±1.07b</b>	<b>3.2±0.34b</b>	<b>0.015±0.0017b</b>	<b>0.18±0.026b</b>	<b>0.15±0.024b</b>	<b>0.029±0.0037b</b>	<b>1.6±0.35b</b>	<b>2.2±0.62b</b>

Table 5.7. Effect of P supply and cropping system on the amount of mineral elements mined by cowpea planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (bold type) are significant at  $P \leq 0.05$ . nd = not determined.

Treatment	P	K	Mg	Ca	S	Na	Cu	Zn	Mn	B	Fe	Al
kg ha <sup>-1</sup>												
<b>Year 1: Effects of P on amounts of mineral elements</b>												
P0	8.6±1.4b	38.5±5.2b	6.9±1.0a	31.7±5.2a	5.3±0.8b	1.44±0.21a	0.027±0.004a	0.12±0.020a	0.08±0.016a	0.10±0.014a	1.61±0.25a	nd
P40	10.9±2.0a	44.0±7.3 <b>nb</b>	8.2±1.4a	39.0±7.5a	6.4±1.1a	1.49±0.22a	0.028±0.005a	0.14±0.030a	0.08±0.018a	0.11±0.019a	1.48±0.18a	-
P80	11.6±2.1a	49.3±8.4a	8.4±1.4a	36.7±6.1a	6.9±1.1a	1.68±0.25a	0.030±0.005a	0.14±0.026a	0.09±0.018a	0.11±0.019a	1.92±0.36a	-
<b>Year 1: Effects of cropping system on amounts mineral elements</b>												
Mono cowpea	20.2±2.0a	81.2±7.3a	14.3±1.4a	66.9±7.6a	11.3±1.0a	2.70±0.22a	0.053±0.005a	0.26±0.032a	0.17±0.023a	0.19±0.019a	3.01±0.31a	-
Inter-row cowpea	11.2±1.0b	49.4±3.1b	9.0±0.6b	41.1±2.5b	6.9±0.5b	1.70±0.13b	0.031±0.002b	0.14±0.012b	0.09±0.007b	0.12±0.008b	1.73±0.18b	-
Intra-row cowpea	5.2±0.5c	24.1±1.9c	4.3±0.4c	18.3±1.7c	3.6±0.4c	0.93±0.08c	0.015±0.001c	0.07±0.007c	0.04±0.004c	0.06±0.005c	0.95±0.12c	-
Intra-hole cowpea	4.9±0.6c	20.9±2.1c	3.8±0.5c	16.9±2.3c	2.9±0.3c	0.82±0.09c	0.014±0.001c	0.06±0.009c	0.04±0.005c	0.05±0.007c	0.98±0.14c	-
<b>Year 2: Effects of P on amounts of mineral elements</b>												
P0	7.1±2.3c	57.7±17.6b	8.8±2.2b	38.7±9.2b	7.3±1.7b	2.4±0.54b	0.030±0.009b	0.15±0.034b	0.15±0.031b	0.13±0.033b	1.30±0.29b	1.80±0.35b
P40	13.3±2.8b	87.3±19.7a	13.3±2.6a	65.2±14.0a	10.5±1.9a	3.8±0.77a	0.043±0.010a	0.18±0.043 <b>ab</b>	0.22±0.047a	0.20±0.043a	1.72±0.33a	2.59±0.50a
P80	16.5±4.6a	97.7±28.4a	15.2±3.9a	66.7±18.8a	12.9±3.7a	4.0±0.99a	0.045±0.014a	0.22±0.068a	0.26±0.065a	0.21±0.055a	1.65±0.42a	2.25±0.49a
<b>Year 2: Effects of cropping system on amounts of mineral elements</b>												
Mono cowpea	31.2±4.5a	201.6±28.3a	29.4±3.4a	136.8±17.8a	24.8±3.2a	7.7±0.87a	0.101±0.013a	0.47±0.055a	0.45±0.063a	0.42±0.051a	3.60±0.27a	4.79±0.31a
Inter-row cowpea	10.8±1.3b	75.6±8.4b	12.2±1.0b	53.5±5.6b	9.4±0.8b	3.5±0.36b	0.035±0.003b	0.16±0.013b	0.25±0.037b	0.17±0.017b	1.59±0.18b	2.38±0.29b
Intra-row cowpea	3.9±0.6c	25.2±5.1c	4.4±0.6c	19.7±3.1c	3.7±0.4c	1.3±0.22c	0.012±0.002c	0.05±0.007c	0.08±0.012c	0.06±0.012c	0.56±0.06c	0.89±0.08c
Intra-hole cowpea	3.3±0.4c	21.3±3.7c	3.8±0.5c	17.5±2.4c	3.1±0.4	1.1±0.18c	0.010±0.001c	0.05±0.006c	0.07±0.013c	0.06±0.008c	0.47±0.06c	0.81±0.10c
<b>Year 1: Effects of P on amounts of mineral elements (equal plant density)</b>												
P0	14.3±1.2b	66.1±4.8b	11.7±0.9a	51.9±4.2a	9.0±0.64b	2.5±0.18a	0.045±0.003a	0.20±0.017a	0.13±0.012a	0.16±0.012a	2.68±0.23a	-
P40	17.4±1.7a	71.4±4.9 <b>nb</b>	13.5±1.2a	62.4±6.4a	10.5±0.99a	2.6±0.19a	0.046±0.004a	0.22±0.027a	0.13±0.016a	0.18±0.017a	2.64±0.22a	-
P80	18.7±1.6a	80.5±6.0a	13.7±1.0a	61.2±4.9a	11.4±0.78a	2.9±0.23a	0.049±0.004a	0.23±0.022a	0.15±0.015a	0.19±0.017a	3.22±0.39a	-
<b>Year 1: Effects of cropping system on amounts of mineral elements (equal plant density)</b>												
Mono cowpea	20.2±2.0a	81.2±7.3a	14.3±1.4a	66.9±7.6a	11.3±1.00a	2.7±0.22a	0.053±0.005a	0.26±0.032a	0.17±0.023a	0.19±0.019a	3.01±0.31a	-
Inter-row cowpea	16.8±1.5 <b>nb</b>	74.2±4.6 <b>ab</b>	13.4±0.9a	61.6±3.7a	10.3±0.80a	2.6±0.20a	0.046±0.003a	0.22±0.018 <b>ab</b>	0.14±0.010 <b>ab</b>	0.18±0.013a	2.59±0.27a	-
Intra-row cowpea	15.5±1.5b	72.4±5.7b	12.8±1.2a	54.9±5.1a	10.9±1.09a	2.8±0.25a	0.045±0.003a	0.20±0.021b	0.12±0.012b	0.17±0.015a	2.86±0.36a	-
Intra-hole cowpea	14.7±1.9b	62.8±6.4c	11.3±1.4a	50.7±6.9a	8.7±0.91a	2.5±0.28a	0.041±0.004a	0.18±0.026b	0.11±0.016b	0.16±0.022a	2.93±0.42a	-
<b>Year 2: Effects of P on amounts of mineral elements (equal plant density)</b>												
P0	10.0±2.1b	82.5±16.7b	12.9±1.9b	57.5±7.8b	10.8±1.3b	3.6±0.50b	0.044±0.008b	0.22±0.026b	0.22±0.028b	0.20±0.030b	1.89±0.24b	2.75±0.25b
P40	19.9±2.2a	129.3±17.6a	20.2±1.9a	99.0±11.3a	16.2±1.3a	5.8±0.67a	0.062±0.009a	0.27±0.035a	0.35±0.049a	0.31±0.036a	2.59±0.27a	3.95±0.41a
P80	21.8±3.9a	128.9±24.9a	21.1±3.2a	89.8±16.0a	17.4±3.1a	5.6±0.83a	0.058±0.012a	0.28±0.060a	0.37±0.058a	0.28±0.046a	2.33±0.34a	3.38±0.36a
<b>Year 2: Effects of cropping system on amounts of mineral elements (equal plant density)</b>												
Mono cowpea	31.2±4.5a	201.6±28.3a	29.4±3.4a	136.8±17.8a	24.8±3.2a	7.7±0.87a	0.101±0.013a	0.47±0.055a	0.45±0.063a	0.42±0.051a	3.60±0.27a	4.79±0.31a
Inter-row cowpea	16.2±2.0b	113.3±12.6b	18.2±1.5b	80.2±8.3b	14.1±1.2b	5.3±0.54b	0.052±0.005b	0.24±0.020b	0.38±0.055b	0.26±0.025b	2.38±0.27b	3.57±0.43b
Intra-row cowpea	11.8±1.7c	75.5±15.2c	13.3±1.8c	59.0±9.2c	11.1±1.1c	3.9±0.67c	0.036±0.007c	0.16±0.022c	0.25±0.036c	0.19±0.035c	1.67±0.19c	2.67±0.24c
Intra-hole cowpea	9.8±1.3c	63.9±11.0c	11.3±1.5c	52.5±7.3c	9.2±1.2c	3.2±0.54c	0.030±0.004c	0.14±0.017c	0.20±0.038c	0.17±0.025c	1.42±0.19c	2.42±0.30c

Table 5.8. Effects of P supply and cropping system on the amount of mineral elements mined by maize planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (bold type) for are significant at  $P \leq 0.05$ . nd = not determined.

Treatment	P	K	Mg	Ca	S	Na	Cu	Zn	Mn	B	Fe	Al
kg ha <sup>-1</sup>												
<b>Year 1: Effects of P on amounts of mineral elements</b>												
P0	<b>12.4±1.5b</b>	<b>96.2±8.4b</b>	<b>10.7±1.0b</b>	15.5±1.1a	<b>1.9±0.17b</b>	2.9±0.27a	<b>0.037±0.003b</b>	<b>0.15±0.020b</b>	<b>0.11±0.010b</b>	<b>0.028±0.0021b</b>	3.0±0.21a	nd
P40	<b>12.5±1.1b</b>	<b>97.4±6.9b</b>	<b>10.5±0.8b</b>	16.4±1.1a	<b>2.0±0.14b</b>	2.9±0.34a	<b>0.044±0.008b</b>	<b>0.13±0.017b</b>	<b>0.10±0.009b</b>	<b>0.029±0.0019b</b>	3.0±0.14a	-
P80	<b>17.5±1.3a</b>	<b>122.5±9.6a</b>	<b>13.2±0.9a</b>	17.9±1.0a	<b>2.2±0.13a</b>	3.0±0.18a	<b>0.058±0.009a</b>	<b>0.19±0.020a</b>	<b>0.13±0.010a</b>	<b>0.033±0.0018a</b>	3.2±0.37a	-
<b>Year 1: Effects of cropping system on amounts of mineral elements</b>												
Mono maize	12.8±1.1a	103.7±7.7a	10.8±0.7a	16.3±0.9a	2.0±0.15a	2.8±0.30a	0.042±0.006a	0.14±0.017a	0.11±0.007a	0.029±0.0018a	3.3±0.40a	-
Inter-row maize	14.9±1.8a	109.4±10.7a	12.5±1.3a	17.1±1.4a	2.0±0.17a	3.0±0.19a	0.040±0.004a	0.16±0.023a	0.11±0.013a	0.031±0.0024a	3.0±0.23a	-
Intra-row maize	13.5±1.6a	102.0±9.1a	11.2±1.0a	16.3±1.3a	2.0±0.15a	3.3±0.30a	0.048±0.010a	0.15±0.020a	0.12±0.010a	0.029±0.0020a	2.7±0.18a	-
Intra-hole maize	15.2±2.1a	106.5±13.3a	11.4±1.3a	16.6±1.6a	2.1±0.23a	2.6±0.40a	0.055±0.012a	0.19±0.030a	0.13±0.015a	0.030±0.0030a	3.2±0.33a	-
<b>Year 2: Effects of P on amounts of mineral elements</b>												
P0	<b>5.7±1.0b</b>	<b>57.3±7.6b</b>	<b>7.6±0.9b</b>	8.0±1.2a	<b>2.2±0.25b</b>	2.5±0.38a	0.011±0.0016a	<b>0.13±0.013b</b>	<b>0.10±0.012b</b>	<b>0.020±0.0027b</b>	1.4±0.31a	1.9±0.46a
P40	<b>10.4±1.2a</b>	<b>77.2±7.8a</b>	<b>10.5±1.1a</b>	10.2±1.0a	<b>3.4±0.33ab</b>	2.9±0.29a	0.014±0.0013a	<b>0.18±0.020a</b>	<b>0.15±0.018a</b>	<b>0.027±0.0028b</b>	1.3±0.16a	1.7±0.21a
P80	<b>9.4±1.2a</b>	<b>74.1±7.4a</b>	<b>10.3±1.2a</b>	10.1±1.2a	<b>4.0±0.81a</b>	2.6±0.23a	0.013±0.0013	<b>0.18±0.023a</b>	<b>0.18±0.022a</b>	<b>0.027±0.0029a</b>	1.6±0.24a	2.3±0.44a
<b>Year 2: Effects of cropping system on amounts of mineral elements</b>												
Mono maize	9.2±1.4a	75.7±8.3a	11.1±1.4a	10.8±1.6a	<b>4.6±1.07a</b>	3.2±0.34a	0.015±0.0017a	0.18±0.026a	0.15±0.024a	0.029±0.0037a	1.6±0.35a	2.2±0.62a
Inter-row maize	8.2±1.5a	64.1±11.0a	9.0±1.6a	8.9±1.4a	<b>2.6±0.40b</b>	2.6±0.35a	0.010±0.0014a	0.16±0.030a	0.13±0.024a	0.022±0.0036a	1.2±0.15a	1.6±0.18a
Intra-row maize	8.7±1.5a	71.1±9.1a	8.9±1.2a	8.8±1.1a	<b>2.8±0.33b</b>	2.5±0.33a	0.013±0.0017a	0.16±0.017a	0.13±0.018a	0.023±0.0031a	1.5±0.23a	2.1±0.33a
Intra-hole maize	7.9±1.2a	67.1±8.0a	8.8±1.0a	9.1±1.1a	<b>2.8±0.27b</b>	2.4±0.37a	0.012±0.0015a	0.15±0.018a	0.15±0.023a	0.025±0.0027a	1.4±0.36a	2.1±0.54a

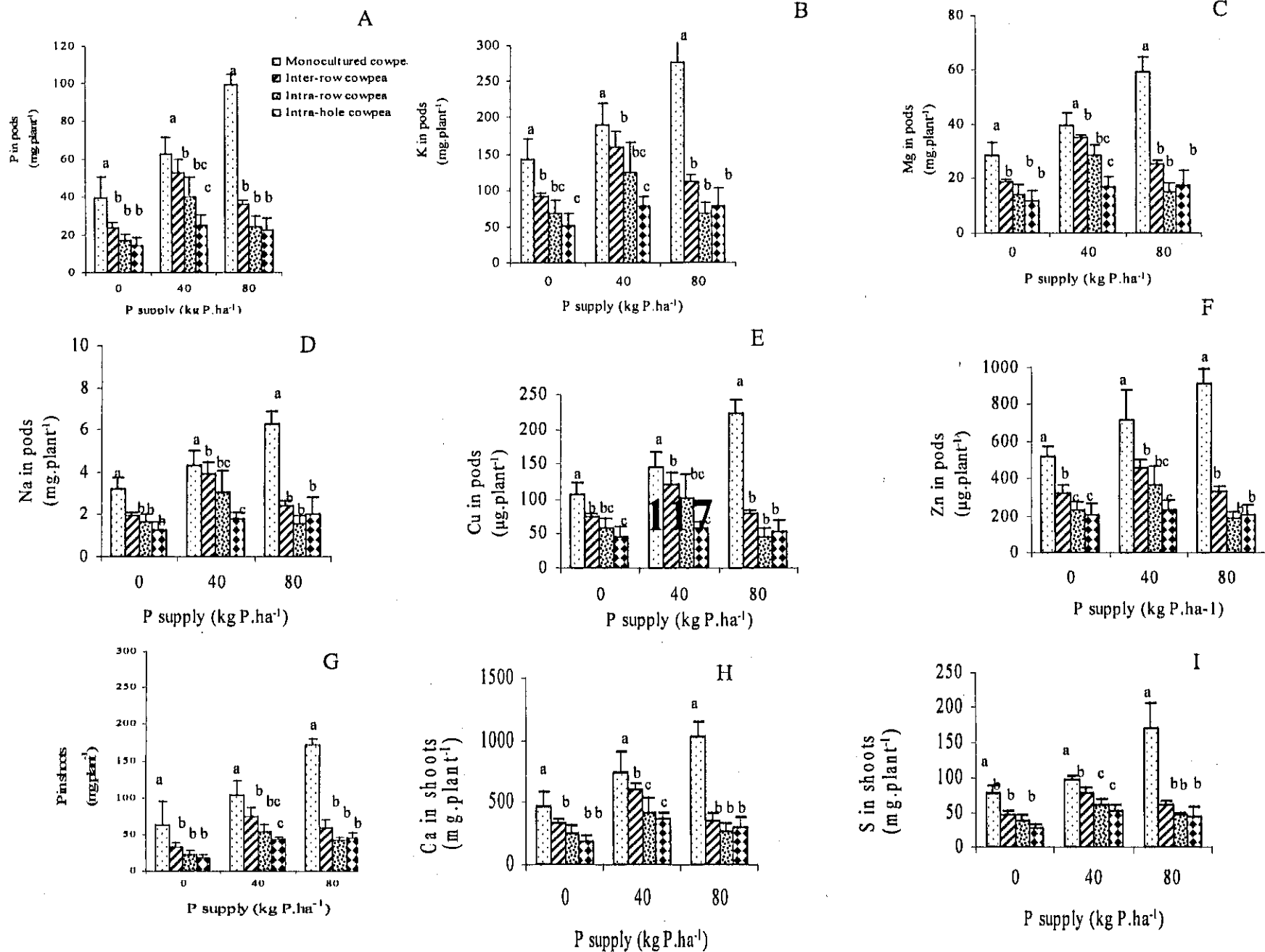


Figure 5.1. Interactive effects of P and cropping system on the amount of mineral elements in pods and shoots of cowpea planted in 2004 (Year 2). A-F= amounts of nutrients in cowpea pods, G-I=amounts of mineral elements in cowpea shoots. Vertical line on bars represent the standard error of mean. Within a P supply rate, different letters on bars indicate significantly different means at P = 0.05 .

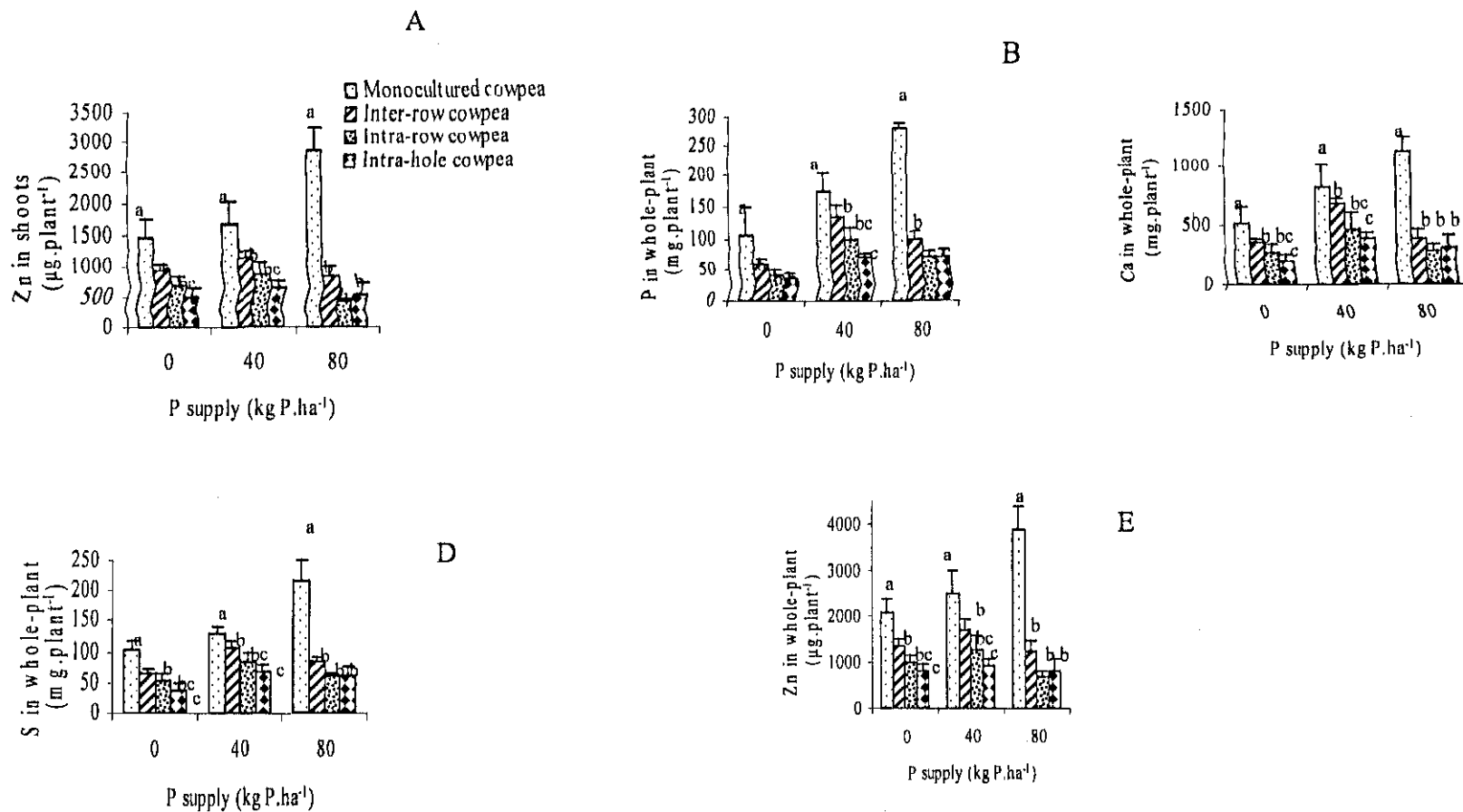


Figure 5.2. Interactive effects of P and cropping system on the amount of mineral elements in pods and shoots of cowpea planted in 2004. (Year 2). A= amounts of mineral elements in cowpea shoots, B -E= amounts of mineral elements in whole-cowpea plant. Vertical line on bars represent the standard error of mean. Within a P supply rate, different letters on bars indicate significantly different means at  $P = 0.05$ .



## CHAPTER 6

CHANGES IN  $\delta^{15}\text{N}$  VALUE AND N NUTRITION IN NODULATED COWPEA (*VIGNA UNGUICULATA* L. WALP.) AND MAIZE (*ZEA MAYS* L.) PLANTS SUPPLIED WITH P IN SOLE AND MIXED CULTURES.

## 6.1 Introduction

Symbiotic N-fixing systems contribute significant amounts of N to cropping systems. The rhizobia-legume symbioses can fix N<sub>2</sub> at rates of 50-300 kg N/ha/year (Dakora and Keya, 1997). As a result, the component species of mixed cultures involving symbiotic legumes and cereals can increase the availability of nutrients to the other species. For example, some studies have shown that the symbiotically-fixed N from the legume may be transferred to the non-legume partner (Giller *et al.*, 1991; Frey and Schüepp, 1993; Elgersma *et al.*, 2000; Høgh-Jensen and Schjoerring, 2000; Chu *et al.*, 2004). Conversely, the cereal can also make other nutrients more available to the legume through the activity of root exudate molecules such as phytosiderophores (Dakora and Phillips, 2002).

Although available evidence suggests that some factors such as crop species, planting patterns, and crop densities can affect biological N<sub>2</sub> fixation (Tothill, 1985; Fujita *et al.*, 1990), few data exist to confirm this view. In farmers' field, planting systems such as intra-row and intra-hole cropping are widely used, yet few detailed studies exist which have addressed the effects of these cropping systems on mineral nutrition in N<sub>2</sub> fixation of symbiotic legumes. Although there is evidence that intercropping can reduce growth of the symbiotic legume via the overshadowing effects of the cereal partner (Willey and Osiru, 1972; Willey, 1979; Mead and Willey, 1980; Horwith, 1985), it is still unclear whether this is genetically pre-determined for the species or only a phenotypic trait. Although a number of studies have been done on cowpea/maize intercropping, these have largely involved the use of inbred lines, whose response is likely to differ from that of landraces.

This study assesses the effects of exogenous P supply and cropping system on plant growth and N<sub>2</sub> fixation in cowpea intercropped with maize in the Western Cape Province of South Africa.

## **6.2 Materials and Methods**

### **6.2.1 Experimental site**

Field experiments were conducted under irrigation at the Agricultural Research Council Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa, during the 2003 and 2004 summer seasons at two different locations within Nietvoorbij. The site description is outlined in chapter 2 section 2.2.1.

### **6.2.2 Experimental design and treatments**

The experimental treatments consisted of three levels (0, 40, and 80 kg P.ha<sup>-1</sup>) of triple superphosphate [46% P, 13.5% Ca, 1.5% S, 0.0007% Cu, 0.008% Zn, 0.021% Mn, 0.053% B and 0.0009% Mo (Cherney, 2003; FAS, 2005)] and four cropping systems (namely, monoculture, *maize/cowpea inter-row*, *maize/cowpea intra-row*, and *maize/cowpea intra-hole cropping*), which were used in both 2003 and 2004. The experimental layout, the crop varieties used and other cultural practices used are described in chapter 2 section 2.2.2.

### **6.2.3 Characterisation of selected soil chemical properties**

The pH of soil was measured in 0.01 M CaCl<sub>2</sub> solution using a 1:2.5 soil-to-solution ratio. Extractable P was determined by citric acid method as described by Dyer (1894) and modified by the Division of Chemical Services (DCS, 1956) and Du Plessis and Burger (1964). A 20 g air-dried soil sample was extracted in 200 mL of 1% citric acid, heated to 80 °C, shaken for 2 min at 10-min intervals over a total period of 1 h and filtered. A 50 mL aliquot was heated to dryness on a water bath, digested with 5 mL of concentrated HCl and HNO<sub>3</sub>, evaporated to dryness on a

water bath, and 5 mL of concentrated HNO<sub>3</sub> and 20 mL of de-ionised water added. The mixture was heated to dissolve the dry residue, and the sample filtered. Measurement of P was then done directly by direct aspiration on a calibrated simultaneous ICP spectrophotometer (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusettes, USA).

#### **6.2.4 Measurement of soil N**

Soil samples were analysed for total N concentrations by a commercial laboratory (BemLab, De Beers RD, Somerset West, South Africa), using a LECO-nitrogen analyser (LECO Corporation, St Joseph, MI, USA) with Spectrascan standards (Drobak, Norway) as described by McGeehan and Naylor (1988).

#### **6.2.5 Plant harvest and sample preparation**

At 60 d after planting, cowpea and maize plants were sampled for nutrient analysis. The procedure followed is described in chapter 4 section 4.3.2.

#### **6.2.6 Analysis of $\delta^{15}\text{N}$ and estimation of plant dependence on N<sub>2</sub> fixation**

The ratio of <sup>15</sup>N/<sup>14</sup>N and the concentrations of N in plant organs were measured using a Carlo Erba NA 1500 elemental analyser (Fisons Instruments SpA, Strada Rivoltana, Italy) coupled to a Finnigan MAT 252 mass spectrometer (Finnigan MAT GmbH, Bremen, German) via a Conflo II open-split device.

The  $^{15}\text{N}$  natural abundance technique was used to estimate the legume dependence on  $\text{N}_2$  fixation as follows:

$$\% \text{ N derived from fixation} = \frac{\delta^{15}\text{N Reference plant} - \delta^{15}\text{N Legume}}{\delta^{15}\text{N (Reference plant)} - B} \times 100 \quad (1)$$

Where B is the  $\delta^{15}\text{N}$  value of the legume organ relying entirely on  $\text{N}_2$  fixation for its N nutrition. B values used for cowpea in this study were 1.759 ‰ for shoot, 0.94 ‰ for roots, and 1.4713 ‰ for pods. Maize was used as a reference plant. The amount of N per organ was estimated as product of % N and dry mass of the organ.

### 6.2.7 Statistical analysis

A 2-factorial design (2-way ANOVA) was used to analyse for plant growth and symbiotic performance. The analysis was done using the software of STATISTICA program 1997. Fisher's least significant difference was used to compare significant treatment means at  $P \leq 0.05$  (Steel and Torrie, 1980).

## 6.3 Results

### 6.3.1 Soil chemical properties at planting

The soil pH measured before establishing the experiments in Year 1 and 2 was 6.3. Available P was 40 and 8.8  $\text{mg.kg}^{-1}$  in Year 1 and 2 respectively, whereas total N concentration in the soil was 9.0 and 13.7%  $\text{g.kg}^{-1}$  for Year 1 and 2 respectively.

### **6.3.2 Effects of P supply and mixed plant culture on growth of cowpea and maize**

Exogenous supply of P to cowpea plants numerically, but not significantly, increased growth of all organs and whole plants in Year 1 (Table 6.1). However, in Year 2, shoots, pods, roots, nodules and whole-plants of cowpea were significantly increased with exogenous P application at both 40 kg P.ha<sup>-1</sup> and 80 kg P.ha<sup>-1</sup> relative to zero-P control (Table 6.1). Applying mineral P to maize also significantly increased growth of shoots and whole plants in both Year 1 and 2 relative to zero-P control (Table 6.1). The dry matter yield of organs and whole plants of cowpea was numerically, but not significantly, decreased in mixed culture relative to monoculture in Year 1. However, in Year 2, biomass of shoots, pods, roots, nodules and whole plants were markedly decreased in mixed culture relative to monoculture (Table 6.1). However, with maize, plant growth was not affected by planting pattern in both Years (Table 6.1). At harvest, it was found that applying 40 or 80 kg P.ha<sup>-1</sup> significantly increased cowpea grain yields by 59-65% in 2003 and 44-55% in 2004. With maize, the increases in grain yield were 20-37% in 2003 and 48-55% in 2004 relative to zero-P control. In both cropping seasons, the number of pod-bearing peduncles per plant, the number of pods per plant, the number of seeds per pod, and seed yield per cowpea plant were significantly increased with the application of exogenous P. In contrast, these parameters were all significantly depressed by mixed culture relative to mono culture cowpea. Intercropping maize with cowpea produced higher total yields per unit land area than the sole crop counterpart.

### **6.3.3 Effect of P supply and cropping system on N concentration in organs of nodulated cowpea and maize**

The application of P to cowpea had no effect on N concentrations in organs except for nodules where supplying P significantly increased % N of cowpea nodules in Year 1 (Table 6.2). External supply of P also had no effect on N concentration in maize organs.

The cropping system showed a significant effect on the N concentration of only cowpea roots and maize shoots in Year 2. Intra-hole cowpea roots showed significantly higher N concentrations relative to monoculture and inter-row planted cowpea (Table 6.2). Shoot N concentration in maize was significantly more increased in intra-hole plants relative to monoculture (Table 6.2).

#### **6.3.4 Effect of P supply and cropping system on $\delta^{15}\text{N}$ values in organs of nodulated cowpea and maize**

As shown in Table 6.3, supplying P to cowpea plants significantly decreased the  $\delta^{15}\text{N}$  of shoots, roots, pods and whole plants in Year 2, and to a lesser extent in Year 1. The application of P also decreased the  $\delta^{15}\text{N}$  values of maize roots in the two years of experimentation, leading to a significantly lowered  $\delta^{15}\text{N}$  at the whole-plant level (Table 6.3).

The cropping system also affected the  $\delta^{15}\text{N}$  of maize and cowpea organs. Relative to monoculture, intercropping decreased the  $\delta^{15}\text{N}$  of shoots, roots, pods, nodules and whole plants of cowpea in Year 2, and only in shoots and pods in Year I (Table 6.3). Similarly, shoots, roots and whole plants of maize showed significantly decreased  $\delta^{15}\text{N}$  when grown in mixed culture compared with monoculture.

#### **6.3.5 Effect of P supply and cropping system on percent of nitrogen derived from atmosphere (Ndfa) in organs of nodulated cowpea**

Applying P to symbiotic cowpea significantly increased the % N derived from fixation in virtually all organs and at whole plant level in that of legume in the two years of experimentation (Table 6.4). Cropping system similarly altered the % Ndfa in various organs of cowpea. In both Year 1 and 2, significantly more N was derived from fixation in intercropped cowpea plants as

compared to monocultures, with intra-hole cowpea showing the highest dependency on symbiotic fixation for its N nutrition (Table 6.4).

### **6.3.6 Effect of P supply and cropping system on nitrogen content of organs of cowpea and maize**

Plant total N in cowpea increased significantly with P supply as a result of higher N levels in nodules in Year 1, as well as elevated N content of shoots, roots and nodules in Year 2 (Table 6.5). Whole-plant N also increased in maize with P supply due to greater shoot N accumulation. Although cropping system showed insignificant effect in Year 1, the N levels in shoots, roots, pods and nodules were significantly decreased by intercropping relative to monoculture (Table 6.5). As a result, whole-plant N content was markedly reduced in cowpea plants grown in mixed cultures (Table 6.5). With maize, however, there was no effect of intercropping on total N of organs or whole plants (Table 6.5).

### **6.3.7 Effect of P supply and cropping system on amounts of N<sub>2</sub> fixation in cowpea**

Providing external P to cowpea increased N<sub>2</sub> fixation and accumulation of fixed N in this species. P application increased the amount of fixed-N in shoots, pods and/or roots of cowpea, leading to significantly increased amount at the whole-plant level (Table 6.6). Measurements of N<sub>2</sub> fixation, expressed on per-hectare basis, were also markedly greater with external P supply relative to control, irrespective of the plant density (Table 6.6).

Although there was no effect of cropping system in Year 1, fixed-N levels were significantly lower in the intercropping treatments relative to monoculture for all cowpea organs (Table 6.6). As a result, the measured values of N<sub>2</sub> fixation were similarly decreased by intercropping, irrespective of whether the cowpea densities were different or equalised (Table 6.6).



## **6.4 Discussion**

### **Effects of P and cropping system on plant growth**

In Africa, the most limiting mineral nutrients to increased crop production are N and P. Although the former can be obtained cheaply from the legume symbioses with root-nodule bacteria, the latter is exhaustible (as rock phosphate), and its deficiency negatively affects N yield from the legume-rhizobia symbiosis. Understanding P- use efficiency in cropping systems involving legumes and cereals has prospects for overcoming production constraints posed by N and P. Assessing the response of maize and cowpea to exogenous P supply showed no changes to cowpea growth in Year 1, although with maize there was a significant increase in shoot and whole-plant growth. Because the endogenous soil P at the site of Year 2 experiment was 4.5-fold lower than that of Year 1 (see Table 3.1), whole-plant growth and that of all organs except roots were significantly increased by external P application in both cowpea and maize (Table 6.1).

The response obtained here for legume and cereal growth in soils with low endogenous P, but not at higher P concentration, is consistent with reports by (Dakora, 1984; Israel, 1987; Chang and Shibles, 1985b; Ssali and Keya, 1986; Pereira and Bliss, 1987; Giller *et al.*, 1998; Ndakidemi *et al.*, 1998; Buerkert *et al.*, 2001; Tang *et al.*, 2001; Carsky, 2003; Jensen *et al.*, 2003) who showed that increasing external supply increased organ development and overall plant growth.

### **Effects of P on cowpea nodule function and metabolism in maize**

The external supply of P to nodulated legumes is known to improve symbiotic performance and N<sub>2</sub> fixation in these species (Robson *et al.*, 1981; Jacobsen, 1985; Singleton *et al.*, 1985; Israel, 1987). In this study, the application of P to maize and cowpea in the sole and mixed culture had no effect on the N concentration of organs in either species, except for % nodule-N which rose

with P supply in Year 1. However, the  $\delta^{15}\text{N}$  values of cowpea organs and whole plants were significantly decreased with exogenous P supply in Years 1 and 2 (Table 6.3), clearly indicating P related enhancement of  $\text{N}_2$  fixation. Interestingly, the  $\delta^{15}\text{N}$  values of maize roots and whole plants were also significantly reduced by P application, in a manner similar to the legume. Such a decrease in  $\delta^{15}\text{N}$  of tissues could either stem from mycorrhizal transfer of fixed-N from the root zone to the cereal or direct rhizosphere imports of secreted fixed-N by vigorous growing maize roots.

In general, the lower  $\delta^{15}\text{N}$  values, the greater the N derived from fixation (Shearer and Kohl, 1986). As expected, the lowering of  $\delta^{15}\text{N}$  in shoots, pods, roots and whole plants of cowpea resulted in significantly greater proportion of % N derived from fixation in those organs in Years 1 and 2 (Table 6.4). This increase in Ndfa with P supply was reflected as a rise in total plant N, especially in Year 2 where mineral P provision to a soil low in endogenous P (Table 3.1) resulted in markedly greater total N of shoots, roots, nodules and whole plants (Tables 6.5). Shoot and whole-plant N was similarly increased in Year 1 and 2 with P supply, possibly as a result of improved acquisition by mycorrhizal activity or a well developed root system from enhanced P nutrition (Bianciotto and Bonfante, 2002; Rengel and Marschner, 2005).

The  $\delta^{15}\text{N}$  Ndfa and total N values truly reflect nodule function as fixed-N levels in organs were found to closely mirror those of the various symbiotic traits. As reported by Israel (1987),  $\text{N}_2$  fixation in nodules increased with provision of exogenous P to cowpea plants (Table 6.6). Whether measured on the basis of the different cowpea densities used in the field or on an equalised density basis,  $\text{N}_2$  fixation was markedly increased by P supply to cowpea plants (Table 6.6).

### **Effects of cropping system on cowpea nodule function and metabolism in maize**

The  $\delta^{15}\text{N}$  values of cowpea shoots, pods, roots, nodules and whole plants were also decreased by intercropping when compared to those of monoculture, clearly suggesting greater nodule activity (Shearer and Kohl, 1986). As a result, the % Ndfa in these cowpea organs was significantly higher with intercropping relative to sole culture (Table 6.4). Interestingly, the magnitude of this increase in % Ndfa from intercropping was also greater in Year 2 relative to Year 1, possibly due to differences in endogenous soil P levels. However, total plant N was found to decrease in cowpea with intercropping relative to monocropping (Table 6.5), a true reflection of the decrease in whole-plant biomass with intercropping (Table 6.1),

As a result of the observed decrease in total legume N with intercropping, fixed-N per organ or whole plant was also significantly reduced by intercropping compared to sole culture (Table 6.6). Consequently, the contribution of cowpea to N economy of the cropping system was also decreased by mixed culture relative to monoculture (Table 6.6). Irrespective of whether the fixed-N measurements were adjusted, or not, the differences in cowpea density used in each cropping system, there was still a marked variation in the levels of  $\text{N}_2$  fixation. This clearly suggests that the smaller amount of N-fixed with intercropping was not merely due to differences in plant numbers, but rather to the effect of intercropping on plant function.

Although the levels of N-fixed may be low with intercropping, the values obtained in this study are comparable to those of other studies where legumes were intercropped with cereals (Willey and Osiru, 1972; Willey, 1979; Mead and Willey, 1980; Horwith, 1985). The unadjusted data shown in Table 6.6 for fixed-N in intercropped cowpea are probably close to the amounts obtained in farmers' fields, where cowpea is sparsely cropped with maize or sorghum.

Table 6.1 Effect of P supply and cropping system on dry matter yield of organs and whole plants of maize and cowpea planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (bold type) differ significantly at  $P \leq 0.05$ . NB: Total shoot mass in cowpea = Shoot weight + pod weight; Total root mass in cowpea = root weight + nodule weight.

	Plant dry matter (g.plant <sup>-1</sup> )									
	Total shoot mass	Total root mass	Pods	Cowpea nodules	Whole plant	Total shoot mass	Total root mass	Pods	Cowpea nodules	Whole plant
	-----Year 1-----					-----Year 2-----				
<b>A: Cowpea</b>										
<b>P levels (kg P.ha<sup>-1</sup>)</b>										
P0	12.1±1.03a	1.7±0.15a	4.2±0.34a	0.36±0.06a	13.8±1.01a	<b>13.2±2.30b</b>	<b>1.0±0.10b</b>	<b>8.1±1.06b</b>	<b>0.25±0.04b</b>	<b>14.3±2.40b</b>
P40	13.4±1.28a	1.8±0.20a	4.7±0.45a	0.40±0.04a	15.2±1.31a	<b>20.9±2.50a</b>	<b>1.5±0.14a</b>	<b>12.5±1.47a</b>	<b>0.43±0.07a</b>	<b>22.4±2.60a</b>
P80	14.3±1.25a	2.1±0.27a	5.0±0.44a	0.43±0.05a	16.3±1.26a	<b>20.4±3.60a</b>	<b>1.4±0.14a</b>	<b>11.7±2.12a</b>	<b>0.43±0.06a</b>	<b>21.8±3.70a</b>
<b>Cropping system</b>										
Mono cowpea	14.6±1.54a	1.8±0.15a	5.1±0.53a	0.43±0.05a	16.5±1.55a	<b>31.2±4.00a</b>	<b>1.7±0.14a</b>	<b>18.2±1.91a</b>	<b>0.52±0.06a</b>	<b>33.0±4.10a</b>
Inter-row cowpea	13.9±1.14a	1.7±0.18a	4.7±0.39a	0.36±0.04a	15.6±1.27a	<b>18.2±1.60b</b>	<b>1.4±0.16b</b>	<b>10.9±1.08b</b>	<b>0.47±0.08b</b>	<b>19.6±1.80b</b>
Intra-row cowpea	12.9±1.22a	2.1±0.35a	4.5±0.43a	0.43±0.08a	15.0±1.32a	<b>12.5±2.10c</b>	<b>1.1±0.11c</b>	<b>7.6±1.42c</b>	<b>0.32±0.05c</b>	<b>13.6±2.20c</b>
Intra-hole cowpea	11.6±1.55a	1.8±0.27a	4.1±0.54a	0.37±0.06a	13.4±1.70a	<b>10.8±1.70c</b>	<b>0.9±0.10c</b>	<b>6.3±1.01c</b>	<b>0.16±0.04d</b>	<b>11.7±1.80c</b>
<b>B: Maize</b>										
<b>P levels (kg P.ha<sup>-1</sup>)</b>										
P0	<b>64.6±5.62b</b>	16.6±1.66a	-	-	<b>81.1±5.53b</b>	<b>54.1±6.90b</b>	11.1±1.77a	-	-	<b>65.2±8.60b</b>
P40	<b>65.4±4.86b</b>	16.7±1.04a	-	-	<b>82.1±4.70b</b>	<b>72.4±7.90a</b>	13.7±1.16a	-	-	<b>86.1±8.20a</b>
P80	<b>81.8±5.37a</b>	17.1±2.73a	-	-	<b>98.9±6.17a</b>	<b>70.2±9.40a</b>	13.8±0.85a	-	-	<b>84.0±9.00a</b>
<b>Cropping system</b>										
Mono maize	70.2±4.62a	18.9±3.25a	-	-	89.1±6.29a	82.0±12.40a	13.5±1.60a	-	-	95.5±12.20a
Inter-row maize	74.3±7.19a	15.5±0.94a	-	-	89.8±7.72a	57.3±9.60a	12.1±1.43a	-	-	69.4±10.50a
Intra-row maize	67.9±6.30a	15.3±1.48a	-	-	83.2±7.08a	60.6±7.80a	13.2±1.67a	-	-	73.8±8.60a
Intra-hole maize	69.9±7.79a	17.6±2.47a	-	-	87.4±9.31a	62.4±6.90a	12.6±1.59a	-	-	75.0±8.20a

Table 6.2 Effect of P supply and cropping system on N concentration in organs and whole plants of nodulated cowpea and maize sown in 2003 (Year 1) and 2004 Year 2). Values followed by dissimilar letters in the same column (bold type) are significant at  $P \leq 0.05$ .

	Shoots	Roots	Pods	Nodules	Whole Plant	Shoots	Roots	Whole Plant
	-----Cowpea-----					-----Maize-----		
<b>Year 1: Effect of P on % N in cowpea and maize</b>								
P0	2.6±0.05a	0.77±0.06a	3.5±0.06a	<b>4.8±0.18b</b>	2.9±0.05a	0.93±0.05a	0.09±0.03a	0.50±0.03a
P40	2.5±0.07a	0.77±0.06a	3.6±0.06a	<b>5.2±0.20ab</b>	3.0±0.06a	0.93±0.05a	0.09±0.02a	0.50±0.03a
P80	2.6±0.05a	0.75±0.07a	3.4±0.08a	<b>5.4±0.14a</b>	3.1±0.06a	1.04±0.07a	0.09±0.04a	0.55±0.04a
<b>Year 1: Effect of cropping system on % N in cowpea and maize</b>								
Sole cowpea	2.6±0.07a	0.79±0.06a	3.4±0.07a	5.2±0.22a	3.0±0.07a	0.88±0.05a	0.07±0.03a	<b>0.48±0.03b</b>
Inter-row cowpea	2.5±0.07a	0.70±0.09a	3.6±0.11a	5.1±0.18a	3.0±0.07a	0.89±0.05a	0.09±0.02a	<b>0.49±0.03ab</b>
Intra- row cowpea	2.5±0.07a	0.73±0.06a	3.4±0.04a	5.4±0.23a	3.0±0.07a	1.06±0.07a	0.13±0.06a	<b>0.61±0.05a</b>
Intra – hole cowpea	2.6±0.05a	0.83±0.07a	3.5±0.08a	4.9±0.21a	3.0±0.07a	1.03±0.06a	0.07±0.02a	<b>0.56±0.03a</b>
<b>Year 2: Effect of P on % N in cowpea and maize</b>								
P0	1.9±0.08a	0.96±0.04a	3.3±0.12a	3.4±0.22a	2.4±0.07a	1.04±0.04a	0.57±0.03a	0.80±0.03a
P40	2.0±0.07a	0.97±0.02a	3.0±0.13a	3.4±0.34a	2.4±0.10a	1.04±0.04a	0.62±0.05a	0.85±0.04a
P80	2.0±0.07a	0.97±0.03a	3.0±0.11a	3.5±0.32a	2.4±0.08a	1.06±0.03a	0.57±0.03a	0.80±0.03a
<b>Year 2: Effect of cropping system on % N in cowpea and maize</b>								
Sole cowpea	1.9±0.09a	<b>0.93±0.03b</b>	3.1±0.15a	3.0±0.28a	<b>2.3±0.08b</b>	<b>0.90±0.04b</b>	0.54±0.03a	<b>0.70±0.02b</b>
Inter-row cowpea	1.9±0.09a	<b>0.91±0.03b</b>	3.0±0.14a	3.3±0.36a	<b>2.3±0.09b</b>	<b>1.04±0.04ab</b>	0.54±0.03a	<b>0.80±0.03ab</b>
Intra- row cowpea	2.0±0.08a	<b>0.99±0.03ab</b>	3.2±0.12a	3.3±0.36a	<b>2.4±0.10ab</b>	<b>1.10±0.04a</b>	0.61±0.06a	<b>0.85±0.04a</b>
Intra – hole cowpea	2.1±0.08a	<b>1.05±0.03a</b>	3.2±0.18a	4.1±0.31a	<b>2.6±0.09a</b>	<b>1.15±0.02a</b>	0.65±0.05a	<b>0.90±0.03a</b>

Table 6.3 Effect of P supply and cropping system on  $\delta^{15}\text{N}$  values (‰) of organs and whole plants of nodulated cowpea and maize sown in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (bold type) are significant at  $P \leq 0.05$ .

Treatment	Shoots	Roots	Pods	Nodules	Whole plant	Shoots	Roots	Whole plant
	-----Cowpea-----					-----Maize-----		
<b>Year 1: Effect of P supply on <math>\delta^{15}\text{N}</math> of nodulated cowpea and maize</b>								
P0	1.7±0.14a	6.4±0.23a	2.6±0.12a	12.0±0.28a	3.6±0.09a	2.9±0.32a	<b>8.0±0.18a</b>	5.5±0.20a
P40	1.4±0.27a	6.4±0.30a	2.4±0.12a	11.8±0.30a	3.4±0.18a	2.8±0.17a	<b>7.5±0.12b</b>	5.2±0.09a
P80	<b>0.75±0.26b</b>	6.3±0.24a	2.3±0.17a	11.7±0.34a	3.1±0.15a	2.6±0.18a	<b>7.6±0.12b</b>	5.1±0.12a
<b>Year 1: Effect of cropping system on <math>\delta^{15}\text{N}</math> of nodulated cowpea and maize</b>								
Sole cowpea	1.9±0.20a	6.4±0.27a	<b>2.8±0.16a</b>	11.8±0.31a	3.7±0.17a	<b>3.3±0.31a</b>	7.7±0.13a	5.5±0.17a
Inter-row cowpea	<b>1.5±0.22ab</b>	6.4±0.34a	<b>2.2±0.17b</b>	11.8±0.37a	3.4±0.16a	<b>2.2±0.25b</b>	7.8±0.24a	5.0±0.19a
Intra- row cowpea	1.1±0.29b	6.4±0.27a	<b>2.3±0.12b</b>	12.1±0.26a	3.3±0.16a	<b>2.7±0.21ab</b>	7.6±0.19a	5.2±0.17a
Intra - hole cowpea	<b>0.60±0.30c</b>	6.2±0.32a	<b>2.5±0.15ab</b>	11.6±0.45a	3.1±0.16a	<b>2.9±0.22ab</b>	7.8±0.15a	5.3±0.16a
<b>Year 2: Effect of P supply on <math>\delta^{15}\text{N}</math> of nodulated cowpea and maize</b>								
P0	1.1±0.12a	<b>4.4±0.035a</b>	1.5±0.10a	10.1±0.35a	<b>2.3±0.076a</b>	4.1±0.25a	6.6±0.30a	<b>5.4±0.24a</b>
P40	1.0±0.14a	<b>4.2±0.016b</b>	1.4±0.13a	10.2±0.34a	<b>2.2±0.088a</b>	3.8±0.34a	<b>5.4±0.30b</b>	<b>4.6±0.24b</b>
P80	0.79±0.17b	4.1±0.019c	1.2±0.15b	9.7±0.31a	<b>2.0±0.100b</b>	3.3±0.43a	4.5±0.12c	<b>3.9±0.23c</b>
<b>Year 2: Effect of cropping system on <math>\delta^{15}\text{N}</math> of nodulated cowpea and maize</b>								
Sole cowpea	1.7±0.13a	4.3±0.047a	1.9±0.11a	10.5±0.33a	2.6±0.069a	4.8±0.29a	5.9±0.41a	<b>5.3±0.31a</b>
Inter-row cowpea	0.97±0.13b	4.3±0.047a	1.5±0.14b	10.5±0.41a	2.2±0.086b	3.6±0.43b	5.8±0.39a	4.7±0.31ab
Intra- row cowpea	0.68±0.10bc	4.3±0.043a	1.1±0.10bc	9.6±0.34ab	2.0±0.070c	3.8±0.38ab	5.1±0.34b	4.5±0.28b
Intra - hole cowpea	<b>0.53±0.08c</b>	<b>4.2±0.039b</b>	0.97±0.09c	9.3±0.36b	1.9±0.052c	2.9±0.32b	5.1±0.38b	4.0±0.29b

Table 6.4 Effect of P supply and cropping system on % N derived from fixation (Ndfa) in organs and whole plants of nodulated cowpea sown in 2003 (Year 1) and 2004 Year 2). Values followed by dissimilar letters in the same column (**bold type**) are significant at  $P \leq 0.05$ .

	Shoots	Roots	Pods	Whole plant
<b>Year 1: Effect of P supply on % Ndfa</b>				
P0	<b>72±2.3c</b>	18±2.9a	<b>56±1.9b</b>	<b>48±1.3b</b>
P40	<b>74±3.5bc</b>	19±3.8a	<b>60±2.0a</b>	<b>51±2.5ab</b>
P80	<b>81±2.4a</b>	19±3.1a	<b>61±2.8a</b>	<b>54±2.2a</b>
<b>Year 1: Effect of cropping system on % Ndfa</b>				
Sole cowpea	<b>68±3.3c</b>	18±3.5a	<b>53±2.7b</b>	<b>46±2.5b</b>
Inter-row cowpea	<b>73±3.2bc</b>	18±4.3a	<b>62±2.8a</b>	<b>51±2.4ab</b>
Intra- row cowpea	<b>78±3.4ab</b>	18±3.4a	<b>62±2.0a</b>	<b>53±2.1ab</b>
Intra – hole cowpea	<b>82±2.4a</b>	21±4.1a	<b>59±2.5a</b>	<b>54±2.3a</b>
<b>Year 2: Effect of P supply on % Ndfa</b>				
P0	<b>83±1.9b</b>	<b>38±0.49b</b>	<b>77±1.5b</b>	<b>66±1.2b</b>
P40	<b>84±2.3b</b>	<b>41±0.22a</b>	<b>78±2.1b</b>	<b>68±1.4b</b>
P80	<b>88±2.6a</b>	<b>43±0.27a</b>	<b>81±2.3a</b>	<b>70±1.6a</b>
<b>Year 2: Effect of cropping system on % Ndfa</b>				
Sole cowpea	<b>74±2.1b</b>	<b>39±0.66b</b>	<b>71±1.7b</b>	<b>61±1.1b</b>
Inter-row cowpea	<b>85±2.1ab</b>	<b>40±0.65ab</b>	<b>77±2.2ab</b>	<b>67±1.3ab</b>
Intra- row cowpea	<b>89±1.5a</b>	<b>40±0.60ab</b>	<b>82±1.5a</b>	<b>71±1.1a</b>
Intra – hole cowpea	<b>92±1.2a</b>	<b>42±0.54a</b>	<b>85±1.4a</b>	<b>73±0.8a</b>

Table 6.5 Effect of P supply and cropping system on total N (mg.plant<sup>-1</sup>) in organs and whole plants of cowpea and maize sown in 2003 (Year 1) and 2004 Year 2). Values followed by dissimilar letters in the same column (bold type) are significant at P ≤ 0.05.

Treatment	Cowpea					Maize		
	Shoots	Roots	Pods	Nodules	Whole plant	Shoot	Root	Whole plant
<b>Year 1: Effect of P supply on N content of cowpea and maize</b>								
P0	197.2±18.0a	10.3±1.2a	153.9±13.4a	<b>16.2±2.4b</b>	<b>377.6±30.1b</b>	<b>593.0±44.5b</b>	13.5±3.4a	<b>606.5±44.4b</b>
P40	221.4±21.6a	12.1±1.9a	158.9±15.7a	<b>19.7±2.1ab</b>	<b>412.1±36.6b</b>	<b>748.1±57.2a</b>	15.8±3.8a	<b>763.9±57.1a</b>
P80	246.9±24.4a	9.3±0.9a	167.6±18.1a	<b>23.6±3.0a</b>	<b>447.4±39.3a</b>	<b>657.6±59.8ab</b>	15.9±5.2a	<b>673.4±59.9ab</b>
<b>Year 1: Effect of cropping system on N content of cowpea and maize</b>								
Sole cowpea	236.5±30.2a	10.7±0.8a	186.0±14.0a	22.3±3.1a	455.5±41.8a	602.2±41.2a	10.9±4.1a	613.1±40.9a
Inter-row cowpea	232.1±22.9a	8.7±0.8a	170.4±13.2a	17.7±2.3a	429.0±34.0a	655.8±74.1a	13.5±3.0a	669.3±72.8a
Intra- row cowpea	222.9±21.4a	11.5±2.5a	139.1±15.8a	22.3±3.5a	395.7±37.0a	691.9±48.2a	22.5±7.3a	714.4±47.6a
Intra – hole cowpea	195.9±26.1a	11.2±1.7a	145.1±25.0a	17.0±2.9a	369.2±50.3a	715.0±85.7a	13.2±3.3a	728.3±87.0a
<b>Year 2: Effect of P supply on N content of cowpea and maize</b>								
P0	<b>91.2±19.3b</b>	<b>7.5±0.7b</b>	278.6±44.0a	<b>8.9±1.9b</b>	<b>386.1±64.0b</b>	<b>498.3±70.6b</b>	9.8±3.5a	<b>508.1±70.4b</b>
P40	<b>162.6±21.2a</b>	<b>9.9±0.8a</b>	390.3±56.8a	<b>13.2±2.2a</b>	<b>576.1±74.3a</b>	<b>640.6±64.4ab</b>	10.9±1.8a	<b>651.4±64.3ab</b>
P80	<b>180.8±34.3a</b>	<b>9.2±0.7ab</b>	340.8±63.7a	<b>14.1±2.4a</b>	<b>544.8±97.3a</b>	<b>699.8±84.6a</b>	14.7±5.8a	<b>714.4±83.3a</b>
<b>Year 2: Effect of cropping system on N content of cowpea and maize</b>								
Sole cowpea	<b>249.2±43.5a</b>	<b>11.3±1.1a</b>	567.9±70.1a	<b>15.7±2.6a</b>	<b>844.1±112.2a</b>	722.2±123.0a	9.8±4.1a	731.9±122.1a
Inter-row cowpea	<b>140.8±17.6b</b>	<b>8.3±0.7b</b>	324.0±27.1b	<b>14.7±2.7a</b>	<b>487.8±41.4b</b>	477.4±61.7a	10.2±2.3a	487.6±62.4a
Intra- row cowpea	<b>93.7±12.4b</b>	<b>7.5±0.6b</b>	256.6±61.1b	<b>10.8±2.2ab</b>	<b>368.7±73.4b</b>	619.7±79.7a	18.8±7.8a	638.4±78.8a
Intra – hole cowpea	<b>95.7±19.5b</b>	<b>8.2±0.8b</b>	197.7±30.7b	<b>7.1±2.0b</b>	<b>308.7±43.2b</b>	632.3±64.3a	8.4±1.7a	640.7±63.6a



Table 6.6 Effect of P supply and cropping system on fixed-N in organs and whole plants of cowpea sown in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (**bold type**) are significant at  $P \leq 0.05$ .

Treatment	Shoots	Roots	Pods	Whole plant	N fixed (different plant densities)	N fixed (equal plant densities)
	mg.plant <sup>-1</sup>				kg N.ha <sup>-1</sup>	
<b>Year 1: Effect of P supply on fixed-N</b>						
P0	<b>143.2±14.7b</b>	1.7±0.28a	86.8±9.0a	<b>231.7±21.3b</b>	<b>22.4±3.4b</b>	<b>38.6±3.6b</b>
P40	<b>162.6±18.4ab</b>	2.2±0.43a	94.6±9.2a	<b>259.3±25.7ab</b>	<b>25.6±4.2ab</b>	<b>43.2±4.3ab</b>
P80	<b>200.2±20.9a</b>	2.0±0.47a	99.9±9.6a	<b>302.1±27.6a</b>	<b>29.6±4.5a</b>	<b>50.3±4.6a</b>
<b>Year 1: Effect of cropping system on fixed-N</b>						
Sole cowpea	158.4±19.4a	2.0±0.52a	99.9±10.4a	260.3±27.0a	<b>43.4±4.5a</b>	43.4±4.5a
Inter-row cowpea	175.4±23.4a	1.7±0.51a	106.8±9.3a	283.9±29.6a	<b>31.5±3.3b</b>	47.3±4.9a
Intra- row cowpea	176.3±20.9a	2.0±0.44a	85.2±9.5a	263.4±27.7a	<b>14.6±1.5c</b>	43.9±4.6a
Intra – hole cowpea	164.6±25.0a	2.1±0.41a	83.2±12.6a	249.9±35.8a	<b>13.9±2.0c</b>	41.6±6.0a
<b>Year 2: Effect of P supply on fixed-N</b>						
P0	<b>74.9±15.1c</b>	2.9±0.29b	<b>215.4±35.7b</b>	<b>293.2±50.2b</b>	<b>32.9±8.9b</b>	<b>48.9±8.4b</b>
P40	<b>132.9±15.7b</b>	4.1±0.35a	<b>298.6±43.1a</b>	<b>435.6±54.7a</b>	<b>46.0±9.8a</b>	<b>72.6±9.1a</b>
P80	<b>150.7±25.7a</b>	3.9±0.29a	<b>261.8±43.9a</b>	<b>416.3±67.2a</b>	<b>49.9±13.4a</b>	<b>69.4±11.2a</b>
<b>Year 2: Effect of cropping system on fixed-N</b>						
Sole cowpea	<b>185.0±34.1a</b>	4.6±0.47a	<b>406.1±55.1a</b>	<b>595.7±87.0a</b>	<b>99.3±14.5a</b>	<b>99.3±14.5a</b>
Inter-row cowpea	<b>118.8±14.6b</b>	3.4±0.31b	<b>249.2±21.7b</b>	<b>371.4±31.3b</b>	<b>41.3±3.5b</b>	<b>61.9±5.2b</b>
Intra- row cowpea	<b>84.9±11.7b</b>	3.1±0.28c	<b>210.8±49.6b</b>	<b>298.7±59.5bc</b>	<b>16.6±3.3c</b>	<b>49.8±9.9b</b>
Intra – hole cowpea	<b>89.2±18.7b</b>	3.5±0.32b	<b>168.4±26.8c</b>	<b>261.0±38.6c</b>	<b>14.5±2.1c</b>	<b>43.5±6.4b</b>

## CHAPTER 7

EFFECT OF EXOGENOUS P SUPPLY AND CROPPING SYSTEM ON ACID AND  
ALKALINE PHOSPHATASE ACTIVITY AND MICROBIAL BIOMASS IN THE  
RHIZOSPHERE OF NODULATED COWPEA (*VIGNA UNGUICULATA* L. WALP.) AND  
MAIZE (*ZEA MAYS* L.).

## 7.1 Introduction

Rhizosphere functioning is affected by many biological processes, such as enzyme activity, microbial numbers, root border cells, and mineral nutrient concentrations. Soil enzymes such as acid and alkaline phosphatases are important in the cycling of P and could be good indicators of soil fertility (Dick and Tabatai 1992; Eivazi and Tabatai 1997; Dick *et al.*, 2000). The phosphatases are a group of enzymes responsible for the cleavage of P from organic esters to release inorganic P needed for both plants and microbes. There are two types of phosphatases: acid phosphatase usually produced by plant roots and alkaline phosphatase released by microbes.

Phosphatases play an important role in the regeneration of inorganic P in P-limited soils through the breakdown of organic P esters (Chrost, 1991). The level of their activity in the rhizosphere can differ with crop species (Izaguirre-Mayoral and Carballo, 2002) and crop management strategies (Patra *et al.*, 1990; Staddon *et al.*, 1998; Wright and Reddy, 2001).

Soil microbial biomass is a measure of the size of microbial population in the soil and therefore plays an important role in soil processes (Dalal, 1989; Alvarez and Alvarez, 2000). Microbial biomass in soil could also serve as a major indicator characterising soil health (Nanniperi *et al.*, 1990; Sparling and Ross, 1993). In agriculture, in particular, the interaction between soil microbes and plants plays a significant role in nutrient cycling in the ecosystem (Wahua, 1984; Wiehe and Höflich, 1995; Höflich *et al.*, 2000). As with the phosphatases, microbial biomass in the rhizosphere of plants can differ from species to species. As a result, nutrient management practices such as intercropping and crop rotation can alter soil biological activity through the addition of organic C that serves as an energy source for microbial growth and metabolism (Vaughan and Malcolm, 1985; Alvey *et al.*, 2003). However, whether the supply of mineral

nutrients or cropping system do affect microbial activity and microbial biomass remains to be determined. However, changes in environmental factors such as soil moisture, soil temperature and C inputs from root exudate components (e.g. mucilage and root border cells) have been shown to alter the level of soil biological activity including phosphatase activity and soil microbial biomass (Ross, 1987; Insam *et al.*, 1989). A number of studies have revealed increases in microbial biomass with external supply of minerals (Bolton *et al.*, 1985; Goyal *et al.*, 1993; Höflich *et al.*, 2000), although others have shown the opposite effect (Biederbeck *et al.*, 1984; McAndrew and Malhi 1992; Ladd *et al.*, 1994).

The aim of this study was i) to examine the effects of exogenous P supply and cropping system on acid and alkaline phosphatase activity in the rhizosphere of cowpea and maize, and ii) to assess the effect of P supply and cropping system on the size of soil microbial biomass in the rhizosphere of cowpea and maize.

## **7.2 Material and methods**

### **7.2.1 Experimental site**

Field experiments were conducted under irrigation at the Agricultural Research Council Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa, during the 2003 and 2004 summer seasons at two different locations within Nietvoorbij. The site characteristics were described in detail in chapter 2 section 2.2.1.

### **7.2.2 Experimental design**

The experimental treatments consisted of three levels (0, 40, and 80 kg P.ha<sup>-1</sup>) of triple superphosphate [46% P, 13.5% Ca, 1.5% S, 0.0007% Cu, 0.008% Zn, 0.021% Mn, 0.053% B and 0.0009% Mo (Cherney, 2003; FAS, 2005)] and 4 planting patterns (namely, monoculture, maize/cowpea inter-row, maize/cowpea intra-row, and maize/cowpea intra-hole planting). The

experimental design and layout, and other cultural practices are well described in chapter 2 section 2.2.2

### **7.2.3 Collection and preparation of rhizosphere soil**

At 60 d after planting, “rhizosphere soil”, defined as soil rich in roots and/or adhering to the roots and influenced by root activity, was collected from around maize and cowpea plants for enzyme and microbial biomass analysis. The collection procedure is outlined in chapter 3 section 3.2.4. Collected rhizosphere soil sample was shaken into a labeled plastic bag and stored at 4°C until they were analysed for soil microbial biomass C and acid and alkaline phosphatase activities.

### **7.2.4 Determination of microbial biomass**

The dry weight of each soil sample was first determined. A sub-sample of soil from each plot was analysed for moisture content by means of oven-drying at 107°C for 24 h. The microbial biomass carbon was determined using the substrate-induced respiration technique of West and Sparling (1986) with modifications. Two duplicate rhizosphere soil samples were taken from each plot, and 5 g of the moist field soil placed in 50 mL centrifuge tubes equipped with gas tight lids and fitted with rubber septa for gas sampling. The soil in each tube was then mixed thoroughly with 10 mL of glucose solution. Dissolved glucose was used rather than solid glucose in order to provide proper mixing of glucose with the soil. The tubes were then capped and shaken horizontally at 22°C for 4 h. Ten minutes after sealing the tubes, the initial headspace CO<sub>2</sub> concentration was measured at time zero (T<sub>0</sub>) by injecting 5 mL lab air into the tube, and then pull out 5 mL air from the tube. The headspace CO<sub>2</sub> concentrations were then measured at 1, 2 and 4 h after the T<sub>0</sub> time point by sampling 5 mL of air from the tube with a syringe and then measuring the CO<sub>2</sub> concentrations using gas chromatography (CG). The time that the gas sample was taken was recorded. The slope of the line relating to CO<sub>2</sub> concentration to time was

calculated. The microbial biomass C was calculated as described by Anderson and Domsch (1978):

$$y = 40.04x + 0.37 \quad (1)$$

Where  $y$  = biomass C (mg 100 g soil<sup>-1</sup>),  $x$  = the respiration rate [mL CO<sub>2</sub>. (100 g soil)<sup>-1</sup>.h<sup>-1</sup>], and 0.37 as correction factor for non-respiring microbial populations.

### 7.2.5 Determination of acid and alkaline phosphatase activity

Alkaline and acid phosphatase activities were determined according to the protocol developed by Tabatai (1982). The  $\rho$ -nitrophenyl phosphate tetrahydrate was used in the colorimetric assay of alkaline and acid phosphatase. The  $\rho$ -nitrophenyl phosphate tetrahydrate was dissolved in acetate buffer initially adjusted to pH 6.5 with 0.1M HCl for acid phosphate and to pH 11.0 with 0.1M NaOH for alkaline phosphate. For each enzyme activity, one g of wet soil samples (in duplicate) was added into a flask and treated separately with 4 mL of the pH adjusted solution above. For each soil sample controls were also included. Samples were mixed thoroughly and incubated at 37°C for 1 h. At the end of incubation, enzyme activity was stopped by addition of 1 mL of 0.5 M Na OH and 4 mL of 0.5 M CaCl<sub>2</sub>. The sample was mixed and filtered through a folded Whatman # 2 filter paper. The supernatant was transferred to tubes and measured at 420 nm. Absorbance of filtrates were compared with  $\rho$ -nitrophenol standards bound with phosphate. To account for non-enzymatic substrate hydrolysis, values for control were subtracted from sample replicates. The enzyme activities were expressed on dry weight basis as  $\mu\text{g p-nitrophenol.g}^{-1}$  soil dry wt.h<sup>-1</sup>.

### **7.2.6 Statistical analysis**

A 2-factorial design (2-way ANOVA) involving P and cropping system was used to statistically analyse microbial biomass C and phosphatase enzyme activities in the rhizosphere soil of cowpea and maize separately. The analysis was done using the software of STATISTICA program 1997. Fisher's least significant difference was used to compare treatment means at  $P \leq 0.05$  level of significance (Steel and Torrie, 1980).

## **7.3 Results**

### **7.3.1 Effect of P supply and cropping system on acid and alkaline phosphatase activity in the rhizosphere of cowpea and maize**

Applying exogenous P to the cropping system decreased the alkaline phosphatase activity in the rhizosphere soil of cowpea and maize in Year 1 (Fig 7.1A, B). The acid phosphatase activity was, however, not significant for both species in Year 1 (data not shown).

In Year 2, the provision of P significantly decreased both acid and alkaline phosphatase activity in the rhizosphere of both cowpea and maize especially at the 80 kg P.ha<sup>-1</sup> (Fig 7.1C, D, E and F).

In Year 1, the cropping system had no effect on the acid and alkaline phosphatase activity in the rhizosphere of either cowpea or maize (data not shown). In Year 2 however, acid phosphatase activity in the rhizosphere of cowpea was significantly increased by mixed culture (especially with intra-hole planting) relative to monoculture (Fig 7.2A). Alkaline phosphatase activity was also significantly higher in the rhizosphere of the inter-row planted cowpea relative to monoculture (Fig 7.2B).

### **7.3.2 Effect of P supply and cropping system on microbial biomass in the rhizosphere of cowpea and maize**

The application of P did not alter microbial biomass in the rhizosphere of either cowpea or maize in Year 1 (Table 7.1). In Year 2, however, microbial biomass was significantly increased by P supply in the rhizosphere of both cowpea and maize (Table 7.1). This increase in microbial biomass was further confirmed by the elevated level of microbial respired CO<sub>2</sub> in the rhizosphere of maize in Year 2 (Table 7.2).

In the two years of experimentation, microbial biomass in the rhizosphere of both cowpea and maize was significantly increased by intra-hole planting relative to monoculture and other cropping systems (Table 7.1). The data for Year 2 were similar in pattern, with intra-hole planting again showing significantly increased microbial biomass compared to sole cropping (Table 7.1). As a result of these increases in microbial biomass, the level of microbially-respired CO<sub>2</sub> was also greater in the rhizosphere of intra-hole planted cowpea compared to other treatments (Table 7.2).

### **7.4 Discussion**

In this study, supplying cowpea and maize with exogenous P as triple superphosphate altered acid and alkaline phosphatase activity. Whether because of the relatively high endogenous soil P concentration in Year 1 experimental site ( $40.0 \pm 3.3$  mg P.kg<sup>-1</sup> for Year 1 vs.  $8.8 \pm 0.8$  mg P.kg<sup>-1</sup> for Year 2), soil acid phosphatase activity in the rhizosphere of cowpea and maize was unaffected by exogeneous P supply in Year 1 (data not shown). However, soil alkaline phosphatase activity was significantly decreased by external supply of inorganic P (Fig 7.1A, B). In Year 2, however,



both acid and alkaline phosphatase activities were significantly decreased in the rhizosphere soils of cowpea and maize with increasing P supply (Fig 7.1C, D, E and F). Because phosphatases are implicated in the acquisition of P in low-P soils, their activity generally decreases with P sufficiency (Duff *et al.*, 1994; Yadav and Tarafdar, 2001; Yun and Kaeppler, 2001). The results obtained here with exogenous P supply are therefore consistent with the findings of previous studies (Duff *et al.*, 1994; Yadav and Tarafdar, 2001; Yun and Kaeppler, 2001).

Unlike maize, the activity of acid and alkaline phosphatase in the rhizosphere soil of cowpea was affected by the cropping system, though not in Year 1 possibly due to the relatively high endogenous soil P concentration. Relative to monoculture, intercropping significantly increased the rhizosphere activity of these enzymes, with intra-hole planting exhibiting a markedly large increase in acid phosphatase activity when compared to sole culture (Fig 7.2A, B). The acid phosphatase activity in the rhizosphere soil of cowpea was generally higher than that of maize, a finding consistent with the results of other intercropping studies involving legumes and cereals (Li *et al.*, 2004). This greater acid phosphatase activity in the rhizosphere soil of the legume compared to cereal has been interpreted in other studies to mean that legume facilitates P availability for uptake by the cereal partner in the cropping system (Horst and Waschki, 1987; Ae *et al.*, 1990; Kahm *et al.*, 1999; El Dessoug *et al.*, 2003; Li *et al.*, 2004). This is because rhizosphere with higher phosphatase activity have greater potential to utilise organic P than that with low activity, although it is debated elsewhere that acid phosphatase may not be a major mechanism for P acquisition in low-P environments (Yun and Kaeppler, 2001).

The phosphatases occurring in the rhizosphere could be of microbial origin, although they are generally attributed to plant. Thus, microflora in the rhizosphere also play a major role in nutrient availability to plants, which in turn modifies the rhizosphere microbial community. In this study,

microbial biomass, defined as the living component of the soil organic matter pool that is responsible for organic matter decomposition and nutrient turnover (Sparling and Ross 1993), was altered by external P supply in Year 2, but not in Year 1, possibly because of the relatively good nutrient status of the soil used in the first year of experimentation. Because of the low endogenous P of the soil used in Year 2, supplying mineral P seemed to promote microbial growth and biomass in Year 2 (Table 7.1), which led to increase of CO<sub>2</sub> from microbes (Table 7.2).

The cropping system also affected the microbial biomass in rhizosphere soil. In both Year 1 and 2, intra-hole planting of cowpea and maize significantly increased microbial biomass relative to monocropping of either plant species (Table 7.1). As a result, microbial release of respired CO<sub>2</sub> was also markedly greater when cowpea and maize were co-planted in one hole (Table 7.2). It would seem that microbial biomass is likely to increase where intercropped partners share close root proximity or exhibit intermingling of their roots. A study by Wahua (1984) found that rhizosphere bacterial counts increased with intercropping and was greater in intra-row than inter-row planting. Because legumes and cereals release root exudates with different chemical profiles into the rhizosphere, intercropping and/or crop rotations are likely to cause significant shifts in rhizosphere microbial communities and hence microbial biomass (Alvey *et al.*, 2003).

In conclusion, the data obtained in this study clearly show that careful management of mixed plant cultures supplemented with moderate mineral inputs can optimise soil “health” and produce sustainable yields.

Table 7.1. Effects of P supply and cropping system on microbial biomass C from the rhizosphere soil of cowpea and maize planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (bold type) are significant at  $P \leq 0.05$ .

Treatment	Microbial biomass C (mg 100g <sup>-1</sup> DM <sup>-1</sup> )			
	Cowpea 03	Maize03	Cowpea 04	Maize04
<b>Effect of P microbial biomass C</b>				
P0	425.0±2.9a	421.3±2.8a	<b>442.4±4.5b</b>	439.0±3.7b
P40	426.7±3.6a	423.2±3.9a	<b>451.7±6.7a</b>	<b>444.2±6.3ab</b>
P80	427.9±4.5a	425.9±4.4a	<b>459.6±6.4a</b>	<b>453.5±5.6a</b>
<b>Effect of cropping system on microbial biomass C</b>				
Mono	<b>421.5±3.6b</b>	<b>415.4±2.5b</b>	<b>435.3±4.6b</b>	<b>435.2±6.5b</b>
Inter-row	<b>422.2±3.1b</b>	419.6±3.0b	449.9±6.9ab	439.4±4.5b
Intra- row	<b>424.2±2.7b</b>	<b>420.7±2.1b</b>	<b>452.2±8.0ab</b>	<b>440.1±3.8b</b>
Intra – hole	<b>438.2±5.4a</b>	<b>438.2±5.4a</b>	<b>467.6±5.5a</b>	<b>467.6±5.5a</b>

Table 7.2. Effects of P supply and cropping system on the amount of CO<sub>2</sub> respired from the rhizosphere soil of cowpea planted in 2003 (Year 1) and 2004 (Year 2). Values followed by dissimilar letters in the same column (bold type) are significant at P ≤ 0.05. ns = not significant. The effects of P supply and cropping systems on uptake of some nutrients in nodules and pods were not significant in Year 1 and 2.

Treatment	Microbial respiration (μmol.CO <sub>2</sub> .g soil <sup>-1</sup> .h <sup>-1</sup> )			
	Cowpea 03	Maize03	Cowpea 04	Maize04
<b>Year 1: Effects of P on microbial respiration</b>				
P0	157.77± 8.27a	185.64± 6.78a	202.96± 13.64a	<b>238.20± 5.98c</b>
P40	162.79± 10.25a	191.10± 9.28a	230.51± 19.36a	<b>269.19± 11.40b</b>
P80	166.15± 12.90a	198.57± 9.61a	257.14± 18.38a	<b>311.92± 12.64n</b>
<b>Year 1: Effects of cropping system on microbial respiration</b>				
Mono cowpea	<b>147.86± 10.21b</b>	183.41± 7.32a	<b>187.37± 13.24b</b>	268.32± 17.76
Inter-row cowpea	<b>149.90± 8.88b</b>	192.42± 8.72a	<b>229.27± 19.72ab</b>	268.15± 11.40
Intra- row cowpea	<b>155.53± 7.68b</b>	195.59± 6.12a	<b>235.93± 23.07ab</b>	275.71± 14.83
Intra - hole cowpea	<b>195.66± 15.56a</b>	195.66± 15.56a	<b>268.24± 20.35a</b>	280.24± 15.79

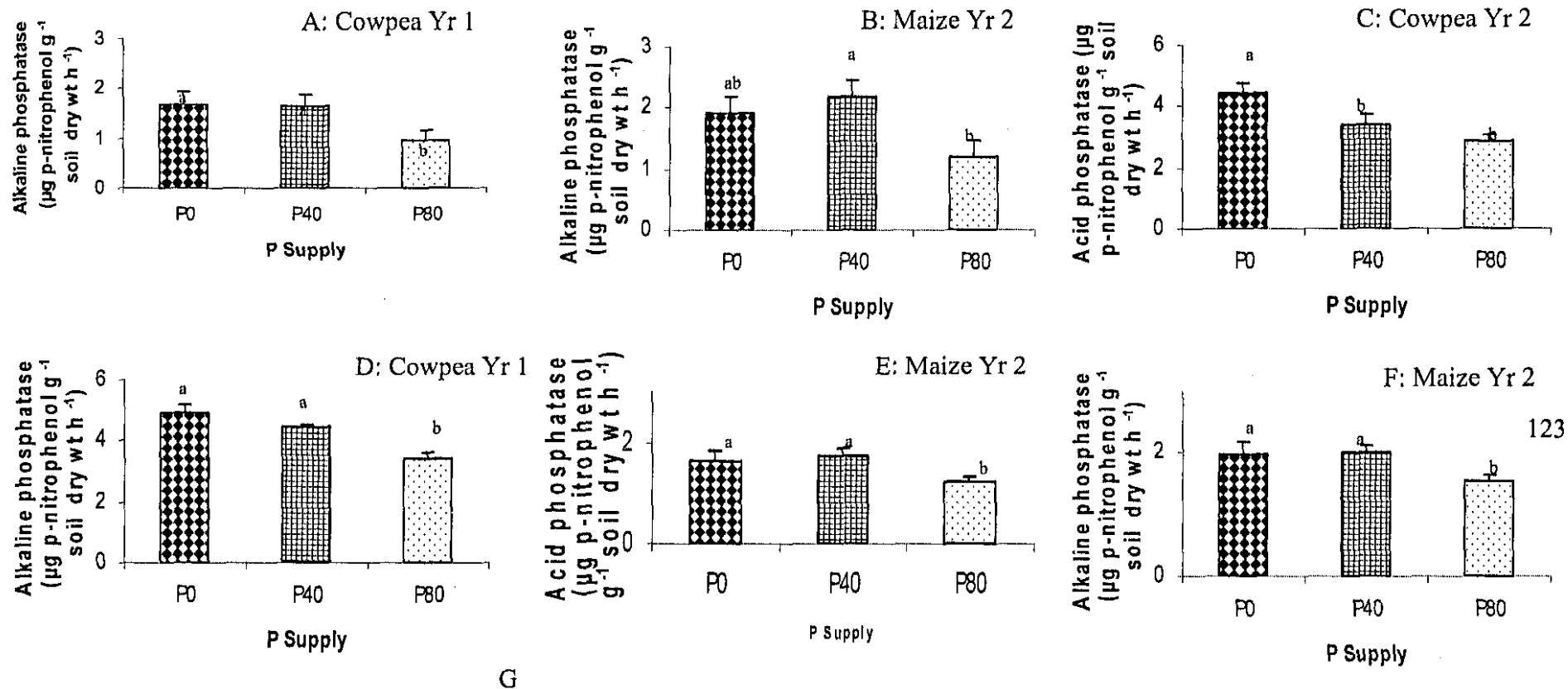


Figure 7.1 .Effects of P supply on the activity of acid and alkaline phosphatase in the rhizosphere soil of cowpea and maize planted in 2003 (Year 1) and 2004 (Year 2). A = alkaline phosphatase in cowpea rhizosphere in Year 1; B = alkaline phosphatase in maize rhizosphere in Year 1; C = acid phosphatase in cowpea rhizosphere in Year 2; D = alkaline phosphatase in cowpea rhizosphere in Year 2; E = alkaline phosphatase in maize rhizosphere in Year 2; F = alkaline phosphatase in maize rhizosphere in Year 2. Vertical lines on bars represent the standard error of mean. For each enzyme different letters on bars indicate significantly different means at P = 0.05. ns = not significant.

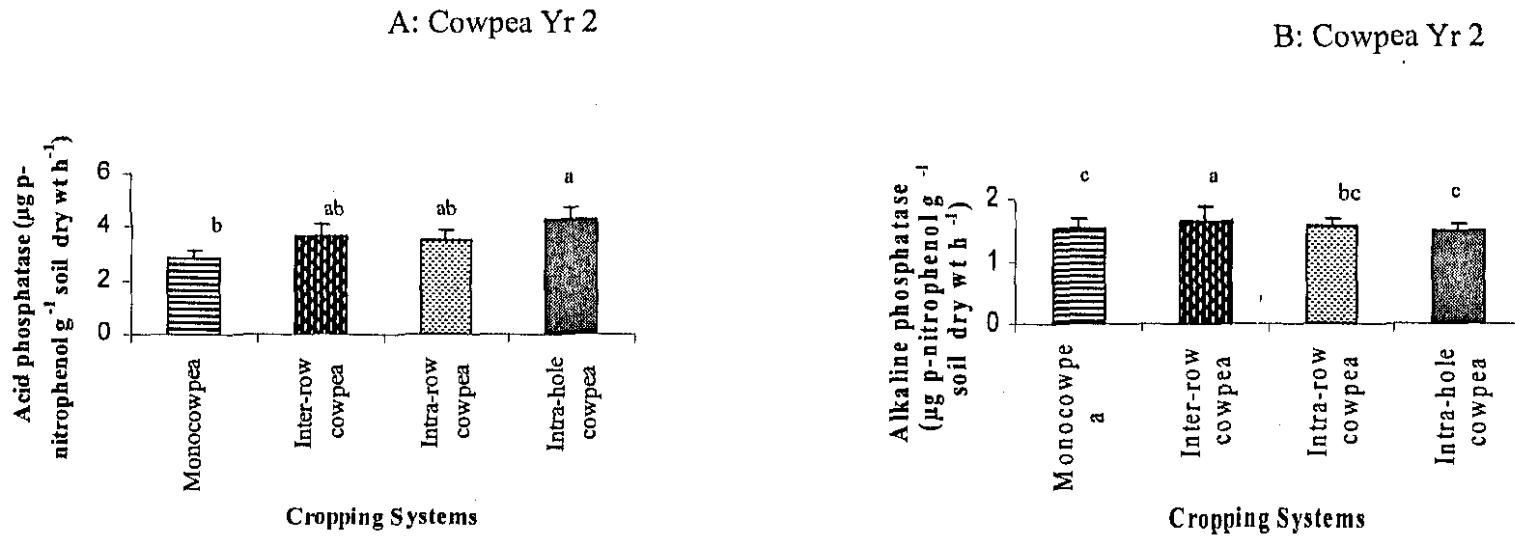


Figure 7.2 . Effect of cropping system on the activity of acid and alkaline phosphatase in the rhizosphere soil of cowpea and maize planted in 2004 (Year 2). A = acid phosphatase in cowpea rhizosphere in Year 2; B = alkaline phosphatase in cowpea rhizosphere in Year 2. Vertical line on bars represent the standard error of mean. For each level, different letters on bars indicate significantly different means at P = 0.05. ns = not significant.

**CHAPTER 8**  
**GENERAL DISCUSSION**

## **General Discussion**

In the global agriculture, N and P are the most limiting mineral nutrients to plant growth and crop production world wide, including Africa (Vance 2001). Results from my 2-year study have shown that the provision of moderate P inputs to low P soil increased plant growth and grain yields of cowpea and maize. This increase in productivity was due to enhanced uptake of P and other mineral nutrients leading to improved symbiotic function and increased nitrogen nutrition of both the legume and cereal. In Africa where rock phosphate is abundant, moderate use of this natural resource is therefore likely to boost crop yields in farmers' fields. However, studies with rock phosphate would have to be undertaken before any exploitation for increasing agricultural yield.

Furthermore, as shown in this study, in a well managed legume/cereal cropping system, P supplementation could improve the production system through organic matter accumulation in the soil and C sequestration. With the increasing CO<sub>2</sub> concentration in the atmosphere, increased legume cultivation in cropping systems could increase crop yields and sequester C with greater economic returns to farmers.

There were strong species differences in nutrient uptake and accumulation by cowpea and maize in this study. At normal plant density monocropping of cowpea showed greater depletion of nutrients from the soil because of its higher plant population relative to maize. This implies that commercial production of cowpea is more likely to deplete the soil of nutrients than maize. However, in traditional African agriculture where intercropping is practiced with very low legume densities, nutrient mining would be expected to be minimal.



The observed changes in mineral content of cowpea organs in this study could have implications in the dietary use of cowpea leaves as vegetables in Africa. Farmers in the tropics, including Africa, grow their crops as mixtures. More specifically, the finding that intercropping can reduce the level of mineral nutrients in leaves and shoots of vegetable crops such as cowpea calls for detailed studies on the effect of mixed cropping on the food quality of crop plants. However, the decrease in mineral content of cowpea with intercropping could also have positive effects on ecosystem functioning, especially where farmers remove crop residues from the field. The decreased content of mineral nutrients in residues of intercropped cowpea implies that the complete removal of such a crop at harvest where intensive cultivation is practiced could potentially reduce nutrient supply to subsequent crops relative to monoculture where the legume accumulated greater amounts of soil nutrients.

A comparison of cowpea with maize revealed large species differences in nutrient accumulation by organs and whole plants. Nutrient levels were generally greater in maize than cowpea, suggesting that the cereal is more likely to exhaust the soil of mineral nutrients than the legume. However, when the plant densities in monocultures and nutrient content per plant were used to estimate the level of nutrient mining by the two species on a per-hectare basis, the cowpea was found to deplete more soil nutrients than the maize because of its greater plant density. This implies that the production of legumes in monocultures, as commonly practiced by commercial farmers, is more likely to exhaust soil nutrients than sole cropped cereals. However, when cowpea is intercropped with maize as done in traditional systems, the nutrient depletion by this legume is likely to be minimal. With the high level of soil degradation in Africa, agricultural practices that reduce soil nutrient depletion would be desirable. The data of this study suggest that intercropping of cowpea with maize or other cereals would be a preferred cropping system for sustainable yields.

Although in this study  $N_2$  fixation was depressed by intercropping, the actual amounts of N-fixed ranged from 14-32 kg N ha<sup>-1</sup> in Year 1 to 15-41 kg N ha<sup>-1</sup> in Year 2 in intercropped cowpea with a very low plant density. Such amounts of N-fixed, though small, would be useful to resource-poor farmers who cannot afford expensive N fertilizers. So, despite the smaller amount of biological N that may be produced by legumes in mixed cultures, legumes remain important in cropping systems because of their contribution to sustainability of the cultural systems. Besides fixed-N, legumes can also make other mineral nutrients more available to cereal partners and vice versa.

In conclusion, intercropping of legumes with cereals is an “old science”. But with new tools and techniques, more data could be generated on belowground processes that would advance our understanding of plant-plant and plant-nutrient interactions in the rhizosphere with potential for increasing crop yields.

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