PHOSPHOROUS DYNAMICS IN SOILS UNDER CONTRASTING LONG-TERM AGRICULTURAL MANAGEMENT PRACTICES IN THE KWAZULU-NATAL MIDLANDS

by

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ABSTRACT

Little is known regarding the effects of land use on soil organic matter and P status of South African soils. For that reason, the effects of the main agricultural land uses in the midlands region of KwaZulu-Natal [maize (*Zea mays*), sugarcane (*Saccharum* spp), annual ryegrass pasture (*Lolium multiflorum*), permanent kikuyu pasture (*Pennisetum clandestnum*), gum (*Eucalyptus grandis*) and pine (*Pinus patula*)] on soil organic matter content, microbial biomass C and P and inorganic and organic P pools derived from a modified Hedley P fractionation was investigated on two sites where the long-term history of land management was known.

In comparison with undisturbed native grassland, permanent kikuyu pasture resulted in an increase in organic C, organic P and microbial biomass C and P. Maize and sugarcane production resulted in a decrease in organic C, organic P and microbial C and P. Under annual pasture, gum and pine forests, organic matter and microbial biomass concentrations remained similar to those under native grassland. Under native grassland, extractable organic P accounted for 50% or more of the total P content of soils but under agricultural management with regular applications of fertilizer P, there was an increase in the percentage of total P present as inorganic P.

Agricultural management greatly affected the distribution of P among the various inorganic and organic P fractions. Resin-Pi and $NaHCO_3$-Pi (the potentially-available forms of Pi) showed similar trends with land use being greatly elevated under kikuyu pasture at both sites and sugarcane and maize at one site. This accumulated Pi was thought to have originated from recent fertilizer applications and possibly recently mineralized organic P. Trends for NaOH-Pi with land use differed greatly from those of the Resin- and $NaHCO_3$-Pi fractions. Concentrations were notably high under maize and sugarcane production. Of the pools of soil organic P, the $NaHCO_3$-Po fraction was most greatly affected by land use, being elevated under kikuyu and decreased under maize and sugarcane. This supports the assertion that it is the $NaHCO_3$-Po fraction that is the most labile soil organic P pool. It was concluded that land use greatly affects soil organic C and P status, soil microbial biomass C and P contents, soil inorganic P concentrations and the distribution of P among the various P fractions.

A short-term (8 weeks) laboratory incubation experiment was carried out to compare the effects of inorganic (KH_2PO_4) and organic (cattle manure, poultry manure and maize crop residues) sources of P, applied at a rate equivalent to 30 kg P ha^{-1}, on soil inorganic and organic P fractions and the potential availability of soil P. Additional treatments consisted of lime [$Ca(OH)_2$] at 5 ton ha^{-1} and lime plus inorganic P. Applications of lime raised soil pH to a similar extent after 1, 4 and 8 weeks incubation. After 8 weeks, a small increase in soil pH was also noted for the cattle and poultry manure and maize residue treatments. For the inorganic P fractions, substantial treatment effects were observed only for the Resin-Pi fraction. The inorganic P source was more effective than the organic ones at increasing Resin-Pi after 1 and 4 weeks incubation and of the organic sources, cattle and poultry manure were more effective than maize residues. Resin-Pi concentrations generally increased between 1 and 4 weeks incubation but then declined rapidly between 4 and 8 weeks incubation. After 8 weeks incubation, treatment effects on Resin-Pi were small. Concentrations of $NaHCO_3$-Pi, dilute HCl-Pi and concentrated HCl-Pi all declined over the incubation period. There was no clear trend with incubation for NaOH-Pi although for the poultry manure and maize treatments, concentrations declined between 4 and 8 weeks incubation. In general, concentrations of $NaHCO_3$-Po were greater for organic than inorganic P sources after 8 weeks incubation suggesting microbial immobilization of P in these treatments. There were increases in $NaHCO_3$-Po and concentrated HCl-Po over the incubation period suggesting progressive immobilization of P from the Pi fractions that declined in concentration during the incubation. Concentrations of NaOH-Po were not greatly affected by incubation period. The lime treatments, however, had lower NaOH-Po concentrations than the others suggesting that liming may have stimulated microbial mineralization of Po. Residual-P concentrations increased over the incubation period. This was attributed to conversion of extractable Pi fractions into recalcitrant, non-extractable Pi forms and/or immobilization of P into intransigent organic forms.

DECLARATION

I declare that this dissertation represents my own research work and has not been submitted for any degree at any academic institution.

Signed......Majaule..........................
Ugele Majaule (candidate)

Signed.M J Savage (for RJ Haynes)..........
Prof. R.J. Haynes (supervisor)

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In the loving memory of my late sister Widzani Vanessa Majaule.

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CHAPTER 1

GENERAL INTRODUCTION AND STUDY OBJECTIVES

The dynamics of soil P are controlled by the interaction of bio-geochemical processes, land-use and cropping practices (Hinsinger, 2001; Oberson *et al.,* 2001; Zheng *et al.*, 2004a). With the increasing emphasis on sustainable agriculture and environmental quality, it is important to quantify the effects of different crop management practices and land use systems on soil P transformations. Declining world P resources and increased pressure on agriculture to feed growing world populations from constantly decreasing agricultural land areas also underscore the need for sustainable P management (Sanyal and De Datta, 1991).

The relative amounts of the different P fractions in soils vary with age of soils, degree of weathering intensity, and land-use. At advanced stages of soil development, the amount of Ca-P held by apatite minerals decreases, whereas organic P, non-occluded Fe- and Al-bound P, Ca-bound P in the form of secondary minerals, and occluded P increases (Matar *et al.,* 1992). However, other factors such as fertilization, liming, irrigation, climate and parent materials also have an immense influence on the relative amounts of the various P forms present. There is a direct relationship between the Ca-P/ (Fe +Al)-P ratio and soil pH (Matar *et al.,* 1992). High levels of Fe and Al oxides in tropical and sub-tropical soils dictate that Fe – and Al-P forms are more abundant than secondary Ca-P forms, except in heavily limed soils.

The total content of P in soils varies greatly, with as much as 5.7 billion hectares worldwide being considered P deficient (Hinsinger, 2001). Sandy savannah soils in arid regions have low total P while prairie soils and those derived from volcanic lavas, some coprolitic limestone, and igneous rocks usually have high content of P (Tisdale *et al.,* 1985). Soil solution P concentration is usually in the 5 – 25 ppm range, which is much lower than that of other macronutrients.

Phosphorus deficiency is a major constraint to crop production on many highly weathered and leached acid soils in tropical and temperate regions of the world (Haynes and Swift, 1988; Hedley *et al.*, 1994). Low P availability is attributed to high

P retention by Al and Fe oxides and amorphous materials (Sanchez and Uehara, 1980; Moller *et al.*, 2000). Consequently, high rates of both lime and phosphate are frequently required for optimal crop yields. Liming of such soils has beneficial effects because Al toxicity inhibits uptake and translocation of phosphate by plants (Haynes, 1984).

Phosphorus uptake by plants is a process limited by the low diffusion rate of P in the soil. Its acquisition by plants is generally proportional to the root surface area in contact with the soil solution (Hinsinger, 2001). Adaptive mechanisms employed by plants on P deficient soils include increased root proliferation, excretion of organic acids and phosphatases, and lowering the rhizosphere pH, all of which enhance P mobility and uptake (Oberson *et al.*, 2001).

Application of P fertilizers can alleviate P deficiency but in the developed nations, soil P surpluses have sometimes resulted in pollution of water resources (Mozaffari and Sims, 1994). The situation is different in most parts of Africa where soil acidity, with associated Al toxicity, as well as P and Ca deficiency, are common growth-limiting factors (Maroko *et al.*, 1999). Deep weathering of these soils due to high rainfall and low evapotranspiration leach out bases (e.g. K, Mg, Ca) and salts from the soil profile, which leaves high concentrations of Al and Fe oxides.

Inadequate return of P to compensate for P losses due to crop and residue removal, leaching or erosion, has caused negative nutrient balances in many tropical soils (Paul and Clark, 1989; Maroko *et al.*, 1999). Increasing productivity solely by high external inputs such as lime, fertilizer, and herbicides, is impractical for most resource-poor farmers because of lack of availability or excessive cost. Fertilizer efficiency is extremely low due to limited soil moisture, or added phosphate is strongly adsorbed by soil surfaces. Organic materials supply soluble P to plants (and other nutrients) and ameliorate Al toxicity by increasing soil solution pH.

In South Africa, acid soils occur extensively in Western and Eastern Cape coastal areas, KwaZulu-Natal, Eastern Mpumalanga and Northern Province (Dekkers, 1971). Large quantities of Al and Fe oxides have been reported for some of representative soil types (Reeve, 1970; Soil Classification Working Group, 1991).

These soils are characterized by severe P limitations for plant growth, high rates of external inputs (e.g. liming, P fertilizers) are required to correct soil acidity and P deficiency, and increase crop productivity in these soils (Bainbridge *et al.*, 1995).

Maize (*Zea mays* L.), sugarcane, dairy, forests, rangeland are the main agricultural land uses in the midlands region of KwaZulu-Natal (KZN) (Dominy *et al.*, 2001). These practices have been shown to affect soil organic matter content and quality (Dominy *et al.*, 2002; Haynes *et al.*, 2003). Generally, South African agricultural industry is successful, particularly in relation to its neighbouring states. Sugarcane is the single largest crop in the KZN province, and this significant industry sees sugarcane plantations along the coastal belt forming the mainstay of the economy and agriculture. KZN is the country's leading timber producer and contributes about 40 percent of the total plantation output for South Africa.

Dairy farming is another important land use in the region. Common pasture grasses are kikuyu grass (*Pennisetum clandestinum*), ryegrass (*Lolium perenne*), browntop (*Agrostis tenuis*) and white clover (*Trifolium repens*). Inclusion of clover in pastures is very popular due to its N_2-fixation potential. Generally, pasture grasses are grown annually, perennially, or in mixed cropping rotation systems involving arable crops. In the Midlands region of KwaZulu-Natal, *P. clandestinum* and *L. perenne* dominate pastures. Kikuyu has higher dry matter yield (Haynes, 1999), and is grown on permanent basis. Due to its susceptibility to winter frost, ryegrass is sown annually to provide animal forage during the winter months.

P turnover and availability are influenced by crop management (Condron, 2004). Sequential extraction procedures such as that developed by Hedley *et al.* (1982) offer the option of examining various inorganic (P_i) and organic (P_o) pools differing in their origin and bioavailability. Fractionation procedures can yield valuable information about P pathways in soils under various agricultural land uses (Tiessen *et al.*, 1982; Beck and Sanchez, 1996; Maroko *et al.*, 1999). However, the assignment of P fractions to pools of differing plant availability, particularly for low P tropical soils is still inconclusive (Buehler *et al.*, 2002).

This project focuses on aspects of soil P dynamics that are influenced by long-term land use and management practices. Understanding the impacts of cropping systems on soil P pools and fluxes will allow for improved P management. The hypothesis of this research is that the distribution of soil P among various pools is influenced by land use and management practices. A modified Hedley *et al.* (1982) fractionation procedure will be used to characterize the soil inorganic (P_i) and organic P (P_o) in different soil P pools at the same depth (0-5 cm), in different land-use systems. P fertilizers, organic manures and lime will be used to manipulate the transformation of P between the various pools in the soil over a period of 8 weeks.

CHAPTER 2

PHOSPHORUS AVAILABILITY IN SOILS: A LITERATURE REVIEW

2.1 Introduction

Soils vary extensively in their capacities to supply phosphorus (P) to plants because a large proportion of total P is not readily available for uptake. Solution P concentration depends on the solubility of inorganic P minerals and desorption of adsorbed inorganic P, but in highly weathered soils, P bioavailability may depend mainly on organic P (P_o) turnover processes (Beck and Sanchez, 1994). The various P minerals present in soil and the concentration of solution P supported by these minerals are highly dependent on solution pH. The most favourable pH for P availability is near neutral to slightly acid (pH 5.5 – 6.5).

The solution concentration required by most plants varies from 0.01 to 0.3 ppm and depends on the crop species and agricultural intensity (Tisdale *et al.*, 1985). Adequate P plant nutrition depends on the availability of labile P forms in the rhizosphere. Labile P is the readily available portion (fraction) of the total P that exhibits a high dissociation rate and rapidly replenishes decreases in solution P due to plant uptake. The remaining portion of adsorbed P that does not readily desorb is termed non-labile, and is not readily available to plants. Bioavailability of P is regulated by rapid replenishment of labile forms by desorption from the solid phase, by mineralization of soil organic compounds, and on diffusion through the soil solution into the rhizosphere.

Soil P reacts extensively with various soil components, essentially by processes of adsorption and precipitation of phosphate minerals (Sanyal and De Datta, 1991). Interactions of phosphates with Al and Fe hydroxides occur at lower pH and with Ca at higher pH. For variable charged soil surfaces, the decrease in phosphate adsorption with increasing pH is attributed to the decrease in electrostatic potential in the plane of adsorption (Ross, 1989). On the other hand, an increase in P adsorption with increasing Ca^{2+} concentration in the soil solution is attributed to several mechanisms such as: Ca phosphate precipitation, formation of surface complexes between P that is held on sorption surfaces and Ca in solution, increase in ionic strength of the background solution, increase in electrostatic potential due to Ca ions, and the adsorption of P by freshly precipitated Fe and Al hydrous oxides (Barrow, 1980; Haynes, 1984; Sanyal and De Datta, 1991).

Soil phosphorus (P) is classified into three pools; solution, labile, and non-labile P (McLaren and Cameron, 1990; Guo and Yost, 1998). The inorganic fraction of soil phosphorus occurs in numerous combinations with Fe, Al, Ca, and other elements. Calcium phosphates become insoluble under alkaline conditions, and Fe and Al phosphates are insoluble under acidic soil conditions. Five classes of soluble organic phosphate compounds commonly found in the soil are; inositol phosphates, phospholipids, nucleic acids, nucleotides, and sugar phosphates. The first three are the dominant groups in soils, but inositol hexaphosphate ester (phytic acid) is more stable than the other phospholipids and nucleic acids (Turner *et al.*, 2003).

Distribution of P among inorganic and organic fractions in soils can be influenced by land-use and cultural practices (e.g. crop rotation, fertilization, irrigation, tillage). These factors influence microbial activity, and therefore the availability of organic and inorganic P (McCallister *et al.*, 2002). Conversion from natural to agricultural ecosystems impacts on soil organic matter pools, soil microbial biomass properties and, hence size of soil P fractions. The fractionation of soil P into various organic and inorganic pools with differing levels of plant availability, coupled with understanding of the P adsorption/desorption properties of soils can indeed provide insights into management practices that influence P bioavailability.

2.1.1 Soil phosphorus pools

Phosphorus pools are characterized by the quantities of P they contain and by the time required for the phosphate ions they contain to enter into the soil solution. P pools range from labile P that can be readily desorbed plus P in solution, to non-labile (stable) P held in insoluble metallo-organic complexes (Guo and Yost, 1998). The three (3) pools of P in the soil are: solution, active (labile) and fixed (non-labile) P (Iyamuremye *et al.*, 1996). Soil solution P is often referred to as the intensity factor, whereas the inorganic and organic labile P are collectively called the quantity factor. The relationship between these pools is defined by the following equilibrium: soil solution P ↔ Labile P ↔ Non-labile P (Figure 2.1).

The quantity of P in any pool depends on the specific characteristics of the soil, its use and management practices applied. Various reactions and processes are involved in the transformation of P between the 3 pools. Results on the effects of soil amendments on P pools can be inconsistent in part because of variability of sites and extraction methods. Guo and Yost (1998) suggested that grouping soil P fractions into these functional pools can enhance the application of nutrient models in P management.

2.1.1.1 Soil solution P

Maintenance of solution P concentration or intensity for adequate P nutrition in the plant depends on the ability of labile P (quantity) to replace soil solution P taken up by the plant. The ratio of intensity to quantity factors is called the capacity factor, which expresses the relative ability of the soil to buffer changes in soil solution concentration (Ige *et al.*, 2005).

Solution P pool represents phosphate that is readily accessible by plant roots (Guo and Yost, 1998). Plants and microorganisms meet their P requirements by drawing phosphate ions from this pool mainly through production of CO_2 and organic acids (Figure 2.1). P is taken up mostly as inorganic orthophosphate (HPO_4^{2-}, $H_2PO_4^-$). Phosphorus taken up by plants and microbes is constantly replaced by release from inorganic and organic forms of P associated with the solid phase (Ross, 1989). Inorganic P can be released to solution by desorption and / or dissolution from mineral P associated with Al, Fe, or Ca. In addition, mineralization of organic P and release of P from the microbial biomass contributes to the solution phase. Thus, organic and microbial P dynamics also play important roles in the control and maintenance of inorganic P in soil solution (Condron, 2004).

Amongst soil microorganisms, mycorrhyzal fungi play a more important role in plant P acquisition. Paul and Clark (1989) have suggested 3 ways for the prominence of mycorrhzae in solubilization of mineral phosphate in soils:

1. excretion of organic acids and production of CO_2;
2. extra surface area and more penetration of soil aggregates, hence more P is drawn; and
3. ability of mycorrhizal hyphae to extract P at lower levels of soil solution concentrations than plant roots.

2.1.1.2 Active P

The active P pool (Figure 2.1) is P in the solid phase, which is relatively easily released to the soil solution. As plants take up phosphate from the solution pool, the concentration of orthophosphate ions in this pool are lowered, and to maintain the equilibrium, phosphate from the active pool is released. The active P pool is constituted of inorganic phosphate adsorbed to clay fraction, organic P that is easily mineralized, and some phosphate that is chemically associated with Ca, Al or Fe McLaren and Cameron, 1990).

2.1.1.3 Fixed P

The fixed P pool contains inorganic phosphate compounds that are very insoluble and organic compounds (organic P, P_o) that are resistant to mineralization by microorganisms (Guo and Yost, 1998). The inorganic phosphate compounds in this fixed pool are more crystalline in their structure and less soluble compared to the active P pool. However, Buehlera *et al.* (2002) demonstrated that for P deficient soils, the fixed P pool can be a significant P source.

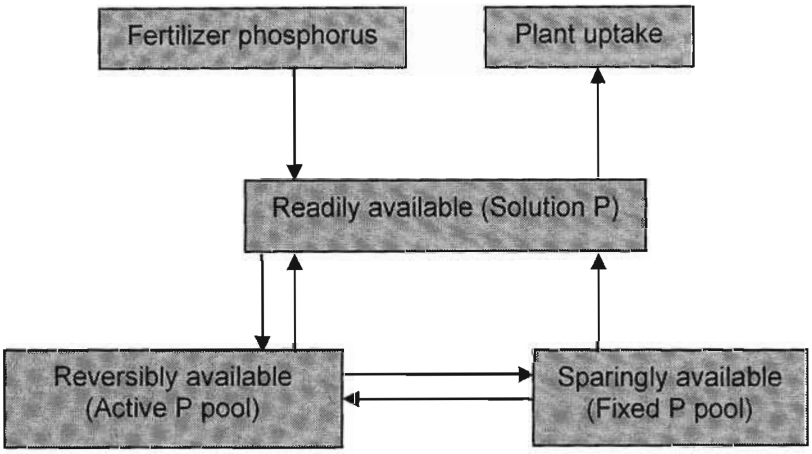


Figure 2.1 A representation of the three functional P pools and their relationships (*Source:* Guo and Yost, 1998)

2.2 Inorganic P (P_i)

Phosphorus exists in sedimentary, metamorphic and igneous phosphate rock deposits in the form of various phosphate minerals such as fluorapatite ($3Ca_3(PO_4)_2.CaF_2$), chloroapatite ($3Ca_3(PO_4)_2.CaCl_2$), and hydroxyapatite ($3Ca_3(PO_4)_2.Ca(OH)_2$). Flourapatite is usually the most important primary P source and exists as crystals of fine sand and silt size in many rocks and soils (Matar *et al.*, 1992). A variety of soil processes, for example Ca^{2+} uptake by plants, influence dissolution of P from apatite minerals. P released from these minerals (and organic sources) can be reprecipitated into various secondary phosphates, incorporated into soil organic matter or adsorbed by soil components (Sanyal and De Datta, 1991).

Inorganic phosphorus compounds present in the soil are orthophosphate ($H_2PO_4^-$ and HPO_4^{2-} and PO_4^{3-}), calcium phosphate, aluminium and iron oxide-phosphates (Cade-Menum *et al.*, 2000). The inorganic P is bound with varying adhesiveness to calcium, iron and aluminium compounds in the soil. The relative significance of each depends primarily on the pH of the soil. Amounts of inorganic, organic and microbial P in soil and the equilibrium concentration of inorganic P in soil solution are determined by a combination of factors. These include soil chemical properties (e.g. clay / hydrous oxide mineralogy, pH, ionic strength) and soil P status as determined by the type, duration and intensity of land use and the associated P inputs (Ross, 1989).

P 'retention' or 'fixation' can be considered as summation of physical adsorption, chemisorption, precipitation and occlusion (Matar *et al.*, 1992). Adsorption/desorption and precipitation/dissolution equilibria control solution P concentration and therefore, its mobility in soil and bioavailability.

2.2.1 Adsorption reactions

Non-specific adsorption or precipitation refers to P retention by coulombic interaction in the outer layer of the adsorbing soil constituent. Under acidic pH conditions – below the point of zero charge (estimated at between pH 7 and 10), polymorphic forms of Al and Fe oxides have a net positive charge and they attract phosphate ions from solution into exchange sites on their surfaces. In contrast to specific adsorption reactions, phosphate and adsorption surfaces form outer-sphere complexes through water molecules – weak bonding.

Important aspects of the chemical or specific adsorption mechanism are: the surface reaction between phosphate and hydroxyl ions, and the coordination of the adsorbed phosphate ions (Tisdale *et al.*, 1985). During specific phosphate adsorption, the oxygen of the phosphate enters into a six-fold coordination with metal cations hence this process is termed ligand exchange. Adsorption of the phosphate ions is also accompanied by release of hydroxyl groups, which in turn may raise the soil pH. Experimental evidence in support of the ligand exchange process – the displacement of hydroxyl (OH^-) groups that are in coordination to hydroxylated mineral surfaces includes kinetics of adsorption and desorption; hydroxyl ion release; infrared spectroscopy; and stereochemical inferences (Goldberg and Sposito, 1985).

The interaction of phosphate and the mineral surfaces is considered specific in reference to the stable bonds formed between these soil constituents (Tisdale *et al.*, 1985). Phosphate ions can be irreversibly adsorbed on many soils through binuclear complex formation process in which a bridging ligand forms from the replacement of two surface hydroxyl groups (Figure 2.2). Adsorption of borate, molybdate and arsenate on amorphous Fe and Al hydroxides, especially in weathered soils occurs by binuclear bridging mechanisms (Ross, 1989).

Binuclear, monodentate and bidentate surface complexes of metal hydrous oxides and the phosphate ions is inferred from crystallographic studies and determinations of hydroxyl ion release (Alejandra *et al.*, 2006). The monodentate reversible complex involves replacement of orthophosphate anions by hydroxyl ions, if the process is energetically favoured. Even though infrared spectroscopic studies show bidentate complex of phosphate on dried acidified goethite, under field conditions, where several factors such as water content, organic acids, and pH become significant, the monodentate complex predominates (Goldberg and Sposito, 1985). Binuclear surface complex – an irreversible process, results from formation of a bridging ligand by making a second replacement of a surface hydroxyl when this is geometrically feasible.

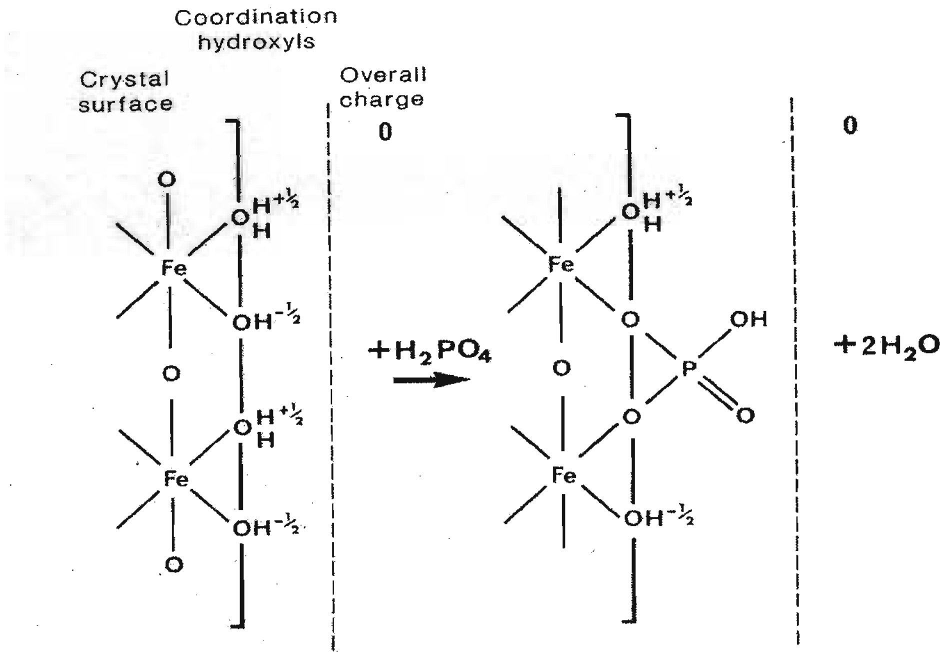


Figure 2.2 Binuclear Fe.OP(O)$_2$O.Fe bridging surface complex, characteristic of phosphate adsorption on goethite (*Source:* Ross, 1989)

2.2.2. Precipitation reactions

Whilst specific adsorption reactions predominate at low-solution P concentrations (Matar *et al.,* 1992), precipitation reactions dominate when the concentration of P and hydroxylated cations (Al, Fe, Ca, Mg) exceeds the solubility product (Ksp) of the newly formed phosphate mineral (Tisdale *et al.*, 1985). Phosphate ions in the soil solution readily precipitate with metal cations to form a range of P minerals. The type of P minerals formed depends on the soil pH because it governs the solution concentrations of metal cations and solubility of phosphate compounds.

In acid soils, the increased solubility of Al and Fe oxides result in high solution concentrations of trivalent Fe and Al (Hinsinger, 2001). Thus, precipitation of Al and Fe phosphates (e.g. strengite, vivianite, variscite) is most favoured between pH values of 4.8 and 5.2. Conversely, in neutral and alkaline soils, dissolution and precipitation reactions associated with calcium (e.g. dicalcium or octacalcium phosphates, apatites) and, and to a lesser extent Mg phosphates, have a large influence on P solubility.

2.2.3 Factors regulating P adsorption

Bioavailability of soil phosphorus depends on several important biological and physicochemical properties which include; ionic strength of the electrolyte solution; soil pH; texture, extent of P saturation, decomposition of organic matter; microbial activity; hydrous oxides of iron (Fe) and aluminium (Al); and the nature and amount of clay (Sanyal and De Datta, 1991; Haynes and Mokolobate, 2001). Charge characteristics of soils are very important because they influence both the cation (CEC) and anion exchange (AEC) properties of soils. Thus, from a soil management perspective, it is very critical to obtain a complete assessment of soil factors that optimise P availability to plants.

2.2.3.1 Clay content and mineralogy

A significant correlation of P sorption parameters with clay content has been reported (Bowden *et al.*, 1980). For example, the Langmuir adsorption maximum is positively correlated to clay content, Fe content, and exchangeable Al (Sanyal and De Datta, 1991). As well as clay content, reactions of P in soils are greatly affected by the mineralogical composition of the clay fraction (Matar *et al.*, 1992)

Phyllosilicate minerals have lower P retention capacity than the amorphous and crystalline oxides (Ross, 1989). P adsorption on these layered silicate clays occurs mainly through Al- and Fe-OH at broken clay lattice edges, where unsatisfied Al^{+3} groups are exposed or, in the case of 1:1 kaolinitic clay, where octahedral Al layers are exposed at the crystal surface. Unsatisfied positive charges may also occur at Si^{4+} groups in tetrahedral clay positions. Phosphate adsorption on 1:1 type minerals such as kaolinite (which has a significant amount of pH-dependent negative charge), is far greater than by 2:1 clays such as montmorillonite. This is mainly due to a higher negative charge density on surfaces of 2:1 clays which repels phosphate anions (Ross, 1989).

Amorphous oxide minerals retain exceedingly high P than do crystalline iron and aluminium hydrous oxides (e.g. hematite and gibbsite) (Ross, 1989). In comparison to crystalline aluminosilicates and calcium carbonate, amorphous oxide minerals can adsorb up to 1000 times more P (Sanyal and De Datta, 1991). Greater adsorption of phosphate by amorphous Al and Fe hydrous oxides (allophane) than crystalline oxides (gibbsite and goethite) is associated to the former having large number of singly coordinated surface hydroxyl ions (Haynes, 1984).

2.2.3.2 Electrolyte strength and species of cation

There is evidence that the ionic composition, both cationic species and concentration of electrolyte solution influence the adsorption of phosphate through their effects on electrostatic potential on soil colloids (Goldberg and Sposito, 1985). An increase in concentration of electrolyte cations near negatively charged soil colloids make the potential in the plane of adsorption less negative and, hence, increase the adsorption of anions and decrease the potential for their release from sorption sites (Bowden *et al.*, 1980; Barrow, 1987). Since divalent cations have relatively smaller hydrated ionic radius than do monovalent cations, the former are more strongly attracted to lattice surfaces (Ross, 1989). The decrease in electrostatic potential is more marked if the counter cations are polyvalent (Sanyal and De Datta, 1991). Replacement of a divalent ion (e.g. Ca^{2+}) by a monovalent electrolyte cation (e.g. Na^{+}) at colloid surface increases the lattice negative charge of the exchange surface.

As a result, adsorption of phosphorus by soils is influenced by the species of cation in the system. Divalent cations enhance phosphorus sorption relative to monovalent cations. For example, clays saturated with Ca^{2+} can retain greater amounts of phosphorus than those saturated with sodium or other monovalent cations. Barrow (1987) found that P sorption was consistently greater in $CaCl_2$ than in NaCl solutions (Figure 2.3). The difference between the two salts was strongly pH-dependent – sorption in the Na solutions declined more rapidly than in Ca medium. This observation has been corroborated by Naidu *et al.* (1990) who reported increases in P sorption by a limed soil in dilute $CaCl_2$ supporting medium. Curtin *et al.* (1992) also found that P sorption in Na^{+} saturated acidic clay loam soil was less than that in Ca^{2+} saturated soil. At pH 6.7, the Ca-soil sorbed 4 times as much P as the Na-soil.

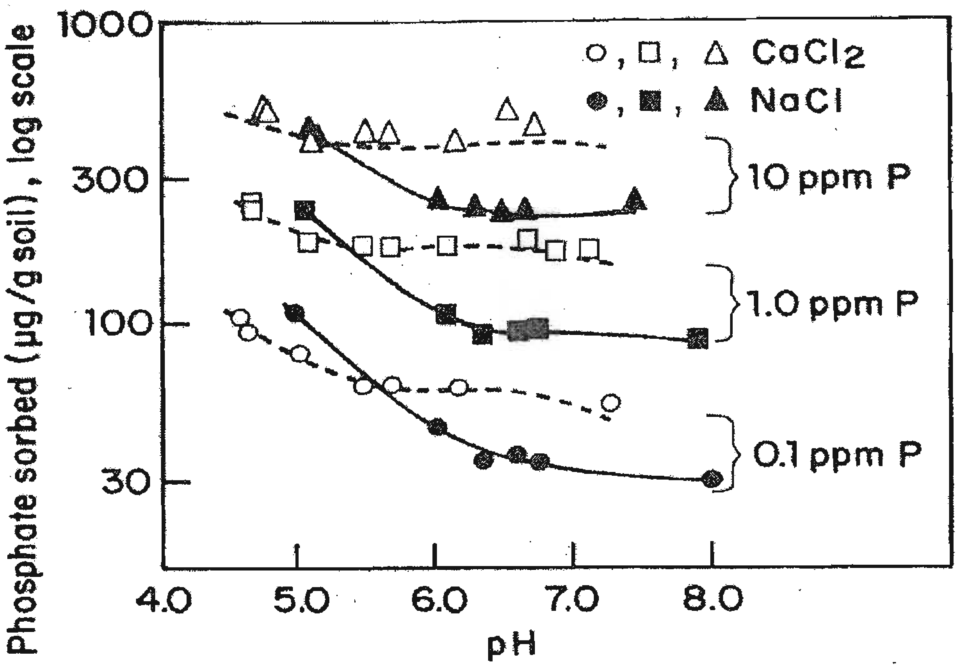


Figure 2.3 Effect of background electrolyte on phosphate sorption at various solution P concentrations (*Source:* Barrow, 1987)

2.2.3.3 Soil pH

Among the various factors, pH has the largest effect on the adsorption of phosphate, especially by variable charge surfaces. This property governs the reaction mechanisms between soil and phosphate and quantity of phosphorus retention on soil constituents. This is thought to be due to the large effects of pH on P speciation, dissociation of hydroxyl groups, and charge of the variable charge surfaces involved in P fixation (Sanyal and De Datta, 1991). The P species present in the soil affects plant uptake because P is mainly adsorbed as HPO_4^-, which prevails in slightly acidic medium (pH < 7). The soil pH level largely controls the solubility of Fe, Al and Ca compounds and, hence, pH directly affects the availability of phosphorus for plant growth.

The potential on the oxides arise from the adsorption of OH^- and H^+, and there is no inherent charge on the metal oxides themselves. The surface charge of these $MOH_2^{0.5+}$ and $MOH^{0.5-}$ groups varies significantly with changes in ionic strength, pH, point of zero charge (pH_o), and valency of the counter ion (Bowden *et al.*, 1980;

Barrow, 1984). At low soil pH (acidic conditions), the activity of H^+ is higher than that of hydroxyl ions (OH^-). At this pH level, the surface charge on amphoteric surfaces is positive (Figure 2.4), which coincides with high P sorption and low CEC. At the pH_0, the net charge on the adsorption surfaces is zero (Figure. 2.4). Maximum phosphate adsorption by iron and aluminium hydrous oxides in acid soils occurs between pH 2 and 4 (Sanyal and De Datta, 1991). As the pH increases, the surface charge on amphoteric surfaces becomes increasingly negative (Figure 2.4) and results in electrostatic repulsion between these surfaces and phosphate ions (Bowden *et al.*, 1980; Barrow, 1984). The adsorption affinity of sorption sites is decreased at high pH values, with possible net P movement of weakly held P from the soil colloids into solution (Haynes, 1984).

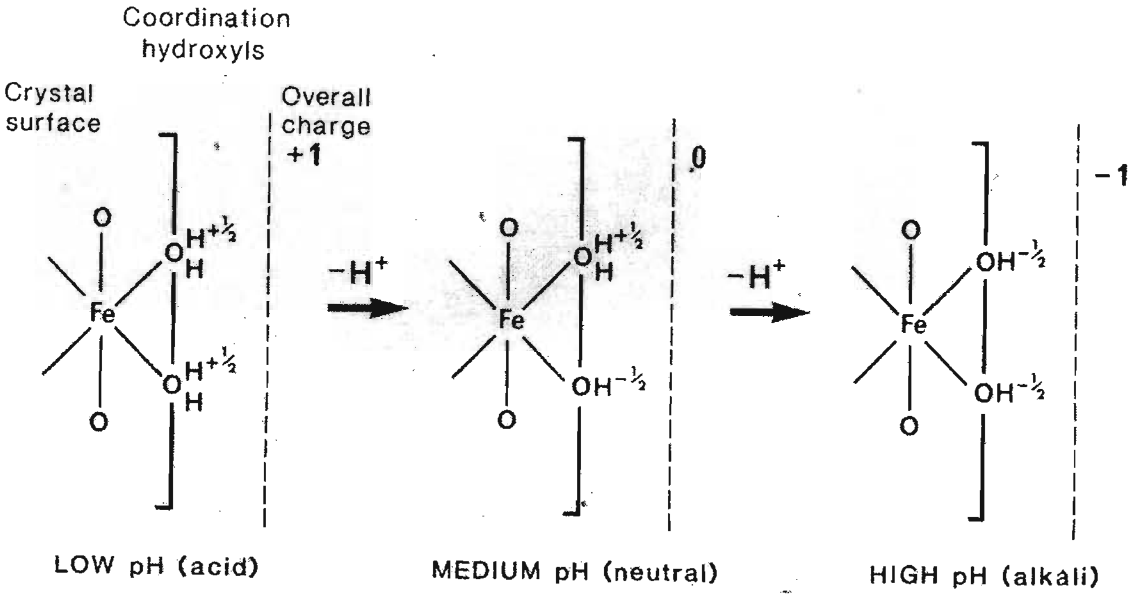


Figure 2.4 Effect of solution pH on the dissociation of unsatisfied hydroxyls at the surface of Fe and Al hydroxides. (*Source:* Ross, 1989)

2.2.3.4 Redox potential

Soils subjected to fluctuations in water content due to irrigation or rainfall come under the influence of another variable: the redox potential (Lindsay, 1979). This variable is controlled by the concentration of oxygen. During dry periods, the soils become re-oxidized. The decrease in redox potential following flooding by rain or irrigation water can cause transformations of crystalline Al and Fe minerals to the amorphous forms (Matar *et al.*, 1992), which in turn affects P retention properties of soils. The development of plough pans in cultivated soils may also promote reducing conditions. The associated changes in soil pH variously affect P availability depending on the initial pH level and organic matter content of the soil. As the potential of the system decreases, its pH increases. Willet (1989) reported that soils with the greatest increases in extractable P during flooding also had the largest increases in pH.

In reduced systems, Fe and Al phosphates are hydrolyzed by chelatants formed by anaerobic decomposition of organic matter, and P is released to the soil solution becoming available to the plants (Sanyal and De Datta, 1991). Increased P availability due to flooding is also been attributed to the reductive dissolution of Fe^{3+}, which releases both sorbed and occluded P into solution. Willet (1989) showed that reduction of the Fe^{3+} to Fe^{2+} oxide was the main P source under flooded conditions. In addition, the positive effect of soil pH on P availability was limited to soils recently fertilized with mineral P. The reductive dissolution of manganese (Mn) oxides can also contribute to P release in flooded soils (Lindsay, 1979).

2.2.3.5 Inorganic anions

The competitive ability of inorganic anions against phosphorus for sorption sites depends on the bond strength of the anion with the sorption surface (Alejandra *et al.*, 2006). Competition between these anions and phosphate determines their ability to release adsorbed P into the soil solution therefore increasing it bioavailability. Specifically, sorbed inorganic anions and acids such as hydroxyl, silicic acid, sulphate, and molybdate can be competitive against phosphate whereas weakly held inorganic anions such as nitrate and chloride do not affect P sorption. In practical terms, application of compounds containing silicate can block adsorption sites on soil colloids (Haynes, 1984). In deed, specific adsorption of silicate has been reported to desorb previously adsorbed phosphate, which causes increases in solution P (Hue, 1991).

2.2.3.6 Organic matter content

Products of organic matter decomposition may react directly with P sorption sites in the soil resulting in increased solution P concentration and, hence, P plant availability. Sorption of decomposition products of OM may also increase the negative charge of the adsorption surfaces, or lower the point of zero charge (PZC), thus resulting in phosphate repulsion (Bowden *et al.*, 1980).

Evidence that organic matter can modify the sorption of inorganic phosphate (P) by soils and soil components has been drawn from several competition experiments involving P and various organic acids/anions (Hue *et al.*, 1994; Guppy *et al.*, 2005). Although most of these experiments have compared competitive sorption of P to mostly low-molecular weight organic acids/anions (e.g. malate, citrate, acetate), higher molecular weight organic acids (e.g. humic and fluvic acids) are also effective in ion competitive inhibition of P sorption by soil colloids (Guppy *et al.,* 2005). Organic acid anions such as oxalate, citrate, and poly-galacturonate have been shown to be specifically sorbed at soil surfaces in a similar manner to phosphate (Nagarajah *et al.*, 1970). Figure 2.5 shows specific adsorption of oxalate onto metal hydrous oxide surfaces.

In some instances, positive correlation between soil OM content and P sorption has been found in previous studies (Mizota *et al.*, 1982; Sanyal *et al.*, 1993). Therefore, there are contradictory opinions. This has been attributed to Al and Fe organic complexes adsorbing P. The formation of metal bridges results in increased number of sorption sites, decreasing solution P concentration (Owusu-Bennoah and Acquaye, 1989). The metal ligands increase the PZC and, hence, the amount of phosphate anions that can be adsorbed. Complexes involving organic matter may maintain Fe, Al, Mg and Ca ions in solution when their insoluble precipitates (e.g. $CaCO_3$) could have formed, thereby decreasing potential for P fixation. These facts account for the contrasting views on effects of organic matter in decreasing P adsorption.

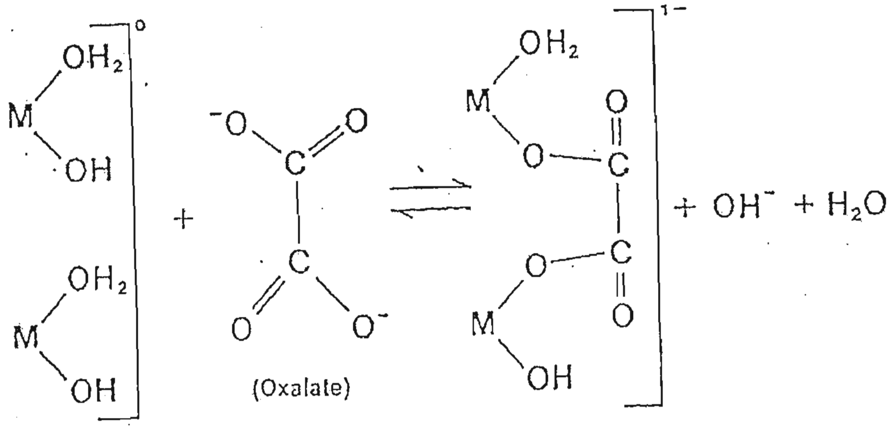


Figure 2.5 Specific adsorption reactions of oxalate and metal (Al or Fe) hydrous oxides at mineral edges (*Source:* Hue, 1992)

2.2.3.7 Time

Exchangeability of adsorbed inorganic P with the soil solution decreases with time as the adsorbed P become more strongly bound to mineral surfaces (Condron, 2004). Consequently, its supply to plants is reduced because of decreased solubility and increased adsorption. The reduction in P exchangeability between solution and solid phase is variously referred to as 'fixed' or 'sorbed' P. Hypothetic migration of phosphate into the adsorbing colloids (Matar *et al.,* 1992), is variously regarded as chemisorption, occlusion or absorption. In general, the terms relate to the removal of P from solution and its retention on soil surfaces. This P fixation is the major reason for the continued application to maintain crop productivity in agricultural soils.

2.2.3.8 P saturation

When a P deficient soil is fertilized, P is initially rapidly adsorbed on soil colloids, followed by slower adsorption reactions and retention within soil colloids (Hedley *et al.,* 1994). Adsorption of additional P decreases as the quantity of P already adsorbed increases, so that repeated additions increase solution P. That is, as P adsorption surfaces become saturated, applied P is subsequently held less tightly causing an increase in the proportion of P in the labile fractions. P saturation is the ratio of the amount of sorbed P to the P sorption capacity (Dao and Cavigelli, 2003). A buffer coefficient (BC) for added P is the increase in extractable soil P per unit of applied fertilizer P (Dobermann *et al.,* 2000). Evaluation of fertilizer efficacy requires a detailed knowledge of the adsorption and desorption characteristics of the soils for phosphate.

2.3 Organic P

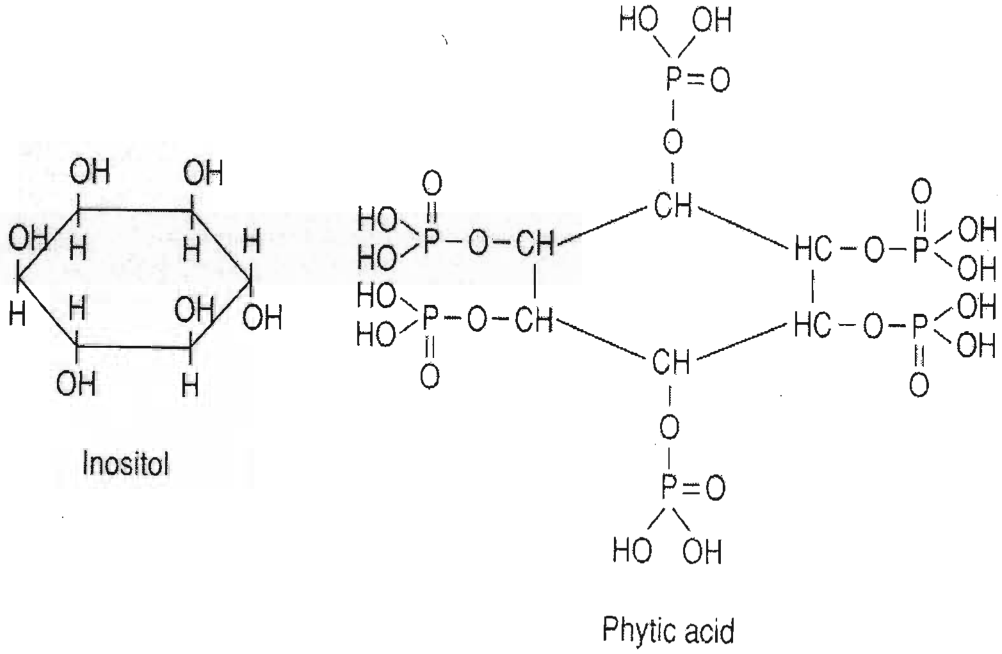
2.3.1 Nature of soil organic P

The organic forms of P (P_o) are found in humus and other soil organic fractions originating from plant and animal residues. Although the inorganic P content of soils is generally higher than that of organic P, it constitutes a large proportion of the total P (up to 80%) in some soils and provides a source of P for plant uptake (Chen *et al.*, 2001). Mineralization (decomposition) of organic matter, primarily plant residues, releases soluble organic P compounds, and organic anions and as already noted, these compete with P for adsorption sites, thus increasing its bioavailability. In weathered tropical soils, especially in low-input agricultural systems, the organic phosphorus (P_o) is important in replenishing the plant available P (solution) pool (Tiessen *et al.*, 1984).

Organic P content of soils and the ability of soils to recycle this P fraction are largely dependent on the soil organic matter (SOM) content and associated microbial biomass. The P_o content of soils generally increases with increasing organic C and/or N (Tisdale *et al.,* 1985). P contents of soil organic matter ranges from about 1 to 3% (Matar *et al.,* 1992). Although phosphorus is generally considered immobile in the soil, repeated applications of manure can move organic P compounds to a greater depth than can inorganic P in solution (Letkeman *et al.*, 1996).

Agronomic practices that enhance soil organic matter accumulation also lead to increases in organic phosphorus contents. The benefits of P_o in replenishing available P_i can be increased by adopting management practices that maintain or increase the size of the P_o pool. The build-up in P_o can be achieved, for example, under zero tillage systems because crop residues are retained on the surface, which ensures slow mineralization and release of P into the soil solution. In contrast, conventional tillage promotes rapid mineralization due to greater soil aeration and soil temperatures, and therefore cultivated soils usually contain lower organic P than equivalent uncultivated soils.

Although the nature of many of the soil organic (P_o) compounds is not yet fully clear (Tisdale *et al.,* 1985), the common ones include: orthophosphate monoesters (e.g. inositol phosphates, mononucleotides, sugar phosphates); orthophosphate diesters (e.g. phospholipids, RNA, DNA); teichoic acids and phosphonates (Cade-Menum *et al.,* 2000). Other P-C, P-N and P-S linkages exist, but phosphate esters (C-O-P) (Figure 2.6) are the predominant form of organic phosphorus in soils.


Inositol

Phytic acid

Figure 2.6 Chemical structure of inositol and inositol phosphate (phytic acid) (*Source:* Tisdale *et al.*, 1985)

Phosphate esters, such as inositol phosphates (up to 60% of the total organic phosphorus), nucleic acid phosphorus (5 to 10%) and phospholipid phosphorus (<1%), are bonded to soil organic and inorganic components by mechanisms such as adsorption and are generally less a part of soil humic materials than organic nitrogen or sulphur (Turner *et al.,* 2003). Phytin, the calcium-magnesium salt of inositol hexaphosphoric acid, is the most common form of organic P; constituting 30

– 80% of the total soil organic phosphorus. Condron *et al.* (1985) determined that over 90% of the organic P fraction was in the inositol phosphate or phytic acid form. Phytic acid is an orthophosphate monoester which is biologically stable in soils (Tisdale *et al.*, 1985). Some organic forms of phosphorus can be assimilated by plants, either directly or after breakdown by enzymes occurring in the root exudates of plants (Horst *et al.*, 2001).

2.3.2 Soil microbial biomass

Soil microbial biomass is composed of fungi and bacteria, and these may increase, decrease, or remain unaffected by management practices (Bardgett *et al.,* 1996). Although the soil microbial biomass comprises only about 2-3 % of the total organic carbon in soil, it is reported to play a central role in biochemical processes and serve as a crucial repository for plant nutrients (Chen and He, 2004).

Soil microbes immobilize solution P as microbial P, eventually releasing both readily mineralizable organic P compounds (labile organic P) and more stable organic P forms (Tisdale *et al.,* 1985). The microbial biomass P represents a rapidly cycling P pool because microbial biomass is readily decomposable (Dalal, 1998). Immobilization of P by soil microbes and its slow release through microbial turnover protects P from physico-chemical reactions and, hence, increases its bioavailability because a significant proportion of microbial P is labile (Oberson *et al.*, 2001). Thus, the microbial biomass acts as a transformation agent and as a source and sink for various nutrients (Figure 2.7).

Biological transformation of P is very important as the inorganic transformation in regulating P availability especially in highly weathered soils (Tiessen *et al.,* 1984; Stewart and Tiessen, 1987). Increased emphasis on biological activity in low-input farming systems means that organic and microbial P dynamics are likely to play an important role in overall P availability (Oberson *et al.*, 2001). A direct relationship between organic P and Bray 1 extractable P was reported by Chen et al. (2001).

Soil microbial biomass P and C are very dynamic and fluctuate more over time than total P or organic C (Brookes *et al.*, 1982). The ratio of microbial C and P (C_{mic}, P_{mic})

to total organic C are therefore sensitive indicators of changes in soil organic matter under contrasting management systems (Weigand *et al.*, 1995). Consequently, measurements of soil microbial nutrient pools may reflect effects of different crop management practices on nutrient cycling, even before such effects can be detected by measurements of other nutrient pools (Omay *et al.,* 1997). Chen *et al.* (2001; 2002) used microbial biomass P as an index of soil P availability, but caution should be exercised in applying soil quality indices, especially when considering soils developed from different parent materials (Chen and He, 2004).

Zero-tillage and legume based crop rotations enhance soil microbial diversity (Doran, 1980). After withdrawing management inputs (fertilizers, liming, grazing), both total microbial biomass and activity in the surface soil horizons are significantly reduced (Bardgett *et al.,* 1996). Although microbial P turnover was not determined in this particular study, the decrease in microbial activity is likely to have resulted in lower microbial P turnover.

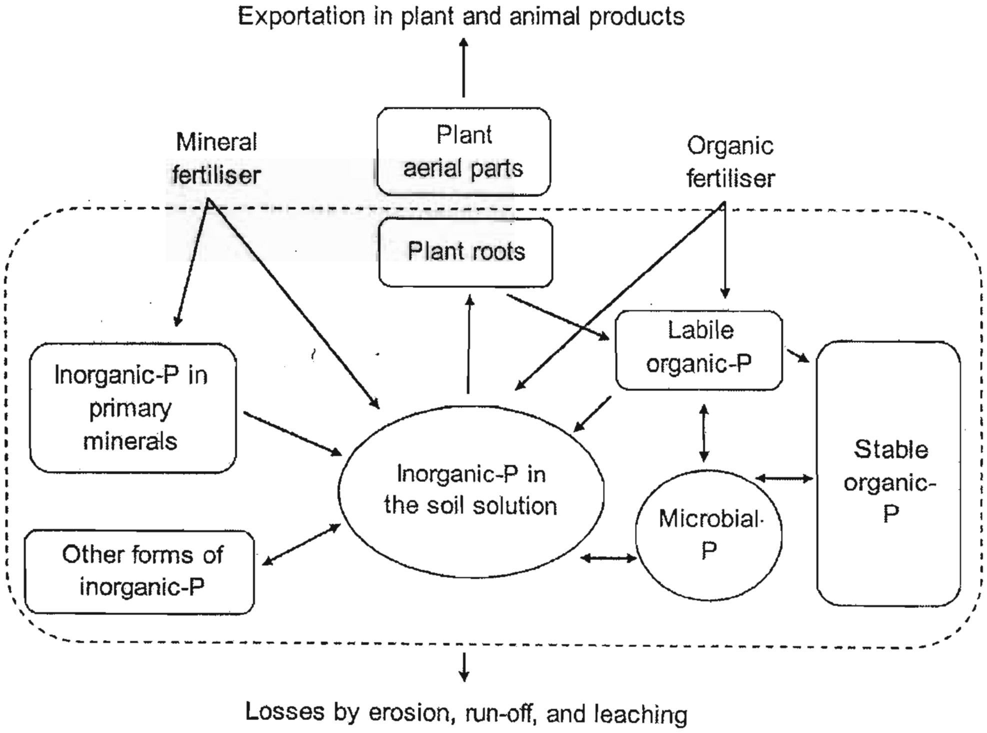


Figure 2.7 Relationships between phosphorus fractions in the P cycle (*Source:* Stewart and Sharpley, 1987)

2.3.3 Soil phosphatases

A wide range of microbes produce phosphatase enzymes that mineralise organic P and release inorganic P needed by microorganisms and plants (Hinsinger, 2001). Specific organic P hydrolysis reactions of some phosphatases are presented in the following reactions (Bishop *et al.*, 1994):

- Orthophosphoric acid monoester + H_2O $\xrightarrow{\text{acid phosphatase}}$ alcohol + orthophosphate
- Orthophosphoric acid monoester + H_2O $\xrightarrow{\text{alkaline phosphatase}}$ alcohol + orthophosphate
- Orthophosphoric acid diester + H_2O $\xrightarrow{\text{phosphodiseterase}}$ alcohol + orthophosphate monoester
- *myo*-inositol hexaphosphate + H_2O $\xrightarrow{\text{phytase}}$ *myo*-inositol pentaphosphate + orthophosphate

The phosphatases catalyze the hydrolysis of both esters and anhydrides of H_3PO_4. The enzymes are classified as acid and alkaline phosphatases because their maximum activities occur at low (pH 6.5) and high (pH 11) pH ranges, respectively. In general, soil enzyme activities are used as an indirect measure of microbial activity (Weigand *et al.*, 1995). Acid phosphatase enzymes are released by both microorganisms and higher plants as part of root exudates, whereas alkaline phosphatases are produced mainly by microorganisms (Tabatabai, 1994). To assay for phosphatase activity, a substrate (disodium *p*-nitrophenyl phosphate) is added to soil, and a buffer is used to maintain the pH at the desired value (pH 11 for alkaline phosphatase). The rate of formation of a hydrolysis product (*p*-nitrophenol) is proportional to phosphatase activity.

Some crop species (e.g. lupins) have greater efficiency of P acquisition and utilization than cereal crops due to their ability to produce more phosphatases than other crops (Horst *et al.*, 2001). The induction of both extra and intracellular acid phosphatases (ACP) and alkaline phosphatases is a common adaptive strategy by plants to phosphorus deficiency (Hinsinger, 2001). The rapidly dwindling supply of rich phosphate reserves underlies the requirement for crop variety improvement to increase phosphate acquisition and utilization by crops.

2.3.4 Mineralization and immobilisation of organic P

During decomposition, some of the P and other nutrients are assimilated by microbial biomass (immobilization) and some are converted into organic forms (P_o). Eventually, both readily mineralizable organic P compounds (labile organic P) and more stable organic P forms are released into the soil (McLaren and Cameron, 1990). Mineralization reactions also release organic anions that compete with P for adsorption sites with Fe, Al and Ca solution species (Hutchison and Hesterberg, 2004). Land-use systems that increase soil organic P enhance soil biological activities, and therefore mineralization of P_o is also greater. In deed, in general, P mineralization increases as the total soil organic content increases (Figure 2.8).

Nonetheless, the mineralization of soil organic P is also determined by its chemical nature and associated reactivity. For example, the predominance of orthophosphate monoester inositol phosphates in soil may be partly attributed to its association with the structural components of senescent plant material, which limits its susceptibility to mineralization (Stewart and Tiessen, 1987). In contrast, orthophosphate diester forms of organic P (nucleic acid and phospholipid P) are relatively soluble in the soil and are therefore rapidly mineralized. In addition, the susceptibility of soil organic P to mineralization is influenced by physical factors, including particle size aggregate stability and the associated effects of wetting and drying (Adams and Odom, 1985). Mycorrhizae fungi may also increase the solubility and consequent mineralization of soil organic P (Chen *et al.*, 2002).

The mineralization of organic P is determined by its chemical nature and associated reactivity (Condron *et al.*, 1985; Turner *et al.*, 2003). Organic P content in soil extracts is thus characterized by its susceptibility to hydrolysis by phosphatase enzymes. For example, the predominance of orthophosphate monoester inositol phosphates in soil may be partly attributed to its association with the structural components of senescent plant material, which limits its susceptibility to mineralization (Stewart and Tiessen, 1987). In contrast, orthophosphate diester forms of organic P (nucleic acid and phospholipid P) are relatively soluble in the soil and are therefore rapidly mineralized. In addition, the susceptibility of soil organic P to mineralization is influenced by physical factors, including particle size aggregate stability and soil moisture regimes.

During decomposition of soil organic matter, soil microbes assimilate phosphorus and other nutrients – immobilisation, and this depends on the C:P ratio of the organic material. Net P mineralization has been reported for residues with a C: P ratio below 100, whilst a C: P ratio above 300 results in net immobilization. Results presented in Figure 2.8 show that as the C/Po ratio of residues increased, so did P immobilization. Bishop *et al.* (1994) suggested that soil microbes are able to utilize labile and less available forms of P, and leads to increases in organic P fractions at the expense of inorganic fractions.

C/P Ratio	***Mineralization/Immobilization***
> 200	Net mineralization of organic P
200 – 300	No gain/loss of inorganic P
< 300	Net immobilization of inorganic P

As well as the C/P ratio, the N/P ratio of decomposing residues regulates the predominance of P mineralization over immobililization. A decrease of either N or P in soil can result in increased mineralization of the other (Tisdale *et al.*, 1985). The ratio of N to P in manures is less than that of crop removal. Thus applications of organic amendments based on plant N requirements result in accumulation of P in soils.

Determination organic P mineralization is difficult because inorganic P (Pi) released from mineralization can be removed from solution (1) P retention on clay and other soil surfaces, and (2) P precipitation as secondary Al^{3+}, Fe^{2+}, or Ca^{2+}-P minerals (Tisdale *et al.,* 1985). Very few experimental procedures can directly measured changes in soil organic P concentration during mineralization (Bishop *et al.*, 1994). Oehl et al. (2001) applied the principles of isotope exchange kinetics to examine soil organic P mineralization and P cycling through the soil microbial biomass in a soil that was cropped for 20 years.

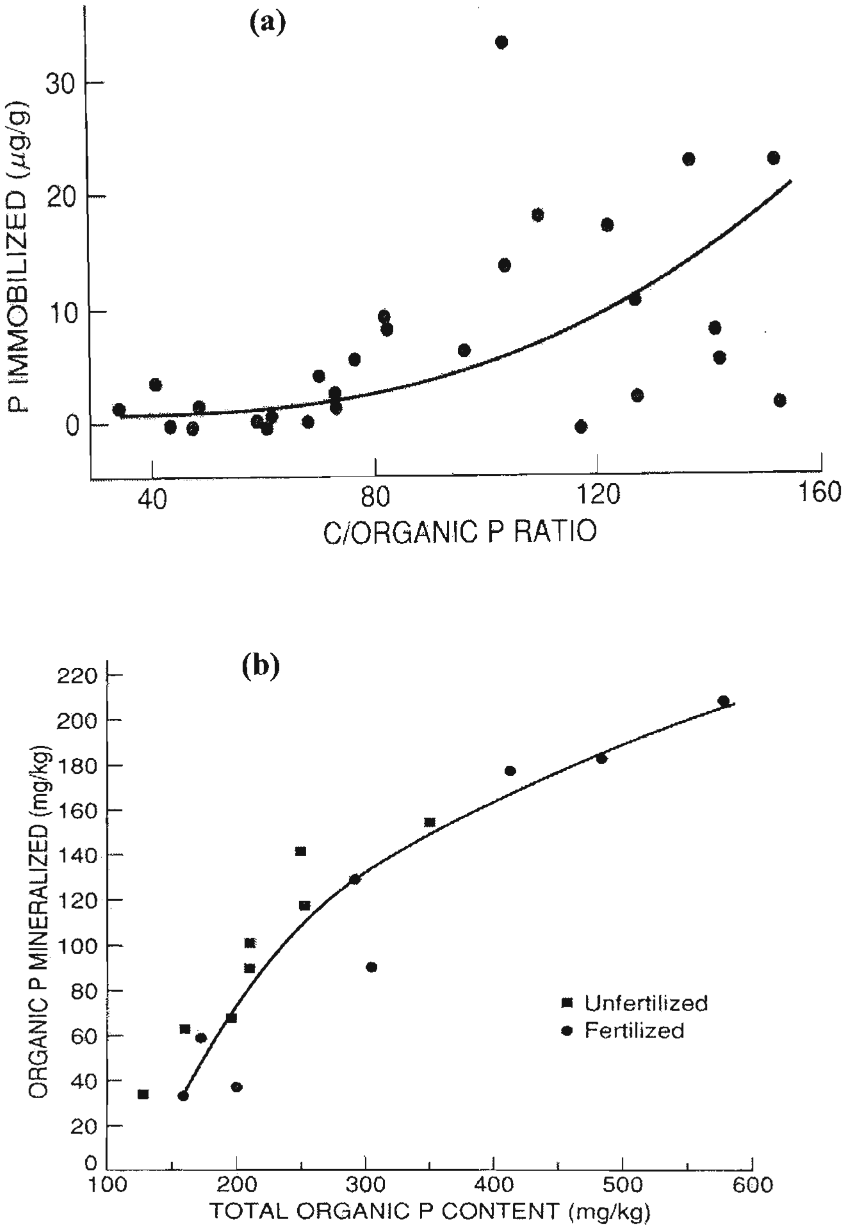


Figure 2.8 Relationships between (a) inorganic P immobilization and C/P ratio, and (b) mineralization of organic P and the total organic P content in the soil (*Source:* Tisdale *et al.*, 1985)

2.4 Extraction and fractionation of soil P

Chemical sequential extraction has been widely used to divide soil phosphorus (P) into different inorganic (P_i) and organic (P_o) fractions (Oberson *et al.*, 1999; Owusu-Bennoah and Acquaye, 1989). Fractionation procedures (e.g. Chang and Jackson, 1957; Hedley *et al.,* 1982) divide the soil P continuum into fractions based on various chemical extractants. Further, isotopic exchange and sequential P extraction techniques can be combined to investigate the interactions between the labile P pool and the chemical P forms that influence the equilibrium between the solid phase, surface and solution equilibria.

The importance of different P fractions in replenishment of available P_i depends on soil properties (Tiessen *et al.,* 1983), weathering intensity and management system (Guo and Yost, 1998). P fractionation methods provide an alternative for characterizing soil P availability and may elucidate some of the problems regarding lack of crop response to P. Measurements of the various soil P fractions are based on extracting solutions which remove inorganic and organic P of increasing chemical stability (Guo and Yost, 1998). Increasingly stronger solutions extract P fractions that are believed to be increasingly less available to plants. The important assumption in the sequential extraction is that readily available soil P is removed first with mild extracting solutions, while less available P pools are extracted with stronger acids and alkali solutions (Hedley *et al.*, 1982).

2.4.1 Chang and Jackson method

The fractionation procedure proposed by Chang and Jackson (1957) extracts mainly strongly retained P and therefore may not be appropriate for studying soil P dynamics that influence uptake by plants (Agbenin and Goladi, 1998). In this method, selective extracting solutions of increasing harshness are used, so that P associated with Al, Fe and Ca is extracted by a combination of ligand exchange and dissolution reactions. The advantage of this method is that extracts used in P fractionation parallel those used for Al and Fe, which allows for relation of P fractions to Fe and Al forms (Cade-Menum *et al.*, 2000), thus characterizing the chemical linkage of P and its degree of availability. However, it does not account for the organic fraction of soil P (P_o) (Agbenin and Goladi, 1998), despite the significant proportion of this fraction in some soils.

2.4.2 Hedley fractionation method

The method developed by Hedley *et al.* (1982) and modified by Tiessen and Moir (1993), fractionates the soil P into five inorganic P (P_i), three organic P (P_o) fractions, and one residual P fraction (Figure 2.9). The procedure utilizes extracts of differing strengths and composition, and recognizes readily available P forms as resin P_i, $NaHCO_3$-P_i, and $NaHCO_3$-P_o. Other fractions are considered as less labile or stable forms, but in highly weathered soils, these fractions may contribute significantly to plant available P (Beck and Sanchez, 1994).

Currently, variants of the Hedley fractionation, which mostly exclude determination of microbial P, have been used extensively to separate forms of organically bound soil phosphorus from the inorganic fractions (Agbenin and Goladi, 1998; Beauchemin and Simard, 2000). In these approaches, microbial P fraction is assumed to be distributed between the bicarbonate and hydroxide extractions. Further, some methods use concentrated HCl instead of H_2SO_4 (Tiessen and Moir, 1993). Certain authors (Cross and Schlisinger, 1995; Cade-Menum *et al.*, 2000), have raised concerns about the suitability of the Hedley procedure for carbon-rich acid soils because of the difficulty in precipitating organic matter.

2.4.2.1 Resin – P

Extraction using anion exchange resin in bags or on membranes is generally assumed to closely estimate P bio-availability than chemical extractions because they accumulate nutrients from soils through exchange reactions by a similar mechanism to the soil-root interface (Tiessen and Moir, 1993). The resin-extractable pool (resin-P) represents inorganic P (P_i) from the soil solution and that which is weakly adsorbed on sesquioxides, crystalline minerals or carbonates (Tiessen *et al.,* 1984; Richards *et al.,* 1995). Resin – P originates from the most labile P pool, and therefore a good estimator of plant available P, particularly for acid soils (Hedley et al., 1982; Beck and Sanchez, 1994).

P fractions that interact with resin-P fraction are likely to act as sources or sinks of solution P. When the resin P fraction decreases, phosphatase enzyme activity can increase, with a corresponding increase in P mineralization of organic P (Schmidt *et al.,* 1996). Conversely, fertilizer P application rates above crop removal increase resin P content, which suppresses phosphatase activity, and consequently suppresses mineralization of organic P.

2.4.2.2 Bicarbonate – P ($NaHCO_3$ - P)

Sodium bicarbonate (0.5 M at pH 8.5) extracts weakly adsorbed P_i and easily mineralizable organic P (P_o). $NaHCO_3$ extracting solution (pH 8.5) can affect hydrolysis of surface Al and Fe, therefore releasing Al- and Fe-bound phosphate. The bicarbonate P_o fraction consists of compounds like ribonucleic acids and glycerophosphate and may be assimilated by plants (Hedley *et al.,* 1982; Buehler *et al.,* 2002). $NaHCO_3$ – P is considered as labile P_o sorbed on the soil surfaces plus a small amount of microbial P (Schmidt *et al.*, 1996; Zhang and MacKenzie, 1997).

2.4.2.3 Sodium hydroxide – P (NaOH- P)

Sodium hydroxide extractable P_i is assumed to be non-occluded P associated with amorphous and some crystalline Al and Fe hydroxides (Fe- and Al-bound P_i) (Beck and Sanchez, 1994; Buehler *et al.,* 2002). Since the hydroxide P_i is held strongly by chemisorption to Fe and Al components of soil colloids, it is considered as moderately labile soil P (Sharpley, 1991). It is considered to be slowly available to plants by desorption (Zhang and MacKenzie, 1997). In acid soils, this P fraction can act as a sink for P during excess P fertilization, but is also a P source when resin P levels are low (Hedley *et al.*, 1982). In highly weathered P deficient soils, the hydroxide-extractable P_i fraction may be substantially increased by P additions because the high exchangeable Al in these soils (Buehler *et al.*, 2002) results in precipitation of Al phosphates. However, when applied to relatively fertile soils, other P fractions may accumulate P instead of the NaOH-P.

Organic P (P_o) associated with chemically and physically protected organic compounds (e.g. fulvic and humic acids) are also extracted by sodium hydroxide

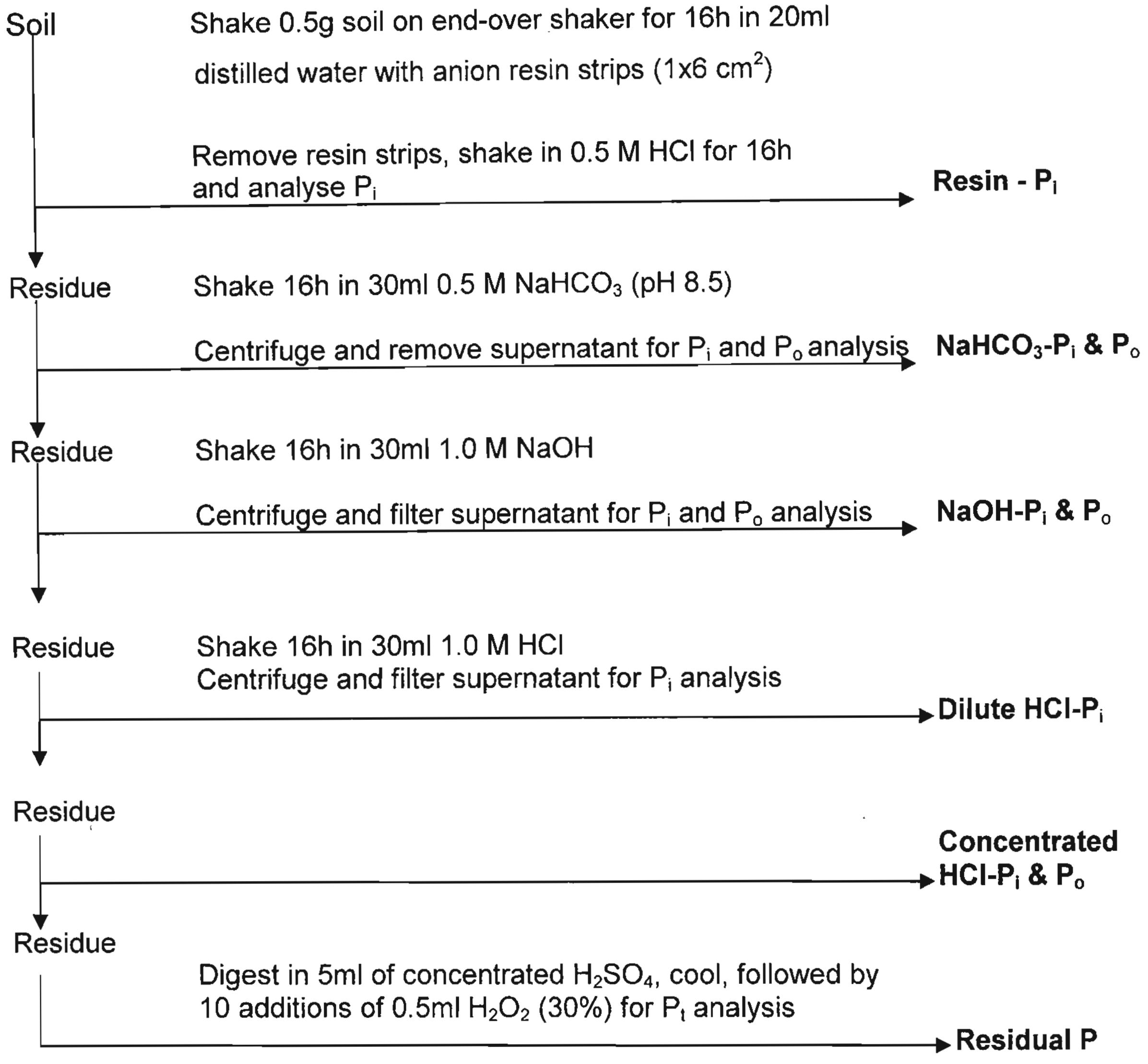
(0.1M NaOH). These organic materials are reported to be stable and involved in long-term soil P transformations (Guppy *et al.*, 2005). Some workers have identified NaOH extractable P_o as an important source of P in unfertilized tropical soils (Tiessen *et al.*, 1982; Schmidt *et al.*, 1996). Beck and Sanchez (1994) used path analysis to compare the role of P fractions in replenishing solution P concentration in non-fertilized and fertilized treatments of a long-term continuous cultivation experiment on a Typic Paleudult. The study determined that organic P (particularly NaOH-P_o) was the primary source of plant available P in the non-fertilized system.

2.4.2.4 Hydrochloric acid – P (HCl – P)

A considerable part of soil phosphorus can be dissolved by hydrolysis of soil with hydrochloric acid. HCl – P_i is relatively stable and bound to Ca (Ca-P) and can be associated with apatite minerals (Lehmann *et al*., 2001). The P_o removed by this extracting solution may originate from more stable pools from particulate organic matter and as such are considered unavailable to plants (Zhang and MacKenzie, 1997).

2.4.2.5 Residual - P

Residual P constitutes both inorganic and organic P (Condron and Goh, 1989; Agbenin and Goladi, 1998) and is usually extracted with concentrated sulphuric acid. Long term application of P fertilizer, especially in excess of crop needs, often leads to enrichment of this fraction through occlusion of P_i. In addition, residual P can be converted soil organic P via plant, animal, and microbial residues. There is often high correlation between residual P fraction and plant available P (Agbenin and Goladi, 1998; Guo and Yost, 1998). However, such a correlation does not mean that residual P contributes directly to plant uptake (solution P), because the less labile pools can exist in equilibrium with each other, or with moderately labile pools. For example, Agbenin and Goladi (1998) determined that residual-P contributed to resin-P indirectly through bicarbonate-P_o ($NaHCO_3$-P_o) and hydroxide P_o (NaOH-P_o) on a savanna Alfisol under continuous cultivation. The failure by conventional soil test P (STP) methods to extract NaOH-P_o, for example, can result excessive fertilization rates recommended. Several other studies have shown that this P fraction can serve as a sink and reservoir for soil phosphorus (Beck and Sanchez, 1994).



Shake 0.5g soil on end-over shaker for 16h in 20ml distilled water with anion resin strips (1x6 cm^2)

Remove resin strips, shake in 0.5 M HCl for 16h and analyse P_i

Shake 16h in 30ml 0.5 M $NaHCO_3$ (pH 8.5)

Centrifuge and remove supernatant for P_i and P_o analysis

Shake 16h in 30ml 1.0 M NaOH

Centrifuge and filter supernatant for P_i and P_o analysis

Shake 16h in 30ml 1.0 M HCl
Centrifuge and filter supernatant for P_i analysis

Digest in 5ml of concentrated H_2SO_4, cool, followed by 10 additions of 0.5ml H_2O_2 (30%) for P_t analysis

Figure 2.9 Modified Hedley sequential fractionation procedure for soil phosphorus (Pi, Po and Pt refer to inorganic, organic and total P, respectively) (*Source:* O'halloran *et al.*, 1987)

2.4.3 Effects of soil management on P fractions

Crop management practices and land management can greatly affect the partitioning of soil P between the inorganic and organic phases and between the different P_i and P_o fractions. Some of the main effects are outlined below.

2.4.3.1 Arable systems and tillage methods

Under arable agriculture, conventional tillage typically results in a decline in soil organic matter content (Selles *et al.*, 1999; Dominy *et al.*, 2001; Dominy and Haynes, 2002). Tillage breaks up soil aggregates and exposes organic matter to microbial attack which was previously physically protected within the aggregate structure. As a result, arable soils under conventional tillage generally have lower organic matter contents than their equivalents uncropped counterparts (Agbenin and Goladi, 1998). Beck and Sanchez (1994) observed a decline in $NaHCO_3$-P_o and NaOH-P_o in soils under cultivation with a concomitant increase in P_i the pools. This reflected the mineralization of organic P and its accumulation in P_i fractions.

Compared to conventional tillage methods, under reduced and zero tillage systems there is an accumulation of organic matter in the surface few cm of soil because the main organic matter inputs are at, or near, the soil surface (Selles *et al.*, 1999). Crop residue management is also an important consideration. Cropping systems where large amounts of crop residues are retained promote accumulation of P in P_o fractions (Agbenin and Goladi, 1998).

2.4.3.2 Pasture systems

Accumulation of organic P fractions under improved pasture systems when compared to the cultivated sites is common (Condron and Goh, 1989). This is expected because of the large organic matter inputs particularly via turnover of the large, ramified grass root system (Tidsale *et al,* 1985). Large quantities of plant debris and animal faeces returned to the soil may increase soil organic residues hence, stimulating biological immobilization of soil inorganic P (P_i) (Condron and Goh, 1989; Agbenin and Goladi, 1998). Some of the inorganic P taken up during the growth phase of pasture plants is returned to the soil and accumulates in soil organic P forms such as inositol phosphates, nucleic acids and phospholipids (Turner *et al.*, 2003).

In comparison with undisturbed grassland systems, plant productivity is increased under improved pastures due to the use of improved pastures species and the application of lime, fertilizers and irrigation (Condron and Goh, 1989). As a result, there is increased accumulation of soil organic P whilst fertilizer P applications tend to elevate P_i levels. Oberson *et al.* (1999) observed that in comparison with native savanna, improved pastures maintained higher labile P_o compounds (phosphanates and diester P). The hydroxide –extractable P fraction was found to be most affected by pasture improvement.

2.4.3.3 Phosphate fertilizers

Phosphate fertilizer applications improve soil P status and as a result P_i fractions generally increase (Stewart and Tiessen, 1987; Beck and Sanchez, 1994). In some cases, where soil organic matter is also accumulating, there is also an accumulation of P in P_o fractions.

Stewart and Tiessen (1987) concluded that the inorganic P fertilization results in P_o accumulation only when organic C and N supplies are abundant, whereas inorganic P_i accumulation is a general occurrence. Application of inorganic P fertilizer was found by O'halloran *et al.* (1987) to result in significant increases in some labile P fractions (resin- and $NaHCO_3$ – P_i), but to have no effect on $NaHCO_3$ – and NaOH-P_o in a Brown Chernozemic loam soil. Schmidt *et al.* (1996) observed accumulation of fertilizer P in the resin- P_i pool at moderate fertilizer rates. Where fertilizer P inputs were in excess of crop uptake, they also observed P accumulation in the $NaHCO_3$-P_i and NaOH-P_i fractions. The significance of soil texture in P distribution in the soil was confirmed by Tiessen *et al.* (1983). They observed that in cropped soils with limited P inputs, the decline in P status was mainly characterized by decreases in the inorganic P fraction in a coarse textured soil, whereas in fine textured soil, the decline was exclusively linked to decreases in the organic P fraction.

2.4.3.4 Organic amendments

Increased P availability resulting from organic manure addition is widely reported (Iyamuremye *et al.*, 1996; Zhang and MacKenzie, 1997). Several mechanisms have been implicated in the increase in resin extractable P by organic amendments; (1) mineralization of the residues releases inorganic and labile organic P (Tisdale *et al.*, 1985), (2) during decomposition processes, soil microorganisms release organic molecules which can form complexes with soil solution Al and Fe, thereby reducing its solubility, (3) adsorption of Al on organic residues surfaces, and (4) precipitation of Al with hydroxyl (OH^-) ions from redox and ligand exchange reactions (Hue, 1992).

Results from several studies have shown that manure addition tends to decrease NaOH-Pi (Agbenin and Goladi, 1998; Zhang and MacKenzie, 1997). This trend can be explained by considering that hydroxide extractable Pi (NaOH-P_i) is associated with Fe and Al components of soil surfaces. As already noted, manure addition would tend to decrease P sorption on these surfaces, mainly because of the competition between phosphate ions and organic materials for adsorption sites (Hue, 1991).

Additions of manure often result in accumulation of soil organic matter and thus an accumulation of organic P (O'halloran, 1993). Qian and Schoenau (2000) found that a single application of liquid swine manure on an Orthic Black Chernozem (pH 7.3) had no effect on the most labile P fractions (resin- and $NaHCO_3$-P), but significantly increased the moderately labile and stable fractions. As they increased incubation time, the concentrated HCl-P_o and residual P significantly increased at the expense of NaOH-P_o and dilute HCl-P_i. In a related study, Hedley *et al.* (1982) also reported increases in residual P with incubation time.

2.4.4 Soil test extractants for P

Soil test procedures for P determine the amount of potentially plant available P present in the solution and in labile P_i pools. Plant available P can be inorganic and partly organic, and is usually estimated using bicarbonate solution or dilute acid extracting solutions (Olsen and Sommers, 1982; Paul and Clark, 1989). Commonly used extraction methods are Mehlich 1 and 3, Bray 1 and Olsen procedures are widely used (Olsen and Sommers, 1982). However, P_i and P_o fractions not measured by these extraction methods contribute to solution P levels and influenced by various soil amendments (Anderson, 1980; Richards *et al.*, 1995).

The Bray 1 extractant (0.03 M NH_4F and 0.025 M HCl) is designed to remove the easily acid-soluble P, largely calcium phosphates, and iron and aluminium phosphates. In general, the method is suited for acidic soils (Olsen and Sommers, 1982). The extraction procedure promotes desorption of soil P by increasing soil Fe and Al activity and formation of FeF_4^- and AlF_4^- (Chang and Jackson, 1957). Re-adsorption of soluble P onto colloids is reduced by the action of F^- in the extraction solution (Bray and Kurtz, 1945). Although this extractant performs well in acidic soils, its effectiveness in calcareous soils and soils of high CEC is limited. Under calcareous conditions, $CaCO_3$ neutralises the acid in the extraction solution and CaF_2 formed by the reaction between Ca^{2+} and F^- rapidly immobilises P.

The Mehlich III is a modification of the Mehlich II extractant (0.015 M NH_4F, 0.2 M CH_3COOH, 0.25 M NH_4NO_3, 0.013 M HCl) and also serves as a multi-element soil extracting solution. The reaction of this extractant in the soil is similar to the Bray I method in that F^- in the extracting solution forms AlF_4^-, allowing release of phosphates associated with aluminium into solution. Acetic acid in the Mehlich III reagent reacts with calcium phosphates in the soil to remove P but is less aggressive than equivalent mineral acids. Additionally, it is neutralised less by calcium phosphates than the Bray I extractant.

The Olsen method of soil P determination is effective in both saline and acidic soils. The extractant is 0.5 M $NaHCO_3$, buffered at 8.4 (Olsen *et al.*, 1954). In calcareous soils CO_3^{2-} interacts with Ca^{2+} and precipitates as $CaCO_3$. The change in equilibrium causes a release of P from calcium phosphates, very much similar to P uptake by plant roots under calcareous soils. In acid soils an increase in OH^- concentrations causes Al complex formation and precipitation of iron to Fe_2O_3. The decrease in solution Al and Fe causes a disequilibrium, which prompts the release of phosphate from oxides minerals.

2.5 Conclusions

P can be strongly adsorbed on sesquioxide surfaces, but P deficiency also occurs in alkaline soils through fixation on calcium carbonate surfaces. In acid soils, P bioavailability is regulated by the chemistry of variable charged sesquioxide surfaces, and to a lesser extent by phyllosilicates. Phosphate ions are strongly adsorbed on these surfaces through specific and/or non-specific mechanisms.

Adsorption of soluble humic materials and organic acids to Al and Fe oxides surfaces decreases the amount of P adsorption sites, and therefore reduces P fixation capacity of acid soils. Efficiency of organic anions on reduction of phosphorus sorption is in part related to their molecular structure, but is also related to the pH of the system. Release of soluble P during organic matter decomposition saturates adsorption sites so that added fertilizer P is taken up by plants.

Long-term fertilizer and manure inputs enhance accumulation of labile P_i in the surface soil (McKenzie *et al.*, 1992; Beck and Sanchez, 1994; Buehler *et al.*, 2002). Continuous cropping systems without P fertilization decrease soil P fertility. Soil microbial P and organic P (which are very important P sources especially under organic farming systems) are enhanced by agronomic practices that promote soil organic matter accumulation.

Comparative studies of soil P dynamics in forest, pasture and arable sites are limited. Most studies have individually investigated the impacts of cropping practices such as fertilization, crop rotations and tillage, on soil P distribution in pools. There are relatively few studies on the combined effects of cropping sequences and nutrient sources on P forms, especially on a long-term basis. The Hedley P fractionation procedure uses reagents to isolate various pools of inorganic and organic soil P. It extracts readily-available P_i and labile P_o fractions and then sequentially extracts the less labile or stable forms. It can be a powerful tool in estimating the interaction of land use systems and management on soil P status. Previous workers (Dominy *et al.*, 2001; Dominy and Haynes, 2002) have identified sites with long-term histories that represent the main agricultural land uses present in the Midlands of KwaZulu-Natal. Thus, application of the Hedley fractionation technique to soils at the above sites could provide valuable information on the P status of agricultural soils of KwaZulu-Natal.

CHAPTER 3

PHOSPHORUS FRACTIONS IN SOILS UNDER CONTRASTING LONG-TERM AGRICULTURAL MANAGEMENT PRACTICES

3.1 Introduction

Long-term agricultural management practices have a significant impact on soil biological, physical and biochemical properties, usually through the changes in soil organic matter (SOM) and nutrient balances (Lupwayi *et al.*, 2005). Focus on understanding phosphorus (P) fluxes in soils in terms of plant availability is mainly because of its agricultural importance and potential to pollute water resources (O'halloran, 1993). Consequently, effects of contrasting agricultural systems on the distribution and forms of P have been investigated for a large variety of soils under different climatic conditions (Sharpley, 1985; O'halloran *et al.*, 1987; Iyamuremye *et al.*, 1996; Agbenin and Goladi, 1998; Buehler *et al.*, 2002).

Without P fertilization, plant available P is maintained primarily by the microbially mediated mineralization of SOM (Stewart and Tiessen, 1987). However, only small amounts of organic P (Po) are mineralized annually in P deficient soils (Tisdale *et al.*, 1985), so that only a small portion of P bound to organic matter is plant available annually. Lime and P applications are key components for increasing crop productivity on acidic soils (Haynes and Mokolobate, 2001). Long-term application of P fertilizer and manure to agricultural soils increases organic (Po) and inorganic (Pi) P near the soil surface (McKenzie *et al.*, 1992), but the effects can also extend deep into the soil profile (Zheng *et al.*, 2004a). Improvement in soil fertility increases microbial activity and the potential for SOM mineralization and, consequently, may induce transformation of P into microbial, organic, or inorganic forms (Sharpley, 1985).

Maize growers in the midlands region of KwaZulu-Natal are rapidly adopting conservation-tillage systems due to enhanced soil water conservation and loss in soil quality associated with the conventional tillage practices. Well managed arable crops under zero-tillage tend to favour an accumulation of SOM and organic P in the surface layer of soil due to large quantities of crop residues being returned and the residual effect of fertilizers (Condron and Goh, 1989). Long-term crop rotations which include large N and P fertilization inputs can also have a positive effect on P cycling in cultivated soils through accumulation of P in the soil as well as stimulation of microbial activity (Dalal, 1998; McLaughlin *et al.*, 1988).

A method developed by Hedley et al. (1982) fractionates soil P into five inorganic and three organic fractions. Such a fractionation procedure makes it possible not only to discern the distribution of P between inorganic and organic forms but also to understand the distribution of P within these broad categories into labile, moderately labile and recalcitrant forms. The use of such fractionation procedures has led to an improved understanding of the fate and transformations of added P in agricultural soils (Ball-Coelho *et al.*, 1993; Buehler *et al.*, 2002).

Maize (*Zea mays* L.), sugarcane, dairy farming, forestry and rangeland are the main agricultural land uses in the midlands region of KwaZulu-Natal (Dominy *et al.*, 2001). The management and cycling of P is likely to differ among these land use forms because the main crops have different P requirements and patterns of uptake and they have different effects on SOM content (Haynes *et al.*, 2003). There are also differences in the amounts of P exported at harvest, and returned to the soil through crop residues. Thus, developing appropriate P management strategies will require a good understanding of the effects of land use forms and management practices on soil P pools. Efficient P cycling and synchronization of P release from organic sources with plant P requirements could reduce fertilizer requirements. In this study, the effects of land use and management practices on soil P fractions were measured using a modified Hedley P extraction procedure (Tiessen and Moir, 1993).

3.2 Materials and methods

3.2.1 Sites description and location

The surface layers (0-5 cm) of soils used in this study were sampled in January 2005 from long-term agricultural fields situated at "Baynesfield Estate" (27°22'S, 30°45'E) and Cedara Agricultural Research Station (29°32'S, 30°17'E), which were designated location 1 and 2, respectively. The climate in the region is sub-humid, warm and consists of summer rainfall and cool, dry winters. The mean annual rainfall for both locations is approximately 880mm. The two locations have the same soil type which is classified as Hutton form (Farmingham series), with a clay content of about 62 % (Soil Classification Working Group, 1991). This well-drained soil is an equivalent of the Rhodic Ferralsol (FAO), or Oxisol (USDA). Its mineralogy is dominated by kaolinite and halloysite, but also has high proportions of crystalline sesquioxides, gibbsite and inter-layered chlorite. Some chemical properties of the soil at each site are presented in Table 3.1.

Undisturbed native sites were identified on unused parts of each farm by Haynes *et al.* (2003). These sites consisted of shrubs and native grasses, and the burnt grass residue on the soil surface indicated that veld fires had periodically passed through both sites. All of the agricultural fields have a history of fertilization and liming. Typical annual application rates to pasture and arable fields are approximately 100 – 300 kg N ha^{-1}, 50 – 150 kg K ha^{-1} and 25 – 50 kg P ha^{-1}. Typical application rates for forest soils are approximately 50 – 100 kg N ha^{-1}, 10 – 60 kg K ha^{-1} and 10 – 25 kg P ha^{-1} (Haynes *et al.*, 2003). The maize, sugarcane (burnt before harvest), ryegrass and kikuyu pasture sites were irrigated during the dry winter months. At Baynesfield, the permanent kikuyu (*Pennisetum clandestinum*) and ryegrass (*Lolium perenne*) pasture were no longer intensively managed or grazed, but at Cedara, pastures were rotationally grazed with dairy cows. Annual ryegrass at both sites was annually cultivated and reseeded whereas kikuyu pasture remained uncultivated for a long time. A summary of the nature of the experimental fields at the two sites is presented below.

Site 1 (Baynesfield 'Estate' Farm)

1. >50 years permanent kikuyu (*Pennisetum clandestinum*) pasture
2. >50 years annual ryegrass (*Lolium multiflorum*) pasture
3. >30 years continuous sugarcane (*Saccharum spp.*) burnt before harvest
4. >30 years continuous maize (*Zea mays*) (conventional tillage, CT)
5. >20 years gum (*Eucalyptus grandis*) forest
6. >20 years pine (*Pinus patula*) forest
7. Undisturbed native grassland

Site 2 (Cedara Agricultural Research Station)

1. >50 years permanent kikuyu (*P. clandestinum*) pasture
2. >50 years annual ryegrass (*L. multiflorum*) pasture
3. >25 years continuous maize (*Zea mays*) (conventional tillage, CT)
4. >25 years continuous maize (zero tillage, ZT)
5. >20 years gum (*E. grandis*) forest
6. >20 years pine (*P. patula*) forest
7. Undisturbed native grassland

3.2.2 Soil sampling and analyses

Three separate, randomly chosen plots (approximately 120m x 30m) were selected within each field or forest area. Within each plot, 20 soil samples were taken randomly to a depth of 5 cm. Samples from each plot were bulked to give three replications per land use. The bulked field moist samples were sieved (<0.5 mm), air-dried and then stored for subsequent laboratory analysis. For microbial analyses, samples were re-moistened to field capacity and incubated for 2 weeks prior to analysis.

Soil pH was measured with a glass electrode in 0.1M KCl, using a 1:2.5 (w/v) soil:solution ratio (Rowell, 1994). Total organic C was determined by the Walkley and Black oxidation method (Olsen and Sommers, 1982). Extractable P and exchangeable cations in soil samples were analysed by routine soil testing methods at the KwaZulu-Natal Department of Agriculture Cedara laboratory. Exchangeable Ca and Mg were extracted with 1M KCl (1:10 soil:solution ratio for 10 minutes).

Exchangeable K and extractable P were extracted with AMBIC reagent [0.025M NH4HCO$_3$; 0.01M NH$_4$F; 0.01M EDTA (ethylenediaminetetra-acetic acid) at pH 8.0] using a 1:10 soil: solution ratio for 10 minutes. In the extracts, Ca, Mg and K were analysed by the atomic absorption spectrophotometry and P by the molybdenum blue method.

Microbial biomass P (P_{mic}) and C (C_{mic}) were estimated on pre-incubated samples (2 weeks), based on the difference between extractable P and organic C contents of chloroform-fumigated and unfumigated soil samples. Soils were extracted with the Bray 1 P extractant (0.03M NH$_4$F, 0.025M HCl) (Olsen and Sommers, 1982) and 0.5M K_2SO_4 (C_{mic}) (Vance *et al.*, 1987) respectively. K factors of 0.34 and 0.38 were used to calculate the microbial P and C pools, respectively.

A modified version of the Hedley et al. (1982) procedure was used to fractionate soil P and is schematically presented in Figure 2.9. After shaking the soil solutions with the various extracting reagents for 16 h and centrifugation at 10,000 rpm for 10 minutes, the aliquots were filtered through 0.45mm filter discs. Aliquots of the NaHCO$_3$ and NaOH extracts were acidified with 0.1 – 0.3 ml 0.9 M H_2SO_4 to precipitate the extracted organic matter and the inorganic phosphate in all the extracts was determined colometrically with the molybdate-blue method. Aliquots of the 0.5M NaHCO$_3$ (pH 8.5), 0.1M NaOH, 0.5 M HCl, and concentrated HCl extracts were digested in the autoclave (103.4 kPa, 121 °C) by acidified ammonium persulfate oxidation and analysed for total P (Tiessen and Moir, 1993). Organic P in the extracts was calculated from the difference between total P and Pi. All laboratory analyses were carried out in duplicate. Concentrated H_2SO_4 and H_2O_2 were used to digest the residue containing insoluble P_i and the most stable P_o forms (Residual-P). P in the resulting digested solution was analysed as outlined above.

Statistical analyses of data were carried out using the Genstat 8 package. The data were analysed as a completely randomised design with three replications (Nsabimana, 2002) although the land use types were not truly replicated within a formal experimental design.

3.3 Results

Some chemical properties of the soils are presented in Table 3.1. Land use forms and cropping practices had significant effects on soil organic matter status and soil pH. Pine forest soil had the highest pH in Baynesfield, and the lowest in Cedara. In general, the majority of soils collected from Cedara had a higher pH than those from Baynesfield. Soil pH in arable fields (maize and sugarcane) at site 1 was lower than that in the native grassland or other land uses. However, at site 2, pH values under maize, kikuyu and native grassland tended to be higher than those under the other land uses.

In both sites, arable crop production (sugarcane and maize) decreased organic C content by 30-50% relative to the control plots (Figure 3.1a and 3.2a). Cultivation (maize, CT and sugarcane) decreased organic C more than the zero-tillage (maize, ZT) (Figure 3.1a). Permanent pasture plots and forest soils (at site 1 only) showed significant accumulation of organic C above the undisturbed native grassland soils. At site 2, there was a loss of organic C under gum forest but not under pine. At both sites, extractable P was lowest under native grassland and of the other land uses, lowest under gum forest (Table 3.1). At site 1, sugarcane had very high extractable P levels. There were no obvious trends for exchangeable Ca and Mg with land use. At site 1, sugarcane and to a lesser extent kikuyu had high exchangeable K levels while at site 2, kikuyu had extremely high exchangeable K values (Table 3.1).

Microbial biomass C and P contents generally showed a similar trend with land use to organic C, and at both sites, permanent kikuyu pasture had the highest microbial biomass (Figures 3.1 and 3.2). At both sites, Pmic was considerably higher under native vegetation (24 – 27.2 mg P kg^{-1}) than maize cultivation (8.8 – 15.5 mg P kg-1). Annual pasture in Cedara significantly reduced microbial biomass relative to the native soil, and maintained Pmic and Cmic levels similar to those under maize (ZT) (Figure 3.5a and b). Maize (CT) resulted in the lowest microbial biomass contents at both sites. Values for the microbial quotient (Cmic:Corg) followed a similar pattern with land use as microbial biomass C, and ranged from 0.52 to 1% (Figures 3.1b and 3.2b).

At Baynesfield, large differences in Resin-Pi, $NaHCO_3$-Pi and NaOH-Pi were observed due to differences in land use (Figure 3.3). For Resin-Pi and $NaHCO_3$-Pi kikuyu pasture and sugarcane clearly had the greatest values and values for maize were also elevated above those of the other land uses. For NaOH-Pi, trends with land use differed greatly from those of Resin-Pi and $NaHCO_3$-Pi. That is, greatest values were observed for maize although values for kikuyu pasture and sugarcane were still elevated above those for the other land uses. Kikuyu pasture had the greatest values for dilute HCl-Pi and concentrated HCl-Pi. Differences due to land use were less pronounced for concentrated HCl-Pi than for the other Pi fractions.

In comparison with the other land uses at Baynesfield, labile organic P ($NaHCO_3$-Po) was very high under kikuyu pasture (Figure 3.4a). There were also significant differences in NaOH-Po due to land use with sugarcane having the greatest values and maize the lowest. For concentrated HCl-Po, variations due to land use were not great with highest values being recorded for annual ryegrass and lowest values for maize.

At Cedara, Resin-Pi and $NaHCO_3$-Pi were clearly greatest under kikuyu pasture and not greatly affected by the other land uses (Figure 3.5a and b). For NaOH-Pi, values were greatest under kikuyu and clearly greater for other land uses than that under native grassland. A similar trend was observed for concentrated HCl-Pi. For the dilute HCl-Pi fraction, kikuyu pasture and pine forest soils had values that were elevated well above those of other land uses.

For $NaHCO_3$-Po, at Cedara, values followed the order: kikuyu pasture > native grassland > ryegrass pasture = maize (ZT) > maize (CT) = gum forest = pine forest (Figure 3.6a). For NaOH-Po, levels under maize (CT) and maize (ZT) were notably low and highest values were observed under native grassland. By contrast, for concentrated HCl-Po, values were highest under kikuyu pasture and maize (ZT) and lowest under ryegrass pasture, gum and pine forest.

A summary of the data from the P fractionation is presented in Table 3.2. Labile P [defined as easily extractable Pi (Resin-Pi plus $NaHCO_3$-Pi) plus readily mineralizable Po ($NaHCO_3$-Po)] was greatest under kikuyu pasture at both

Baynesfield and Cedara. At Baynesfield, lowest values were under native grassland but at Cedara lowest values were recorded under gum forest. Total extractable inorganic P was greatest under kikuyu and maize at Baynesfield and greatest under kikuyu at Cedara. At Cedara, values were also notably high under maize (CT) and (ZT). At both sites, highest values for total extractable organic P were recorded under kikuyu pasture. Organic P was also relatively high under native grassland at both sites and in the soil of these undisturbed sites, organic P accounted for 50% or more of the total soil P content. Residual P was greatest under kikuyu at Baynesfield and ryegrass at Cedara. In comparison with native grassland, residual P was similar or often greater under agricultural land uses at both sites.

The distribution of P between labile, moderately labile and stable P forms for the different land uses at the two sites is summarised in Figures 3.7 and 3.8. It is evident that at both sites there was a shift towards a greater percentage of P being present in the labile fraction (and concomitant decrease in the percentage contribution of the stable fraction) under kikuyu pasture. At site 1, this also occurred to a lesser extent under maize and sugarcane. At site 1 there was a notable increase in the percentage of P present in stable forms under gum and pine forest. However, at site 2, this trend was not evident.

Table 3.1 Some chemical properties of the soils at the two experimental sites

Land use	pH (KCl)	AMBIC extractable P (ppm)	Exchangeable nutrients (ppm) Ca	Mg	K
Site 1 (Baynesfield)					
Native grassland	5.0	5	1120	424	398
Kikuyu	4.9	68	1523	342	552
Ryegrass	4.7	8	1287	515	387
Sugarcane	4.9	161	1281	267	853
Maize (CT)	4.4	95	1103	240	423
Gum forest	4.3	7	674	259	185
Pine forest	6.4	15	2457	464	263
LSD (0.05)	1.3	12	476	57	178
Site 2 (Cedara)					
Native grassland	5.2	6	1358	386	180
Kikuyu	5.2	92	1762	538	1331
Ryegrass	4.3	25	941	195	114
Maize (CT)	5.3	19	931	249	275
Maize (ZT)	5.5	24	1699	433	413
Gum forest	5.0	15	2573	494	303
Pine forest	4.0	53	2783	391	411
LSD (0.05)	1.4	9	530	128	259

Table 3.2 Effects of long-term land use on concentrations of labile P, total extractable organic and inorganic and total P in soils at sites 1 and 2

Land-use	Labile P[1]	Total extractable organic P[2]	Total extractable inorganic P[3]	Residual P	Total P
Site 1 (Baynesfield)					
Native grassland	87	666 (51)[4]	217 (17)	426	1309
Kikuyu	897	1366 (45)	1006 (33)	643	3015
Ryegrass	114	760 (52)	303 (21)	386	1449
Sugarcane	451	586 (28)	968 (47)	513	2067
Maize (CT)	254	247 (13)	1190 (63)	430	1867
Gum forest	113	452 (36)	269 (21)	533	1254
Pine forest	105	516 (37)	347 (25)	550	1413
Site 2 (Cedara)					
Native grassland	113	1115 (63)	240 (13)	411	1766
Kikuyu	464	1119 (27)	2505 (61)	456	4080
Ryegrass	140	783 (33)	972 (41)	644	2399
Maize(CT)	119	516 (26)	1097 (55)	389	2002
Maize (ZT)	157	520 (25)	1089 (52)	467	2076
Gum forest	73	665 (38)	636 (36)	442	1743
Pine forest	112	845 (37)	1025 (44)	411	2281

[1] Labile P = resin P + $NaHCO_3$-Pi + $NaHCO_3$-Po (Schmidt *et al.*, 1996)

[2] Total organic P = $NaHCO_3$-Po + NaOH-Po + conc. HCl -Po

[3] Total inorganic P= resin P + $NaHCO_3$-Pi + NaOH-Pi + dilute HCl -Pi + conc. HCl -Pi

[4] Values in parenthesis are percent contribution of each fraction to total P

Site 1 (Baynesfield)

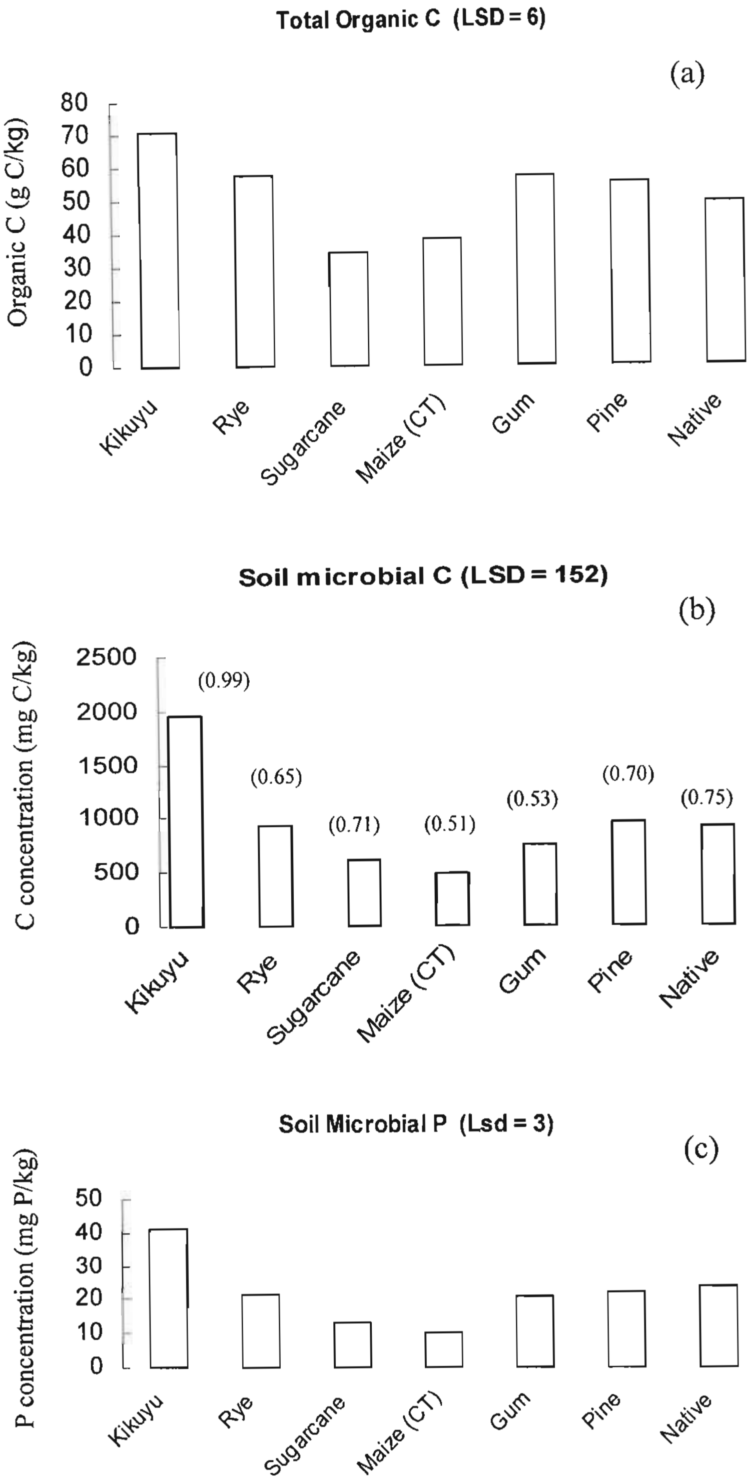


Figure 3.1 Effect of long-term land use on concentrations of total organic C and microbial biomass C and P in soils at site 1. Values for microbial quotient (microbial biomass C/ organic C) are shown in parenthesis above microbial biomass C histograms.

Site 2 (Cedara)

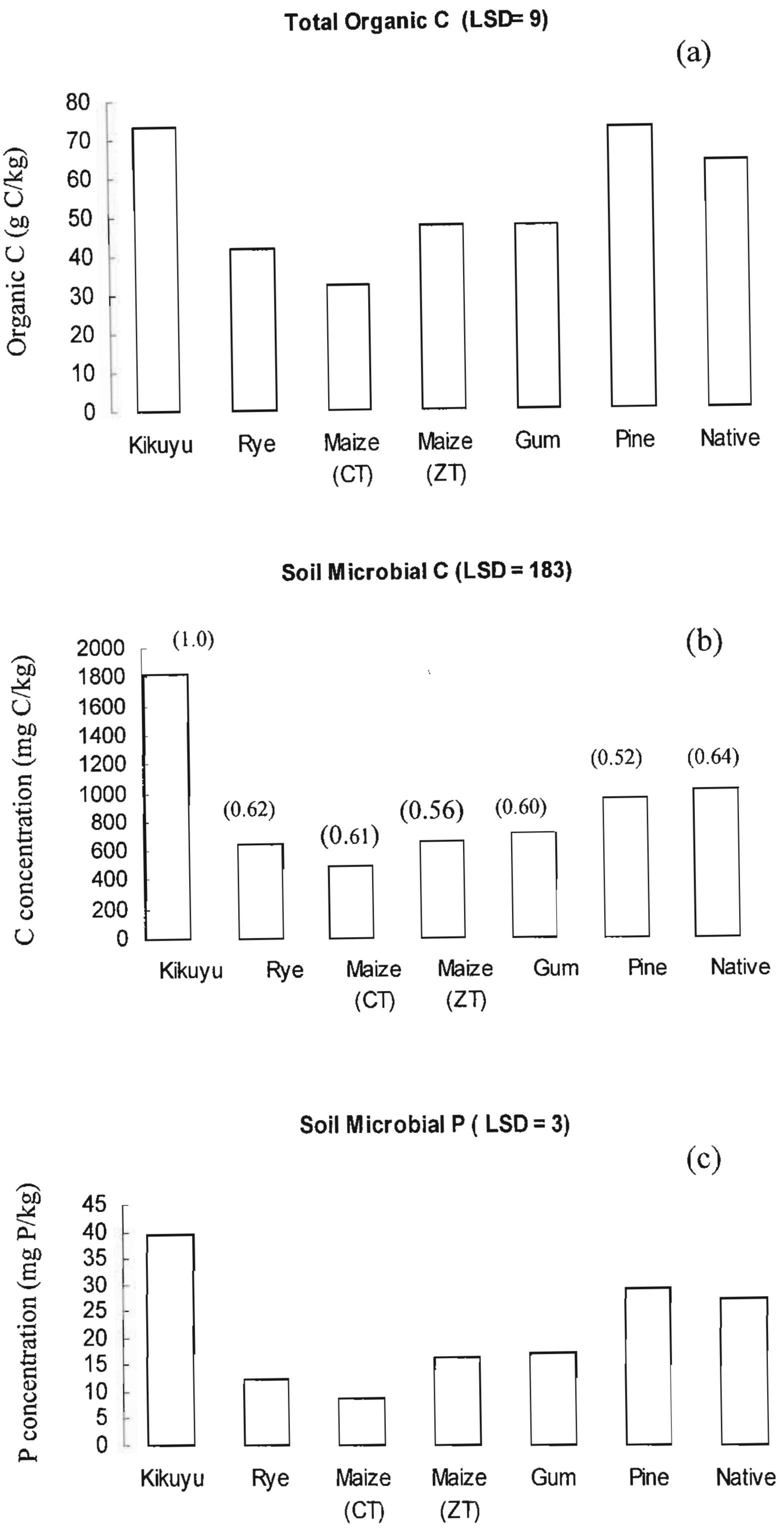


Figure 3.2 Effect of long-term land use on concentrations of total organic C and microbial biomass C and P in soils at site 2. Values for microbial quotient (microbial biomass C/ organic C) are shown in parenthesis above microbial biomass C histograms.

Site 1 (Baynesfield)

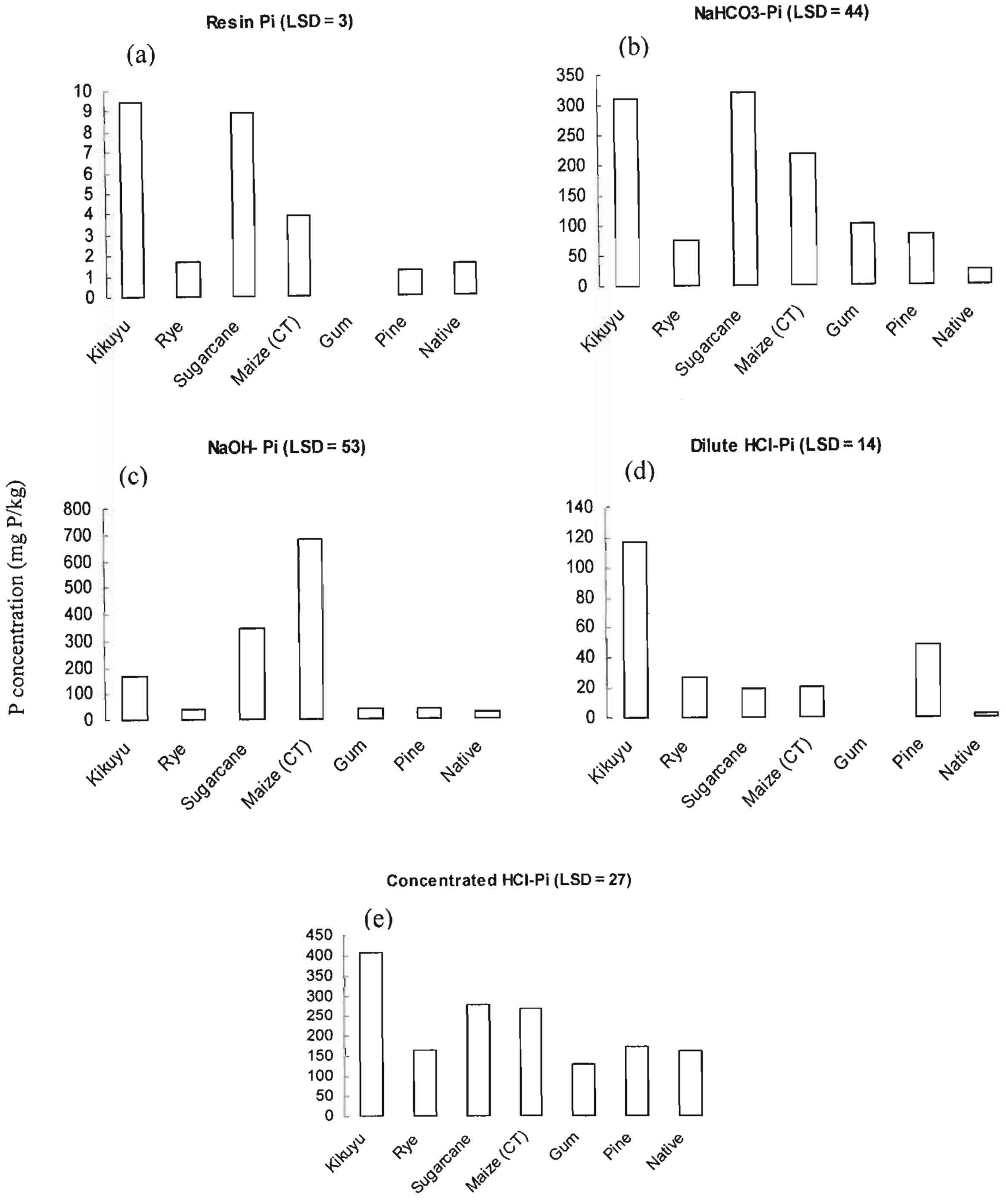


Figure 3.3 Effect of long-term land use on concentrations of inorganic P (Pi) extracted from soils at site 1 using a modified Hedley sequential fractionation procedure.

Site 1 (Baynesfield)

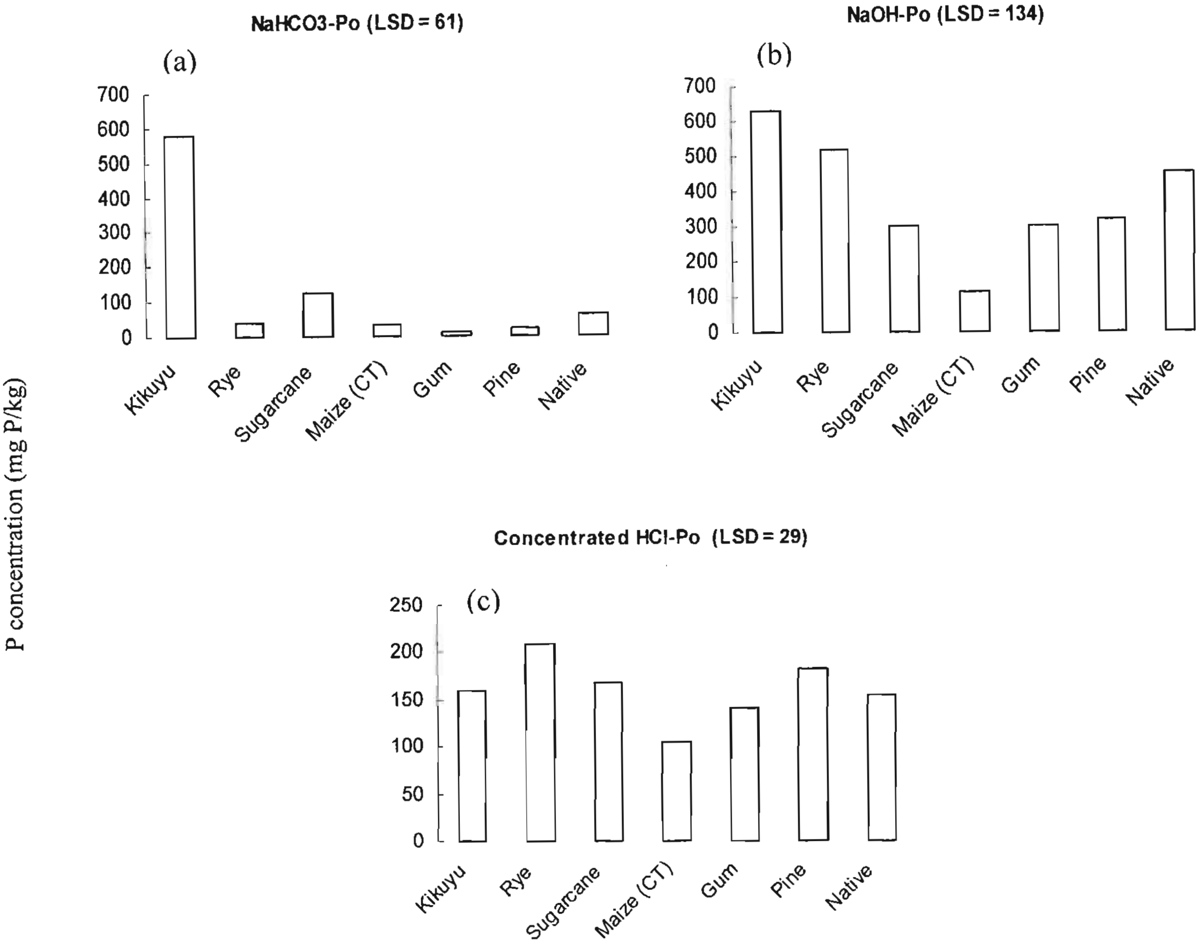


Figure 3.4 Effect of long-term land use on concentrations of organic P (Po) extracted from soils at site 1 using a modified Hedley sequential fractionation procedure.

Site 2 (Cedara)

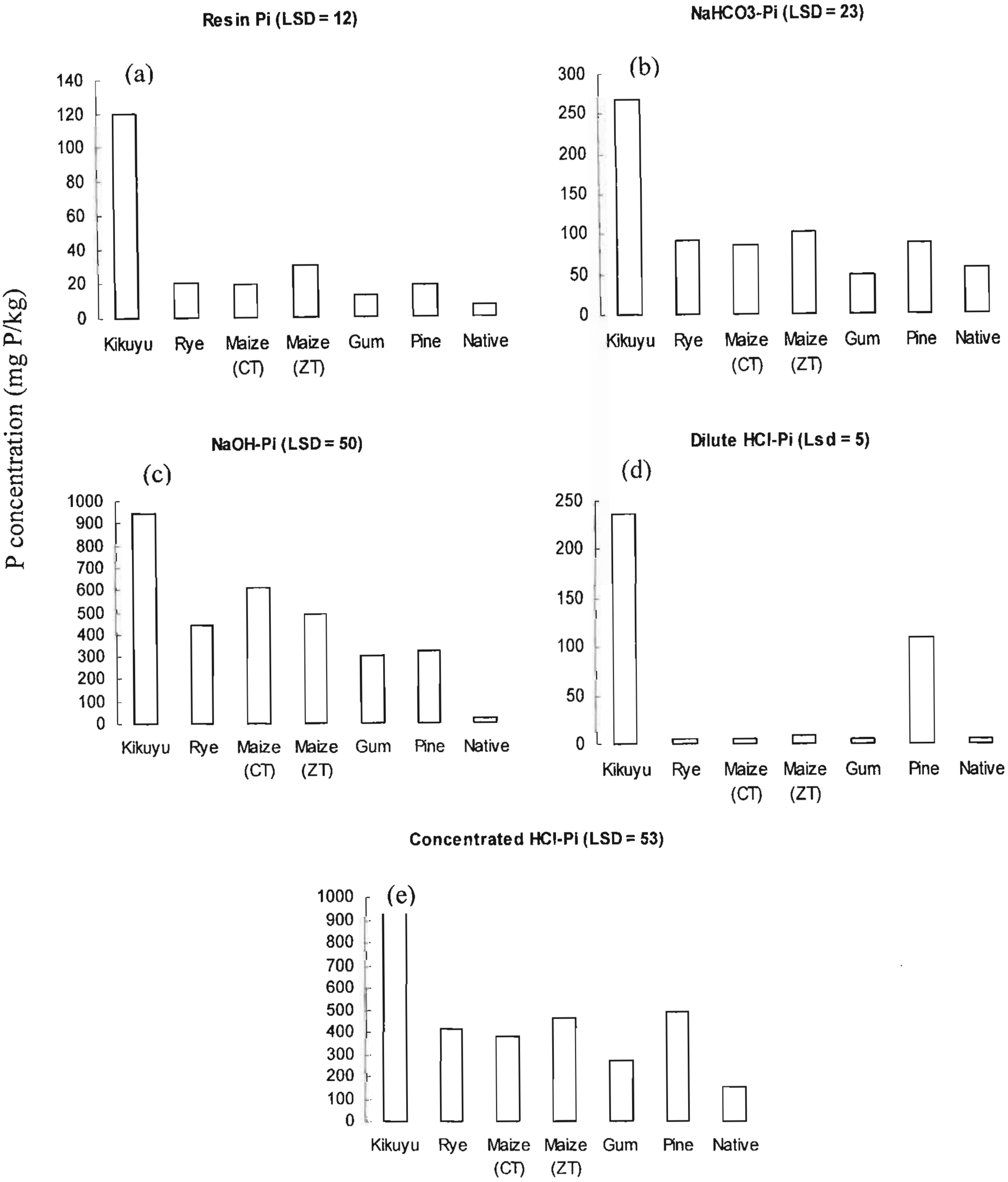


Figure 3.5 Effect of long-term land use on concentrations of inorganic P (Pi) extracted from soils at site 2 using a modified Hedley sequential fractionation procedure.

Site 2 (Cedara)

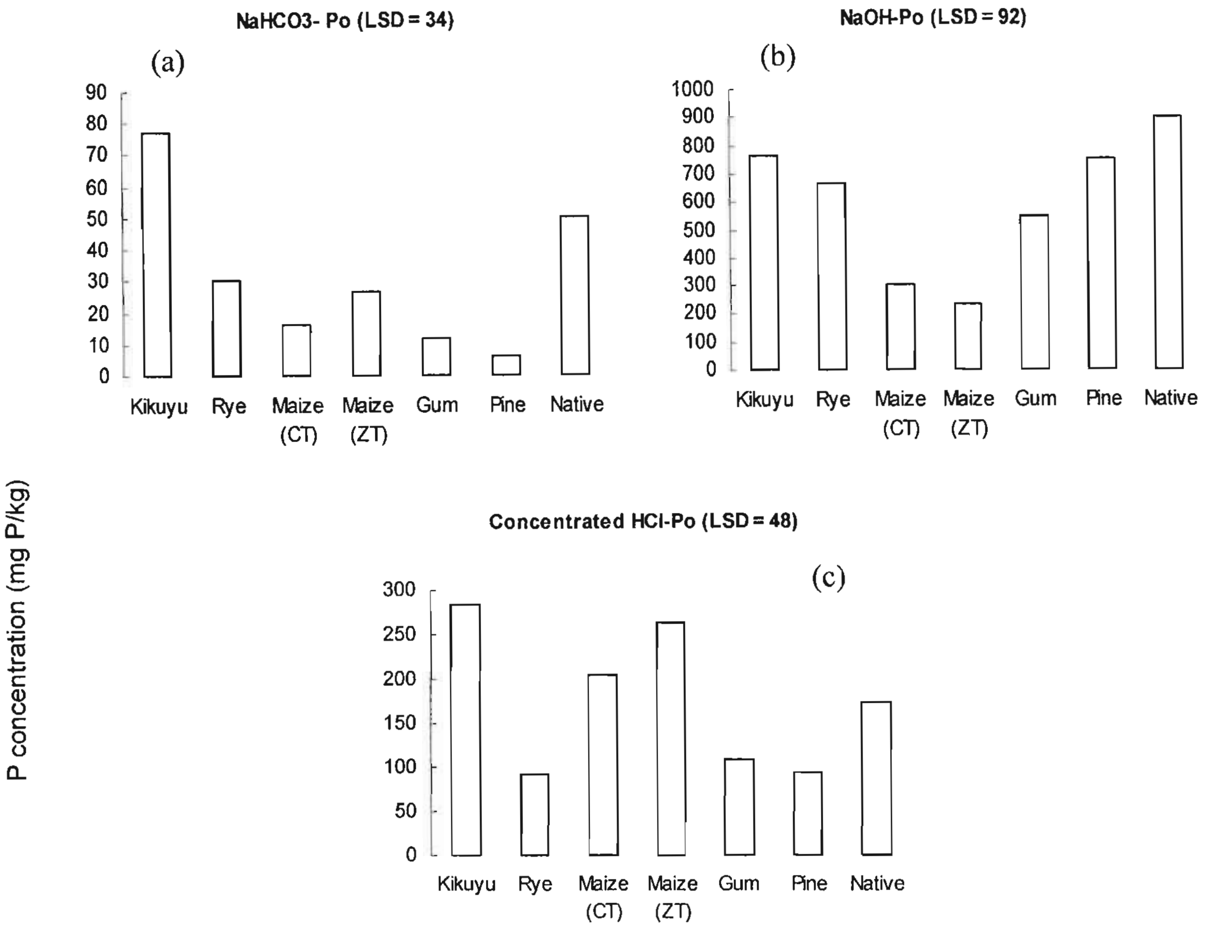


Figure 3.6 Effect of long-term land use on concentrations of organic P (Po) extracted from soils at site 2 using a modified Hedley sequential fractionation procedure.

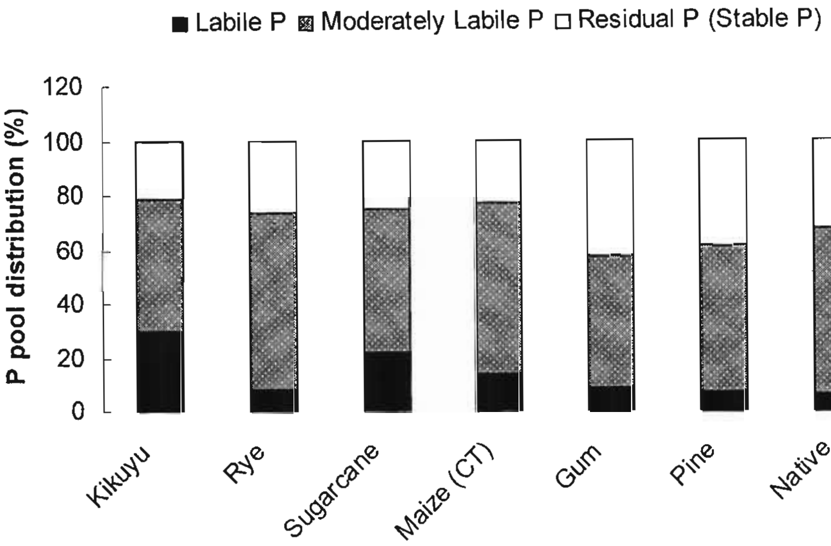


Figure 3.7 Effects of long-term land use at site 1 on the proportions of P present as labile [Resin-Pi + $NaHCO_3$-(Pi+Po)], moderately labile P [NaOH-(Pi+Po) + dilute HCl-Pi + concentrated HCl- (Pi+Po)] and stable P (residual P).

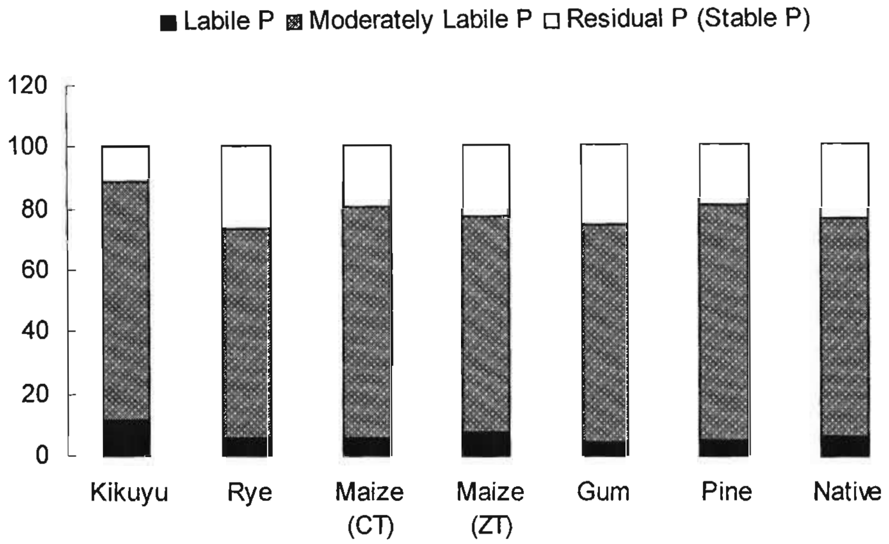


Figure 3.8 Effects of long-term land use at site 2 on the proportions of P present as labile [Resin-Pi + $NaHCO_3$-(Pi+Po)], moderately labile P [NaOH-(Pi+Po) + dilute HCl-Pi + concentrated HCl- (Pi+Po)] and stable P (residual P).

3.4 Discussion

3.4.1 Soil organic matter content

Soil organic matter content (SOM) depends on the balance between the rates of input and losses of carbon from the soil. Differences in the organic C content in the various land uses can be related principally to the quantity and quality of plant material produced, and its removal or incorporation into the soil (Dalal, 1998).

Maize and sugarcane monoculture resulted in least soil organic C content at both sites (Figures 3.1a and 3.2a). Mechanized clearing of native grasslands for agricultural purposes can lead to compaction, reduction of infiltration and increase in surface run-off and erosion of surface soil. This results in a reduction in measured soil organic C content (Lal, 1997). In addition, tillage and intensive cropping following conversion from natural vegetation typically cause a decrease in organic C content through their effects on plant biomass returned to the soil and rate of oxidation and mineralization of SOM (Dominy *et al.*, 2001). Cultivation increases SOM breakdown because it aerates and disrupts soil aggregates which exposes previously protected SOM to microbial degradation (Haynes and Beare, 1996).

Lower biomass input in arable soils is due to wider plant spacing, removal of the harvested crop, residue removal, and/or volatilisation of residue C during pre-harvest burning of sugarcane residues (Wood, 1985; van Antwerpen and Meyer, 1996). Robertson and Thorburn (2001) reported that when the trash is burnt, more than 80% of the organic matter and nutrient in the trash are lost. Despite more frequent cultivation of the maize (CT) field, it still maintained similar SOM (Table 3.1) to that under the sugarcane field probably because the sugarcane was pre-harvest burnt.

Moisture and nutrient deficiency can limit microbial activity, especially during the dry season (Degens, 1998). In contrast, irrigation and the addition of lime and fertilizers (often N and P), undertaken to improve crop yields can increase soil microbial biomass (Neale *et al.*, 1997). Nonetheless, lime-induced increases in organic matter loss have been reported previously (Baldock *et al.*, 1994). Chana and Heenana (1999) found that most of the loss in organic C due to liming was in the light fraction (LF) because, although it was bound to macroaggregates, it was not physically protected from microbial attack. Higher soil pH in maize fields, particularly at site 2 (Table 3.1), possibly enhanced microbial activity and therefore increased the rate of SOM decomposition.

Maize (ZT) had significantly ($P<0.05$) higher organic C content than that of maize (CT) (Figure 3.2a). This reflects the results of 30 years of direct drilling and stubble retention. Other studies also showed greater accumulation organic C and P in surface soils (0-5cm) under zero-tillage than in conventional tillage (Paustian *et al.*, 1997; Selles, *et al.*, 1999). Under zero-tillage crop and weed residues are left at the surface to decompose. As a result there is a build up of soil organic matter close to the soil surface. In contrast, cultivation mixes crop residues into the surface layers and results in a more uniform distribution of organic C and nutrients in the plow layer (Powlson and Jenkinson, 1981; Haynes and Knight, 1989).

Long-term deposition of tree litter at the soil surface prior to harvesting and re-planting of new forests increased organic C contents in forest soils. Relatively little SOM is lost because of infrequent and incomplete harvesting done on forest land. The organic matter on the 0-5 cm surface horizon and in the lower layers is maintained by relatively slow oxidation resulting from cool, shaded microclimatic conditions and from the lack of soil disturbance that occurs in arable soils. These factors may explain why pine and gum forest soils had a higher organic C content than native grassland soil in site 1 (Figure 3.1a). This was true for pine soils at site 2. Some forest litter also contains more lignin than grasses (Parton *et al.*, 1997), hence its decomposition and oxidation occur at slower rates.

As expected, organic C content (Figures 3.1a and 3.2a) under permanent kikuyu pasture was much higher than in other land uses. Irrigation, liming and fertilization applications increase plant dry matter production under improved pastures (Haynes and Beare, 1996). Higher organic matter inputs in intensively managed pasture systems come from scenescing plant materials (e.g. plant roots and tops), exudation of organic compounds from plant roots, and turnover of large microbial biomass found in the rhizosphere (Gregorich *et al.*, 1994; Paustian *et al.*, 1997). Thus, pasture crops provide higher and steady plant residue inputs than do arable crops. When grazed, more than 90 % of the P in the forages is recycled to the soil as animal excrement (Haynes and Knight, 1989; Sparling, 1992). Under permanent pastures annual tillage is not performed so organic matter decomposition is not favoured.

Organic C concentration in the annual ryegrass field at Baynesfield was higher than that at the native grassland, but the opposite was true for the annual pasture at Cedara. The effect of annual pasture on soil organic matter status will be the consequence of a balance between high organic matter inputs (particularly by the turnover of grass roots) and the destructive effect of annual tillage. Evidently, at Baynesfield, the greater organic matter inputs had a dominant effect while at Cedara the tillage effect dominated. Nevertheless, at both sites organic C content was greater under annual ryegrass than maize (CT) which reflects the greater organic matter inputs under pasture than maize.

3.4.2 Soil pH

Soil acidity can be a limitation to crop production in the study soils because they are naturally acidic. That is, at site 1, pH values under annual ryegrass, sugarcane, maize (CT) and particularly gum forest were below those of the native grassland. Similarly, at site 2, values under ryegrass, gum and pine forest were below those under native grassland. Soil acidification in agricultural soils is due to application nitrogen fertilizers and export of basic cations (Ca^{2+}, Mg^{2+}, K^{+}) from the soil through crop harvests (Yan *et al.*, 1996).

Nitrification of NH_4^+ originating from N fertilizers and organic residues releases H^+ ions into the soil solution (Tisdale *et al.*, 1985). As nitrate (NO_3) is leached down the profile Ca^{2+}, Mg^{2+} and K^+ usually serve as the counter-ions. This leaves high concentrations of H^+ in the surface layer, hence the pH drops. Acidification in soils managed under zero tillage is often more pronounced than that under conventional tillage (Haynes and Knight, 1989) due to higher infiltration rates, which accelerate leaching of nitrate and basic counter-ions. However, the data (Table 3.1) shows that the maize (CT) and ZT had similar pH. This may be due to surface application of lime to both systems of tillage.

The low pH (3.77) of pine forest soil at Cedara is probably attributable to accumulation of cations in the tree biomass (Essex and Williams, 1993), leaching of basic cations and its relatively higher organic matter content (Figure 3.5 a). Organic matter decomposition can contribute to soil acidity due to CO_2 evolution and formation of carbonic acid, production of organic acids and chelates. Forest soils, as a rule have favorable soil structure for water infiltration, and this can promote leaching of basic cations to a greater extent than in agricultural soils (Allan and Chapman, 2001).

3.4.3 Soil microbial biomass C and P

Microbial biomass is linked to the availability of C and nutrients from decomposing organic matter (Selles *et al.*, 1999). Consequently, Cmic and Pmic generally followed the same trend as organic C content (Figures 3.1 and 3.2). The accumulation of organic C in pasture and native grassland soils had a positive effect on soil microbial biomass. Fertilization, liming and irrigation of pasture fields have positive indirect effects on microbial biomass by enhancing plant growth and root exudates would increase microbial growth and activity (Merckx *et al.*, 1985; Oberson *et al.*, 1999). When the residues are incorporated either through cultivation or soil faunal activity (e.g. earthworms), plant litter P enters into the soil microbial biomass. Dalal et al. (1991) measured an increase in soil microbial biomass C and N due to N fertilization.

Arable fields had the least microbial biomass contents due to lower organic C contents, annual tillage (soil disturbance), and the fallowing effect during winter months (Alvarez *et al.*, 1998). Regular pesticide, insecticide and herbicide applications could also potentially decrease microbial biomass size and activity (Dalal, 1998). The relatively low pH in arable soils at site 1 could have also contributed to lower microbial biomass size, as the microbial biomass is often related to pH and low biomass values are common in acidic soils (Vance *et al.*, 1987). In an Alfisol, microbial biomass decreased due to 8 years of application of 80 kg N ha^{-1} to wheat, presumably due to lowering of soil pH (Ladd *et al.*, 1994).

Maize (CT) fields generally had the least microbial biomass content at both sites (Figures 3.1 and 3.2). Zero-tillage increased the surface concentration of total organic C, microbial C and P and bio-available nutrients (P, Ca, K, Mg). Deposition of plant residues at the soil surface under ZT favours high soil microbial activity in the surface layers (Selles *et al.*, 1999). Similarly, Doran (1980) showed that populations of bacteria, actinomycetes, and fungi increased 2-to 6 fold as a result of retaining crop residues on the soil surface. Networks of hyphae are more easily established in soil that is minimally disturbed than in soil under conventional tillage, and the microflora of soil under ZT is mainly dominated by fungi (Roper and Gupta, 1995). These factors make soil under long-term ZT systems a more favourable environment for microbial activity.

The microbial quotient can be a useful indicator of the influence of management practices on organic C because it responds more rapidly to changes in soil C than total organic C (Sparling, 1992). At both sites, permanent kikuyu pasture had the highest microbial quotient, which indicates greater labile C input under permanent dairy pasture. Our results show that the microbial quotient was generally low, ranging from 0.52 to 1% (Figures 3.1b and 3.2b), but within the range of reported values of 0.27 – 7% (Omay *et al.*, 1997).

The high microbial biomass under kikuyu pasture, and to a lesser extent native grassland and exotic forests, is important for the productivity and long-term sustainability of these land uses. This is because microbial biomass is a source of labile organic matter, a source and sink of major plant nutrients and an agent for break down of organic residues as well as the formation of humus (Dalal, 1998). Immobilisation of Pi by microbes and its gradual release via microbial turnover protects P from fixation into the less accessible P pools (Magid *et al.*, 1996). Furthermore, a significant portion of microbial P (some obtained from sparingly- soluble Pi forms) is considered readily available for plant assimilation (Oberson *et al.*, 2001).

3.4.4 Labile P (Resin-Pi, $NaHCO_3$-Pi, $NaHCO_3$-Po)

Low resin-P contents (Figures 3.3a and 3.5a) under native grassland in the studied soils support findings of many other studies that phosphorus (P) is an important limiting factor (next only to nitrogen) to the productivity of acidic soils. With no external inputs, the native vegetation relies on mineralization of Po and turnover of microbial biomass P to satisfy its P demands. Plants growing in P limited soils develop adaptive mechanisms to meet their P requirements. They are able to acquire poorly- soluble P by excreting chelating agents (Ae *et al.*, 1990), increasing root surface area, or colonisation with mycorrhizal fungi (Loes and Ogaard, 2001).

The increases in extractable P and labile Pi fractions (resin and $NaHCO_3$-Pi) in soil under arable and pasture cropping systems are attributable to long-term P additions (Zhang and McKenzie, 1997). Repeated fertilizer P additions increase labile P, with more occurring as Pi than Po (McKenzie *et al.*, 1992; Griffin *et al.*, 2003). Zheng et al. (2004b) also reported an increase in the inorganic P (Pi) (resin-P, $NaHCO_3$-Pi, and NaOH-Pi) fractions on a Humic Gleysol under various cropping systems in soils amended with mineral fertilizer and liquid dairy manure.

Higher fertilization rates of pasture swards, and plant P uptake efficiency by forage plants significantly increased $NaHCO_3$-Pi concentration in kikuyu pasture relative to maize cultivated soil. Forage crops exude organic acids and enzymes more than cereals such as barley, which enhances P uptake (Condron, 2004). Since $NaHCO_3$-Pi represents inorganic P weakly absorbed on surfaces (Lehmann *et al.*, 2001), it is possible that forage plants dissolved more Pi from the solid phase into solution. In the long term, more soil microbial biomass under kikuyu soils may also mineralize more Po compared to maize cultivated soils (Table 3.1). It is also probable that fertilizer P rates to kikuyu pastures were greater than those to arable crops and over a 20-50 year period this resulted in greater accumulation of Pi and Po.

The labile $NaHCO_3$-Po fraction was particularly high under kikuyu dairy pasture (Figures 3.4a and 3.6a). The high concentrations of labile Po represent a high potential for mineralization and subsequent release of plant-available Pi (Tiessen *et al.*, 1982). The high values under kikuyu pasture are presumably a reflection of the high rate of P cycling under a highly productive permanent grazed pasture. Not only is the pasture highly productive (above-ground production is about 10-16 Mg ha^{-1} yr^{-1}) but most of the above-ground herbage P is recycled through the grazing cows and returned to the soil in the form of dung.

3.4.5 Moderately labile P (NaOH-Pi, NaOH-Po, dilute HCl-Pi, conc. HCl-Pi, conc. HCl-Po)

The moderately available pool was a significant characteristic of this soil, reaching 77 % of total extractable P in kikuyu pasture at site 2 (Figure 3.8). Accumulation of NaOH-Pi in arable (maize and sugarcane) and pasture soils indicates that annual P applications have resulted in accumulation of P in this fraction (Schmidt *et al.*, 1996). It has been postulated that the chemical nature of the reaction products of water-soluble P fertilizers in soil is influenced by the predominant cations present (Tisdale *et al.*, 1985). Thus, in the studied soils, added Ca phosphate fertilizers are mainly converted to adsorbed and mineral forms of P associated with Fe and Al, whereas in calcareous soils, major reaction products are mainly calcium bound forms of P (Sample *et al.*, 1980). Indeed, the concentration of NaOH extractable Pi is often positively correlated with the Fe and Al oxide contents (Delgado and Torrent, 2000).

Soil NaOH-Pi is considered to be held strongly by chemisorption to amorphous Fe and Al minerals (Tiessen *et al.*, 1984) and is considered as moderately labile soil P (Lehmann *et al.*, 2001). Consequently, large NaOH-Pi contents in the studied soils can be related to their high concentrations of ammonium-oxalate-extractable Al and Fe (Dekkers, 1971; Soil Classification Working Group, 1991).

Figure 3.5c show that the conventionally tilled maize plot had a higher concentration of NaOH-Pi as the zero-tilled plot. Under ZT systems, fertilizer is surface-applied and accumulates near the soil surface whilst under CT fertilizer is mixed intimately with the soil during tillage operations. Lack of fertilizer incorporation minimises contact between fertilizer granules and the soil surfaces, and therefore lowers P fixation of the applied P to oxides and mineral surfaces. Thus soils under maize (ZT) had higher concentrations of Resin-, and $NaHCO_3$-Pi and lower concentrations of NaOH-Pi than those under maize (CT). Additions and retention of lime on the surface layer of a zero-tilled soil may also tend to reduce P fixation hence lower the size of NaOH-Pi fraction. That is, the higher pH under ZT would have tended to inhibit P adsorption and favour P desorption.

The large quantities of NaOH-Pi and HCl-Pi in surface layers of unfertilized forest soils can be partly related to activities of macroorganisms. The burrowing effects of earthworms may deposit unweathered mineral particles near or on the soil surface. Mobilization of these unweathered primary mineral particles from deeper layers of the soil can contribute small amounts of concentrated HCl-P in the upper soil layers because P in this fraction is considered to form part of the structure of primary minerals (Guo and Yost, 1998). P that is released by earthworm activities may also be fixed to Fe and Al hydroxides, and consequently increase NaOH-Pi and HCl-Pi. These processes may well have also been operative in the commercial exotic forests used in the present study.

Nonetheless, accumulation of NaOH-Pi in forest soils, particularly in site 2, likely to have been primarily as a result of fertilizer P additions.

The sparingly soluble Ca-bound P fraction (dilute HCl-P) was typically low in most of soils but its concentration was elevated under kikuyu and pine forest at both sites (Figures 3.3d and 3.5d). The reason for this is unclear since, with the exception of pine forest at site 1, these sites did not have particularly high pH values (which would favour accumulation of Ca-bound P). Perhaps some of this fraction represents residual fertilizer Pi.

Enhanced mineralization of organic Po under cultivation invariably leads to a decline in Po fractions, and in this study the decline was greater under maize (CT) than maize (ZT) (Figure 3.6). For example, the proportion of total extractable Po constituted 25 and 39% of total P in the maize (CT) and maize (ZT), respectively. The decrease in Po pools despite P fertilization of maize and annual pasture systems are consistent with studies from temperate grassland soils reviewed by Stewart and Sharpley (1987) which showed that soils under cultivation decreased Po due to mineralization. Such a finding is not surprising since a decrease in the organic C content under arable agriculture, as noted previously, would be expected to result in an associated decrease in Po. Interestingly, this decline in Po occurred concomitantly with increases in $NaHCO_3$-Pi and NaOH- Pi. Organic P released from decomposing plant residues can be rapidly mineralized in cultivated soils thus increasing $NaHCO_3$-Pi (Zheng *et al.*, 2004b). It is observed that liming increased soil pH in Cedara maize fields (Table 3.1), and in turn, this could have enhanced microbial activity and microbial demands for carbon substrate and nutrients (including P), thereby accentuating mineralization of organic matter and Po (McKenzie *et al.*, 1992). It is, therefore, possible that mineralization of Po resulted in the increases in resin-Pi, $NaHCO_3$-Pi and NaOH-Pi, particularly in kikuyu, maize and annual ryegrass fields (Figures 3.3 and 3.5). Nonetheless, a substantial proportion of these increases in moderately labile Pi fractions are likely to have originated directly from dissolution of fertilizer P and its subsequent reactions with soil components.

The proportion of organic P in the labile and moderately labile P pools in the soil surface increased significantly under kikuyu. This was expected because the annual addition of crop residue to soils in pasture is greater than under arable systems and there was a substantial accumulation of organic matter under kikuyu. Eventually, the net rate of Po accumulation due to long-term fertilization of pasture slows down as a result of increasing mineralization so that in the long-term, the rate of Po formation is balanced by the rate of Po mineralization (Anderson, 1980). According to Sharpley and Smith (1985), the accumulation in moderately labile Po indicates a general improvement in soil P fertility under cultivation.

3.4.6 Stable P pool (residual P)

Fertilizer P applications increase the soluble or labile P pools in the short term, but this P can be transformed into moderately labile and non-labile P pools over a period of time (Sample *et al.*, 1980). In addition, crop utilization of inorganic phosphorus fertilizers in the first year is generally less than 25% (Wagar *et al.*, 1986), which leaves fertilizer residues to accumulate as various forms. Accumulation of residual P (occluded P) above the control occurred in many of the soils (Table 3.2). Increases in residual P are due to slow reactions, which continually remove P from the soil solution (Henry and Smith, 2004). Thus, a build up of residual P may represent a loss of the effectiveness of P fertilizer applications because as this fraction increases, it diminishes the availability of the residues of past P fertilizer applications to plants (Barrow, 1980). Nonetheless, in limed acidic soils, the residual Pi can contribute significantly to crop growth through the formation of unstable labile inorganic Ca phosphates (Lookman *et al.*, 1996). It is important to note here that residual P also includes very stable, complexed organic P (Beck and Sanchez, 1994). Thus, part of the residual fraction that has accumulated is in organic that are not readily mineralizable.

3.4.7 Relative contribution of Po and Pi

Whilst agricultural land use had both positive and negative effects on the total extractable organic P content of soils (depending upon its effect on soil organic matter content) it caused clear increases in total extractable Pi at both sites. This reflects the fact that inorganic P is applied to agricultural soils regularly and that this P tends to accumulate preferentially in the Pi fractions (McKenzie *et al.*, 1992; Selles *et al.*, 1995; Griffin *et al.*, 2003). Under native grassland, organic P made up 51% of total P at Baynesfield and 63% at Cedara (Table 3.2) showing the importance of this form of P in natural systems (Beck and Sanchez, 1994; Dalal, 1998). Slow mineralization of this Po provides Pi for plant growth. Interestingly, total extractable Pi made up only 17 and 13% respectively of total soil P under native grasslands at Baynesfield and Cedara. By contrast, it made up over 50% of total P in some agricultural land uses which again underlines the increased significance of Pi pools under agricultural management.

A similar trend was observed between organic C and NaOH-Po for land uses at both sites (Figures 3.1, 3.2, 3.4, and 3.6). The NaOH-Po is primarily derived from humic compounds generated from plant-residue decomposition (O'halloran *et al.* 1987; Selles *et al.*, 1999). Thus, the amount of organic matter and its rate of humification can be expected to influence the content of NaOH-Po fraction. Organic C declined more under maize (CT) than maize (ZT) and that tendency was also evident in total extractable Po (Table 3.2). For example, total organic P constituted 25 and 38%, respectively of total P in the maize (CT) and maize (ZT) treatments.

3.5 Conclusions

Conversion from natural vegetation to long-term agricultural production led to changes in soil properties. The results provide evidence that when native grasslands are brought under long-term maize and sugarcane production, with no return of residues, there is an appreciable breakdown of soil organic matter

(SOM). In contrast, long term pasture and forestry result in maintenance or accumulation of SOM.

High concentrations of total P have accumulated in the surface horizon (0-5cm) of agricultural soils due to intensive P fertilization, in part because P is generally immobile in soil. In the fertilized systems, total P was higher and P accumulated preferentially in the inorganic fractions. P composition of the native vegetation was dominated by organic P (Po) forms, with a major portion of Po in the moderately available pool. As expected, organic P content of soils showed a broadly similar trend to that for organic C, decreasing under maize/sugarcane, increasing under kikuyu pasture and being maintained or decreased under exotic forests. Application of a modified Hedley fractionation procedure showed that agricultural land management greatly affected the distribution among the various pools. Resin-Pi and $NaHCO_3$-Pi (the potentially available Pi pools) showed similar trends with land use being notably elevated under kikuyu pasture at both sites and under sugarcane and maize at one site. This presumably reflects regular P applications at these sites in the recent past. Concentrations of NaOH-Pi showed different trends to Resin-Pi and $NaHCO_3$-Pi since this fraction represents strongly-adsorbed Pi that may have originated from Pi applied or mineralized some time ago that has become strongly bound to metal oxide surfaces. A large portion of the P that had accumulated in organic form under kikuyu pasture was present in the $NaHCO_3$-Po fraction. This fraction also contributed greatly to the loss of soil organic P that occurred under arable cropping, confirming that it is a labile form of organic P.

The focus of this project was on the 0-5 cm surface layer of the soil. Further investigation is required to the effects of these practices at deeper soil depths, because effects of cropping systems and nutrient sources on P forms have been reported to extend deeper into the soil profile (Zheng *et al.*, 2004a). Short-term (0-5 years) observations can also provide further information on the rate of accumulation and the nature of P applied to soil.

CHAPTER 4

EFFECTS OF ORGANIC AND INORGANIC SOIL AMENDMENTS ON PHOSPHORUS FRACTIONS

4.1 Introduction

Soil acidity is a serious constraint to crop production in the sub-Saharan African soils (Chepkwony *et al.*, 2001). In addition, highly-weathered acid soils are generally rich in Al and Fe hydrous oxides and therefore have a high P fixing capacity (Moller *et al.*, 2000). Thus, applications of lime and fertilizer P (often at high rates) to such soils are frequently necessary for crop production (Haynes and Swift, 1988). When P is added to acidic soils, it is often adsorbed by soil colloids, accumulating in inorganic forms (Pi) with different degrees of plant availability (Sanchez and Uehara, 1980). The high cost and other socioeconomic constraints to the use of inorganic P fertilizers have generated considerable interest in the utilization of organic amendments. Hue (1990) reported that inorganic P fertilizers were more efficiently utilized by crops when applied with organic amendments such as green manure or animal wastes.

Large amounts of animal manure are produced as wastes from housed animal operations in South Africa. For example, poultry production is concentrated in confined feeding operations. There is also an increasing trend for beef production to be carried out in intensive feedlot operations. Large quantities of manures from poultry enterprises and feedlots are therefore available to be applied to local arable and pasture fields. If the plant requirement for N is used as the primary consideration for manure application, P tends to be oversupplied and it accumulates in soils (Tisdale *et al.*, 1985).

The behaviour of P in amended soils is an important component for making nutrient management plans that maximize economic benefits and minimize environmental risks. Often, the tendency is to manage soil organic amendments based on the total P content. This approach would be justified if P in organic residues behaved similarly to that in inorganic fertilizers. However, it is known that P in organic residues is present in a number of forms (Sharpley, 1985).

Incubation experiments with organic and inorganic P inputs have shown that it is possible to study the transformation and distribution of added and native soil P into different pools after various incubation times (Hedley *et al.*, 1982; Adams and Odom, 1985; Sharpley and Moyer, 2000). P transformation in incubated soils depends on factors such as P forms and application rates, incubation time and temperature, and soil properties (McLaughlin and Champion, 1987; Adeli *et al.*, 2005).

P fractionation procedures that divide inorganic and organic P into fractions of various origins and availabilities (e.g. Hedley *et al.*, 1982) are powerful tools in appraising the fate of P added to soils. In the previous chapter, the technique was used on agricultural sites where the long-term history of land management was known. However, such fractionations can also be used in short-term incubation studies to investigate the fate of P added to soils from various sources. For example, Kashem et al. (2003) treated soils with organic amendments and fertilizer P ($NH_4H_2PO_4$) at 0 - 614 mg P kg^{-1} and found that cattle manure resulted in net immobilization of P, whereas biosolids led to net mineralization. They showed that P sorption in the inorganic P fertilizer-amended treatments increased $NaHCO_3$-extractable P at the expense of solution P. Chauhan et al. (1981) found that inorganic P fertilization combined with labile C sources stimulated Po accumulation in incubated soils. Sharpley and Sisak (1997) found that poultry litter leachate P was less available than KH_2PO_4 when incubated with 193 soils for 7 days. An incubation study on clay soils from South Africa showed that 30 - 60% and 80 - 90% of added P (KH_2PO_4) was transformed into less labile pools after 1 and 60 days, respectively (Ochwoh *et al.*, 2005).

P transformations in local soils amended with common organic amendments such as livestock manures and crop residues has received limited study. A measure of the forms of P in soil treated with different soil amendments, and how these forms change with time, could improve our understanding of the management of land applications of these amendments to agricultural soils. The objective of this study was to follow the transformations and distribution of added P in organic and inorganic sources after different incubation periods, by use of a sequential P extraction procedure.

4.2 Materials and methods

The soil used in this experiment was a Hutton form (Farmingham series) [Rhodic Ferralsol (FAO)] and the sample was obtained from a site at Cedara Agricultural Research Station. According to the Soil Classification Working Group (1991), this soil is sandy clay, with a clay content of about 62 %. It had the following properties: organic C = 72 g/kg, pH_{KCl} = 3.8, exchangeable Ca, Mg, and K of 2783, 391 and 411 ppm and AMBIC P = 53 ppm.

The organic P sources used in the study were maize residues (stem portions), poultry and dairy cattle manures. They were selected to represent common alternatives to inorganic P fertilizers, while varying widely in their physical and chemical characteristics. Maize residues were obtained from past trials in the Department of Agrometeorology (UKZN). Dairy cattle and poultry (layers) manure were obtained from the Department of Animal Science, also of the University of KwaZulu-Natal. Maize residues were oven-dried at 80 ^{o}C for 48 hours, whereas cattle and chicken manure were air-dried. Samples were ground (<0.5mm), digested in nitric and perchloric acids (HNO_3 and $HClO_4$), and the total P content was determined by the molybdenum blue method (Olsen and Sommers, 1982). Soluble inorganic P was extracted by the AMBIC method as applied to soil samples.

The incubation experiment consisted of 7 treatments: (i) control, (ii) dairy cattle manure (Cattle), (iii) poultry manure (Poultry), (iv) maize residues (Maize), (v) $Ca(OH)_2$ (Lime), (vi) KH_2PO_4 (PF), and (vii) $Ca(OH)_2$ plus KH_2PO_4 (Lime x PF).

To 100 g air-dried soil samples in 1L plastic containers (fitted with lids), the equivalent of 30 kg P ha^{-1} was added as KH_2PO_4 (12 mg P per 100 g soil) or organic sources, thoroughly mixed, and soils were watered to 70 % field capacity. Lime was added to soil as $Ca(OH)_2$ at 5 ton ha^{-1} (200mg $Ca(OH)_2$ per 100g soil) to the appropriate treatments. The soils were incubated for 8 weeks at room temperature (25°C). Each treatment combination was replicated 9 times in a completely randomized block design. At the end of each incubation period (1, 4 and 8 weeks), 3 replicates were removed and the soils air-dried, ground (<0.5mm) and stored for sequential P extraction (Tiessen and Moir, 1993). Soil analyses were carried out in duplicate. Details of the analytical methods used have been outlined previously (see section 3.2.2).

The data was analysed using analysis of variance (ANOVA) for a completely randomised block design in Genstat 8 software. Differences between means were compared by using the l.s.d. tests at 5% level of significance.

4.3 Results

4.3.1 Characteristics of organic amendments

Total P concentrations in the organic P sources ranged from a low of 1,450 mg P kg^{-1} for maize residues, to as high as 14,500 mg P kg^{-1} for poultry manure (Table 4.1). Extractable inorganic P as a percentage of total P was greatest for poultry manure (30%), followed by dairy manure (17%), and then maize residues (9%).

Table 4.1 P concentrations (mg P kg^{-1}) of animal manures and maize residues

Material	AMBIC extractable P	Total P
Poultry manure	4350 (30)[1]	14500
Cattle manure	892 (17)	5250
Maize residues	131 (9)	1450

[1] Values in parenthesis are percent contribution of each fraction to total extractable P (Total P)

4.3.2 Soil pH (KCl)

The measured pH for the control and the organic residue-amended soils was lower than the original pH value (Figure 4.1). As expected, addition of $Ca(OH)_2$ caused a rapid and significant ($P \leq 0.05$) increase in pH for the duration of the study (Figure 4.1). Incubation of soil for 1 week after the addition of $Ca(OH)_2$ increased soil pH from 3.6 (control) to 4.17 and 4.2.

The addition of KH_2PO_4 alone (PF) did not show the effect soil pH. Addition of organic residues at the rate applied in this study resulted in no significant effects on soil pH after 1 and 4 weeks of incubation but significantly ($P \leq 0.05$) increased it relative to the control after week 8 (Figure 4.1). There was a significant ($P \leq 0.05$) decrease in pH for all soils with increasing incubation time, particularly after 8 weeks of incubation (Figure 4.1). Averaged across the 8 weeks incubation (actual values in parentheses), soil pH increased in the order: PF (3.5) < Control (3.5) < Dairy (3.6) = Maize (3.6) = Poultry (3.6) < Lime x PF (4.1) = Lime (4.1).

4.3.3 Labile P (Resin-Pi, $NaHCO_3$-Pi, $NaHCO_3$-Po)

Differences between P fractions in soil treated with various soil amendments and sampled after 1, 4, and 8 weeks are presented in Figures 4.2 and 4.3 and Appendix 4.1. There was a significant ($P \leq 0.05$) treatment effect on resin-extractable P at all sampling dates. In comparison with the 1 week sampling, Resin Pi concentrations in the control decreased by 11 and 62% after 4 and 8 weeks, respectively. However, for the amended soils Resin Pi concentrations were higher after 4 than 1 week incubation. Concentrations at 8 weeks were markedly lower than those after 1 or 4 week incubation. In comparison with the control, incubating the soils for 1 week increased Resin Pi in the order: Maize < Poultry = Cattle < Lime x PF = PF, whereas Lime decreased it marginally (Figure 4.2a; Appendix 4.1). After 1 week, the calculated percent P recovery in the Resin Pi fraction was 33 and 42% for poultry and dairy cattle manures, respectively

(Appendix 4.2). The calculated percentage P recovery was 50% for the Lime x PF treatment and 58% for PF.

Treatment effects on the $NaHCO_3$-Pi fraction were only significant after 8 weeks. A dramatic decrease in $NaHCO_3$-Pi occurred in all soils including control between 1 and 4 weeks incubation. After 1 week, the amount of $NaHCO_3$-Pi varied from 75 to 95 mg P kg^{-1}, but it ranged between only 13 and 20 mg P kg^{-1} after 8 weeks incubation.

For control, Cattle, Maize, PF and Lime x PF, concentrations of $NaHCO_3$-Po (Figure 4.3a) increased progressively between 1 and 4 and 4 and 8 weeks incubation. However, for Poultry and Lime treatments, concentrations were similar after 1, 4, and 8 weeks incubation. Significant treatment effects on $NaHCO_3$-Po concentrations were only noted at the 1 week sampling time. At this time, concentrations followed the order: Poultry = Lime > Lime x PF > Cattle > Control = Maize.

Total labile P was significantly ($P \leq 0.05$) increased by all treatments, except Maize, after 1 week incubation (Appendix 4.1). After 4 weeks, PF and Lime x PF treatments had greater labile P concentrations than the others but after 8 weeks there were no significant treatments differences. Total percent contribution of labile P to total extractable P decreased with increasing incubation time. Labile P constituted 5 - 6% and 3% of total P after 1 and 8 weeks, respectively.

4.3.4 Moderately labile P (NaOH-Pi, NaOH-Po, dilute HCl-Pi, conc. HCl-Pi, conc. HCl-Po)

Significant treatment effects were noted for NaOH-Pi at all three sampling times (Figure 4.2c; Appendix 4.1). At 1 week, values for Poultry were greater than those for the other treatments. At 4 weeks, Maize and Lime x PF had values greater than control. At 8 weeks, the PF and Lime x PF treatments had greater values than maize. Values for NaOH-Pi were generally lower after 8 than 4 weeks incubation.

Analysis of variance showed that treatment effects for NaOH-Po were significant ($P \leq 0.05$) at all sampling dates (Figure 4.3b; Appendix 4.2). The actual contributions of NaOH-Po to total extractable P were large relative to other labile P fractions. NaOH-Po tended to be lower for the lime and Lime x PF treatments than the others at each sampling time (Figure 4.3b). On average, NaOH-Po accounted for 6 and 8% of the total P in Lime- and animal manure-amended soils, respectively.

Large concentrations of dilute HCl-extractable P (Ca-P) were found after 1 week, but these decreased sharply in all treatments with increasing incubation time, particularly between 4 and 8 weeks (Figure 4.2d). There were no significant treatment effects on dilute HCl-Pi (Appendix 4.2). Dilute HCl-Pi constituted the least percentage of total P (< 1%) in samples incubated for 8 weeks, with or without soil amendments.

Effects of soil amendments on concentrated HCl-extractable P were insignificant ($P \leq 0.05$). HCl-extractable P constituted a significant proportion of total extractable P, with more present as Pi than Po (Appendix 4.1). Both forms were greatly reduced by incubation relative to the original soil, which indicates fixation of P into these fractions.

4.3.5 Stable P pool (residual P)

The residual fraction was significantly increased by incubation relative to the original soil. It constituted the largest P fraction (more than 60% of total P) after incubation and increased steadily with increasing incubation time (Appendix 4.2).

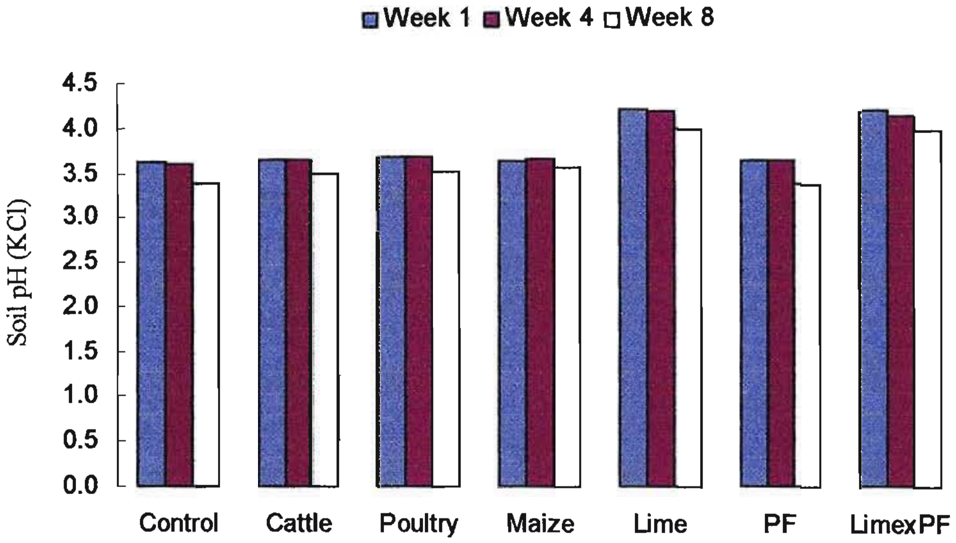


Figure 4.1 Soil pH following incubation (1-8 weeks) with no P addition (control) or with cattle manure (Cattle), or poultry manure (Poultry), or maize residues (Maize), or lime (Lime), or KH_2PO_4 (PF), or lime plus KH_2PO_4 (Lime x PF) applied at 12 mg P kg^{-1} soil.

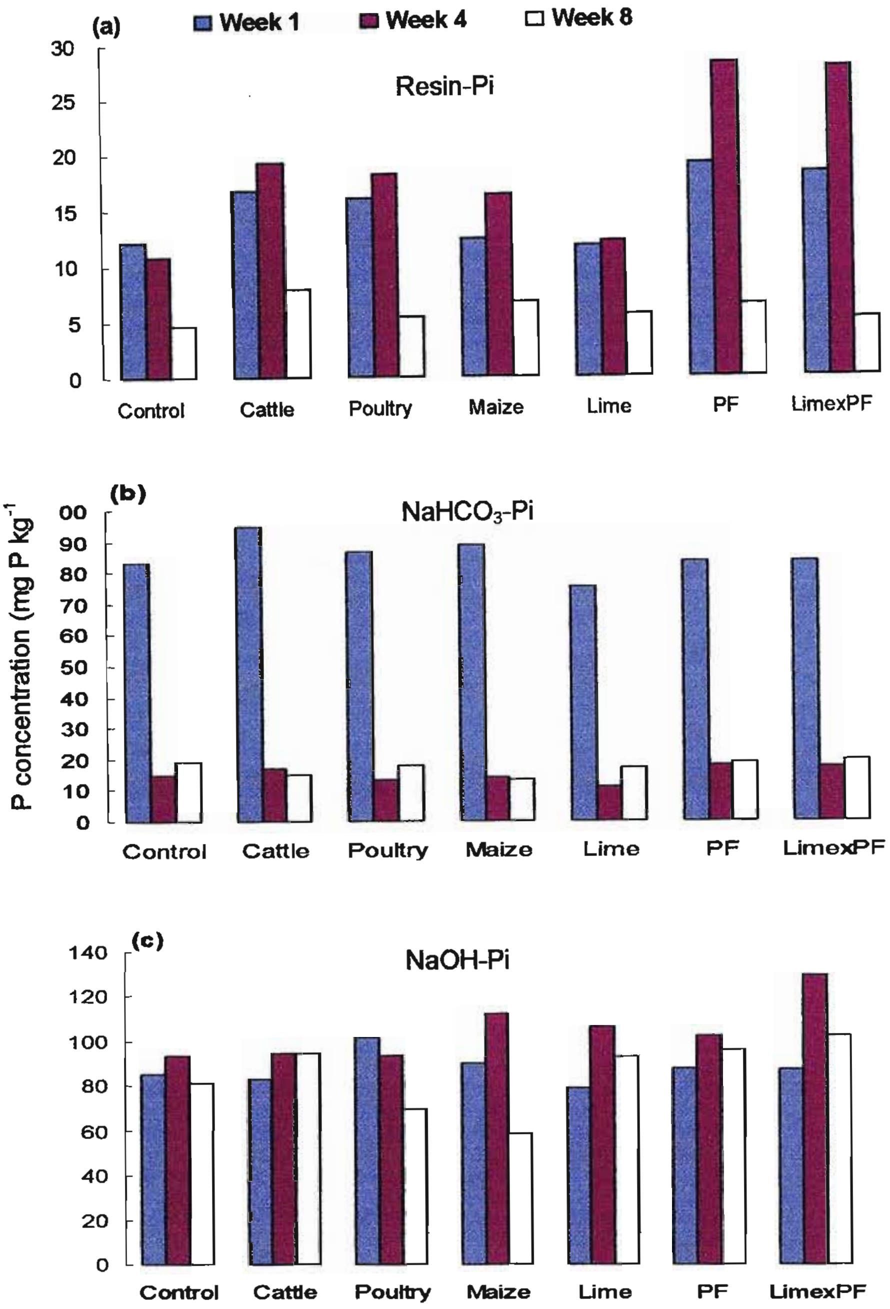


Figure 4.2 (a) Resin –Pi, (b) $NaHCO_3$-Pi, (c) NaOH-Pi, (d) dilute HCl-Pi, and (e) conc. HCl-Pi in soils following incubation (1-8 weeks) with no P addition (control) or with Cattle manure (Cattle), or poultry manure (Poultry), or maize residues (Maize), or lime (Lime), or lime plus KH_2PO_4 (Lime x PF), or KH_2PO_4 (PF) applied at 12 mg P kg^{-1} soil. *(continued)*

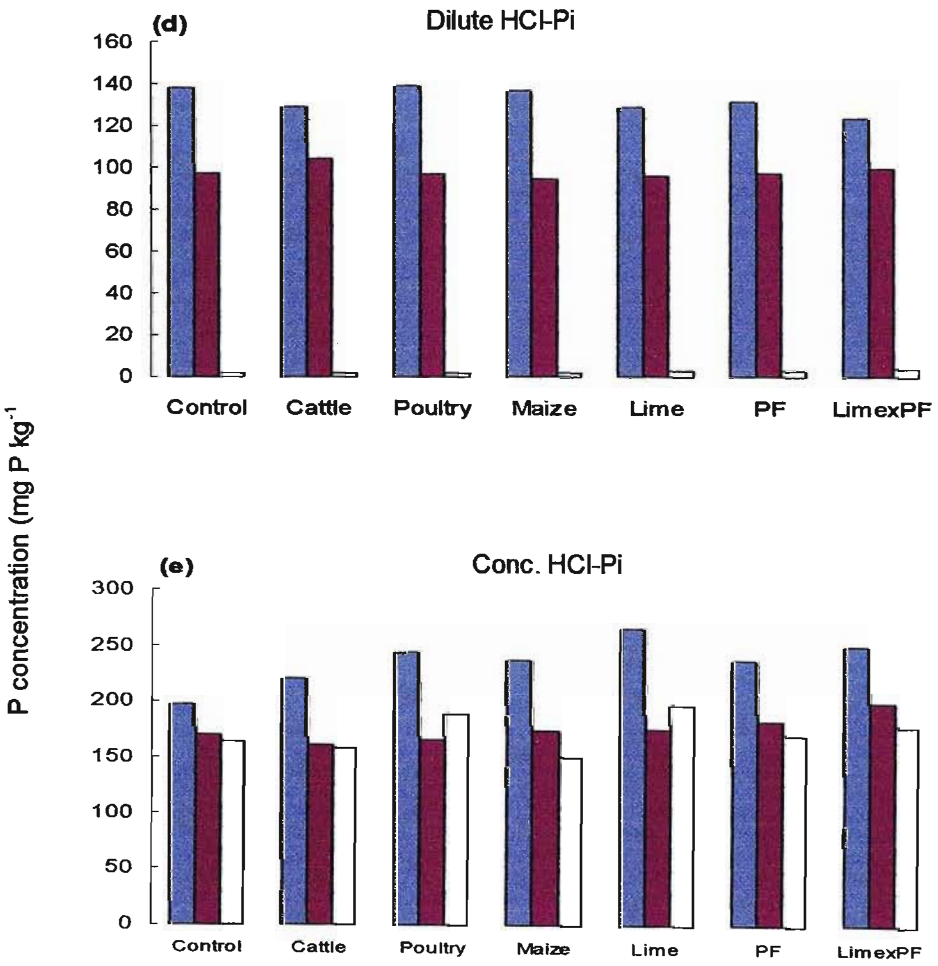


Figure 4.2 Continued.

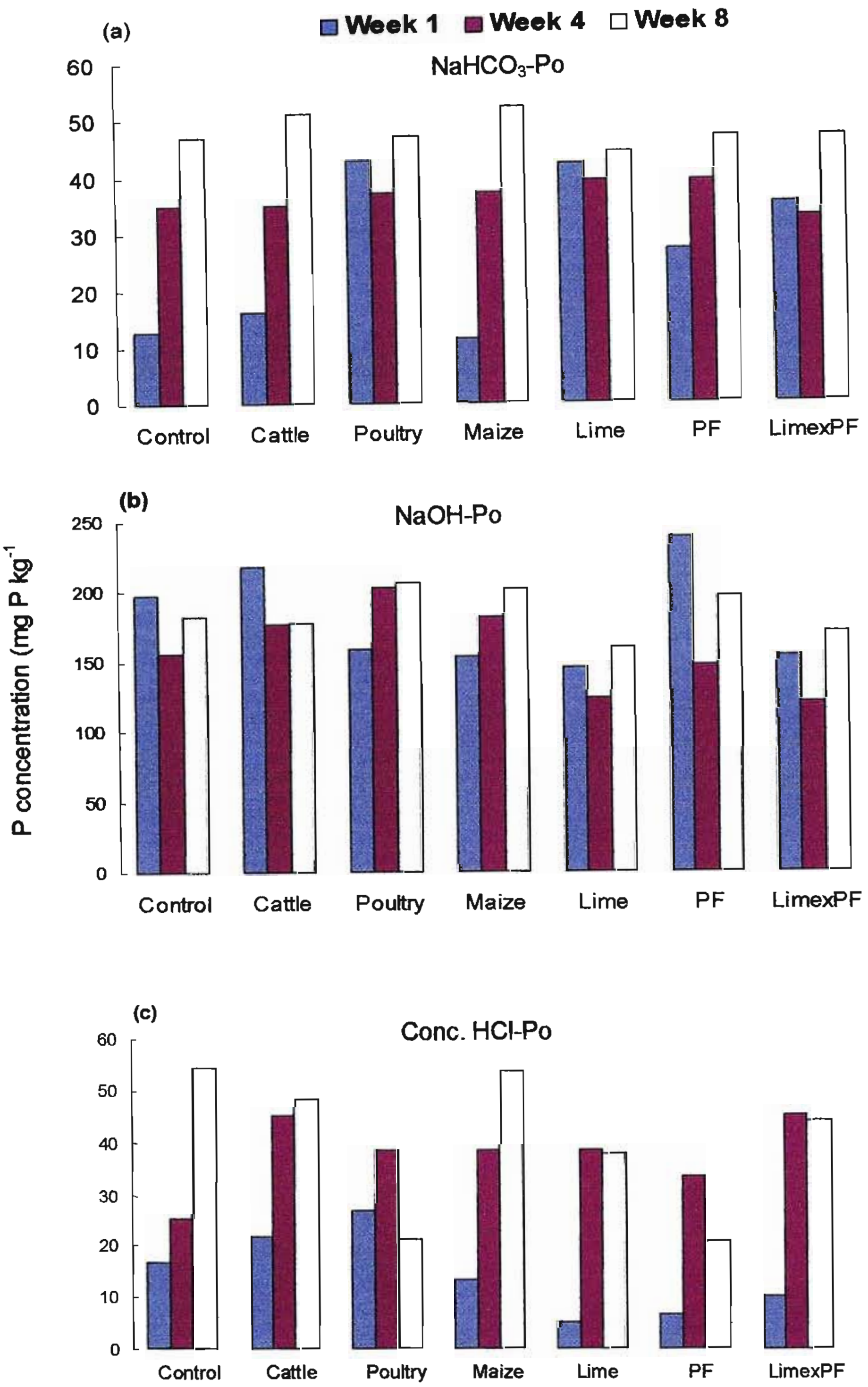


Figure 4.3 (a) $NaHCO_3$-Po, (b) NaOH-Po, and (c) conc. HCl-Po in soils following incubation (1-8 weeks) with no P addition (control), or with cattle manure, or poultry manure, or lime, or lime plus KH_2PO_4, or maize residues, or KH_2PO_4 applied at 12 mg P kg soil.

4.4 Discussion

4.4.1 Effects of soil amendments on soil pH

As expected, application of $Ca(OH)_2$ rapidly and significantly ($P \leq 0.05$) increased soil pH above that in the original soil (un-incubated), control and other treatments. The soil pH increase with lime incorporation involves liberation of OH^- ions, hydrolysis of H^+ and eventually, precipitation and replacement of Al^{3+} and Fe^{2+} ions from adsorption sites by exchangeable cations such as Ca^{2+} (Adams and Odom, 1985).

Addition of organic residues also resulted in small increases in pH above that of the control after 8 weeks incubation (Figure 4.1). The addition of the residues was based on their P content rather than liming potential. Nonetheless, several reports indicate increases in soil pH following application of animal manures and crop residues to soils (Iyamuremye *et al.*, 1996; Nwoke *et al.*, 2004). Several workers (Bessho and Bell, 1992; Noble *et al.*, 1996) have reported soil pH increases and concomitant decreases in exchangeable Al and Al saturation following plant residue incorporation. The decrease in exchangeable Al has been attributed not only to its precipitation due to elevated pH but also to adsorption of Al onto decomposing organic residues (Hoyt and Turner, 1975).

The tendency for pH to decline over the incubation period (Figure 4.1) is probably related to the formation of NO_3-N during the nitrification process (Hoyt and Turner, 1975; Adeli *et al.*, 2005). During nitrification, two H^+ ions are released per unit of NH_4^+ converted to NO_3^-. In addition, during organic matter decomposition, CO_2 and H^+ are produced, which also account for the decrease in soil pH at the end of the incubation (Cabrera *et al.*, 1993).

4.4.2 Effects of soil amendments on labile P

Anion exchange resins extract P from soil solution rather than dissolving soil surfaces or P-containing minerals (Tiessen and Moir, 1993). Resin extractable Pi in this study accounted for less than 2% of total P, indicating that the resins extracted a very small fraction of the total soil P pool. Higher Resin Pi concentrations have been reported for soils with less total extractable P (O'halloran *et al.*, 1987; Smith *et al.*, 2005). Most of the inorganic P in this soil is associated with Al and Fe oxides and apparently held strongly so it is not readily extractable with resin via the removal of P from the soil solution.

For amended soils, Resin-Pi increased significantly between 1 and 4 weeks, but then decreased significantly with further incubation time. Indeed, after 8 wk, labile Pi fractions and total labile P in all soils were reduced to levels below those observed at week 1. This trend has been confirmed by other workers who found that in acidic soils, P application initially increases extractable Pi but it later decreases (Kashem *et al.*, 2003; Ochwoh *et al.*, 2005). The reduction in available Pi over time is not surprising. As time of P contact with the soil increases, P becomes strongly sorbed to Fe and Al oxide surfaces through the formation of stable multidentate ligands (Sanyal and De Datta, 1991). Buehler et al. (2002) found that labelled P transformed from the Resin-Pi to $NaHCO_3$-Pi fraction with increasing time. The decreases in Resin-Pi and $NaHCO_3$-Pi can therefore be attributed to fixation of Pi into the residual pool and also microbial uptake of Pi and its conversion to labile Po forms. Indeed, both the residual P and the $NaHCO_3$-Po pools increased in magnitude during the incubation.

The magnitude of the increases in labile P in amended soils after 1 and 4 weeks incubation (Appendix 4.2) reflects the effectiveness of P sources in furnishing readily soluble P (Sharpley *et al.*, 1984). Application of cattle and poultry manures significantly ($P \leq 0.05$) increased Resin Pi after 1 and 4 weeks, whereas maize residues had no significant effect on Resin Pi at any stage (Appendix 4.1). In addition, the differences between organic amendments on Resin Pi were greatest after I week incubation. These results are probably due to the differing properties of the residues. Table 4.1 shows that 17 and 30% of total P was in extractable inorganic form in cattle and poultry manures, respectively. In contrast, 9% of total P in maize residues was inorganic. The addition of more Pi in the animal manures would have favoured larger increases in Resin Pi.

Gagnon and Simard (1999) found that materials with high P and low C:P ratios released larger amounts of P. Poultry manure generally has low C:P (25) and C:N (11) ratios (Adeli *et al.*, 2005). Nwoke et al. (2004) determined that maize residues had C:P and C:N ratios of 396 and 25, respectively. This was in contrast to a C:P and C:N ratios of 93 and 22, respectively, for farmyard manure. The critical C:P ratio above which there is P immobilization by soil microorganisms is about 300, whereas P mineralization takes place if the ratio is 200 or less (Tisdale *et al.*, 1985). Thus, slower decomposition of maize residues due to relatively higher C:N ratio and immobilization of P due to the high C:P ratio may explain the insignificant effects of maize residues on Resin Pi at all sampling times. By contrast, it is likely that dairy cattle manure and poultry manure stimulated net P mineralization due to their relatively lower C:P ratios.

As already noted, the increases in $NaHCO_3$-Po with increasing incubation time (Appendix 4.1), were probably due to microbial uptake of P mobilized from labile Pi fractions can perhaps also from the less accessible fractions, such as the dilute HCl-extractable Pi (Ca-P), which decreased more than all the fractions after 8 wk of incubation (Figure 4.2d). Most reports indicate that $NaHCO_3$-Po fraction contains P of microbial origin and is labile (Hedley *et al.*, 1982; Tiessen *et al.*, 1993; Schmidt *et al.*, 1996). This immobilization of Pi into labile Po fractions can be very important for increasing P availability, particularly in high P fixing soils (Hedley *et al.*, 1982). Chauhan et al. (1981) effectively demonstrated a positive relationship between solution Pi concentration and microbial P uptake. They also found that inorganic P fertilization combined with labile carbon sources such as cellulose, stimulated Po build-up under laboratory conditions.

The PF and Lime x PF treatments caused greater increases in Resin Pi than did the organic amendments (Appendix 4.1). In addition, application of Cattle, Poultry and Maize generally resulted in greater accumulation of P as total Po than the Lime, PF, and Lime x PF treatments (Appendix 4.1). These observations support the findings of Tran and N'dayegamiye (1995) that mineral P fertilizer additions significantly increase labile P, but with more as Pi than Po.

The benefits of liming on P availability are clearly illustrated by the substantial increases in Resin-Pi above the control in the Lime treatment after 4 weeks incubation (Figure 4.2a). This effect was, however short-lived and not evident after 8 wk. The effect is most likely due to an increased pH decreasing adsorption and promoting desorption of orthophosphate. Increased soil pH causes the charge on the adsorbing surfaces of Fe and Al oxides to become more negative thus favouring desorption of the negatively charged orthophosphate anion (McLaren and Cameron, 1990). There is also the possibility of liming inducing increases in soil microbial activity with a resultant increase in mineralization of Po (Neale *et al.*, 1997). Although there were no discernable decreases in $NaHCO_3$-Po induced by liming, there were notable decreases in NaOH-Po with limed treatments having the lowest values for this fraction at each sampling time (Figure 4.3b).

4.4.3 Effects of soil amendments on moderately labile P

The NaOH-Pi fraction was not greatly affected by treatments although it was surprisingly low in the Poultry and Maize treatments after 8 weeks incubation. The reason for this notable decline in these two treatments is not clear. Nonetheless, there was a tendency for NaOH-Pi to decrease between 4 and 8 weeks incubation in most of the treatments (Figure 4.2c). These results could suggest dissolution/ desorption of P bound to sesquioxides and its enrichment into the residual pool. The NaOH-Po fraction was not greatly affected by treatment except that the Lime, and to a lesser extent Lime x PF treatments tended to have lower concentrations than the others. As already noted, this could well be attributable to lime-induced mineralization of Po.

The high contents of dilute (0.1M) HCl-extractable Pi (Ca-P) in all soils after 1 week increase the probability of Ca phosphates in the soil because this extracting solution mainly removes Ca-bound P (Ochwoh *et al.*, 2005). Significant amounts of Ca (2783 ppm; Table 3.1) in this soil increase probability of Ca-phosphates precipitation. However, Ca-phosphates in acidic soils are not stable (Lindsay, 1979; Pierzynski *et al.*, 1990), which may account for the dissolution of P in this fraction due to incubation (Figure 4.2d). The initial concentrations of dilute HCl-Pi could also be at least partially an error of the fractionation technique. In the sequential fractionation procedure, dilute HCl extraction follows NaOH extraction (Tiessen and Moir, 1993). Due to high pH conditions that occur NaOH extraction, PO_4^{3-} released from Al and Fe minerals can resorb to, or precipitate as calcium minerals, resulting in an underestimation of NaOH-extractable P and overestimation of Ca-P (Benzing and Richardson, 2005). Thus, some of the P initially associated with Al and Fe minerals may have been misidentified as Ca-P. To prevent this problem, use of selective chelating agents (e.g. 0.02 M Ca-NTA with dithionate for extraction of Al- and Fe-P followed by 0.05M EDTA to extract Ca-P) has been suggested (De Groot and Golterman, 1990: *cited in* Bezing and Richardson, 2005).

Concentrated HCl-P represents more recalcitrant Pi and Po forms (Guo and Yost, 1998). An increase in these fractions during incubation would therefore, represent a further loss of P availability. In fact, whilst concentrations of conc. HCl-Po increased over the incubation period, concentrations of conc. HCl-Pi tended to decrease particularly between 1 and 4 weeks incubation. Thus, there was no progressive accumulation of Pi in this pool; in fact, it acted as a source of P for other pools.

4.4.4 Effects of soil amendments on stable P (residual P)

The residual-P fraction was the largest fraction in all incubated soils and increased over the incubation period (Appendix 4.1). Previous reports have indicated that residual-P constitutes both inorganic and organic P (Condron and Goh, 1989; Agbenin and Goladi, 1998). The increase in the residual-P with increasing incubation time in both the control and treated soils can be related to precipitation of insoluble P compounds, reactions of adsorbed P whereby it becomes bound to soil components and immobilization of P into organic forms that are not readily extractable with resin, $NaHCO_3$, NaOH, or HCl.

4.5 Conclusions

In view of the large differences in easily-soluble Pi content between the various P sources, treatment effects on soil P fractions were in general, surprisingly small. The main effect was mainly on the potentially-available Resin-Pi fraction which, as expected, tended to be greater for the inorganic P source than for the organic ones. The declines in Pi fractions during incubation (Resin-Pi between 4 and 8 weeks, $NaHCO_3$-Pi between 1 and 4 weeks and dilute HCl-Pi between 1 and 4 and 4 and 8 weeks) was accompanied by progressive increases in the sizes of the $NaHCO_3$-Po and concentrated HCl-Po fractions. This suggests progressive microbial immobilization of Pi during incubation. An exception was the limed treatments which tended to have low NaOH-Po concentrations suggesting lime-induced net mineralization of Po. The progressive increase in residual P during incubation may also reflect progressive conversion of extractable, adsorbed Pi in recalcitrant, non-extractable, inorganic forms over time.

Overall, the results of this study in terms of the availability of P from various sources were rather inconclusive. Further, studies should grow plants so that the availability can be gauged more decisively via plant uptake.

CHAPTER 5

GENERAL CONCLUSIONS

Conversion of native grassland ecosystems to long-term maize and sugarcane monoculture resulted in significant decline in soil organic matter (SOM). Soil cultivation enhances organic matter loss because it increases soil microbial activity through aeration and exposure of physically protected soil organic C. In contrast, permanent pasture and forestry systems led to accumulation of SOM. Thus, pasture and, to a lesser extent, forestry could form an integral part of management strategies for improving organic matter contents in soil. In particular, the use of pastures in rotation with crops would be an appropriate way of maintaining organic matter under cropping. Nonetheless, as shown here, annual pastures maintain rather than counterbalance the effect of grass in increasing organic matter inputs. Thus, pastures of 3 or 4 years duration in rotation with annual arable crops might be a more appropriate option.

Soil microbial properties were affected by both land use and management practice. In agreement with other reports, permanent kikuyu pasture, forestry and native grassland ecosystem had a favourable influence on soil microbial biomass C and P. The relatively higher microbial biomass in these fields compared to maize fields could be ascribed mainly to high organic matter levels (i.e. more organic substrate for microbial growth). Soil disturbance with an attendent loss of organic matter and possibly also the persistent use of agrochemicals contributed to lower microbial biomass under conventionally-tilled arable systems. The larger substrate and nutrient inputs in the surface layer of soil under zero tillage due to surface retention of crop and weed residues will have contributed to higher microbial biomass relative to conventionally-tilled soil.

Decades of mineral P fertilization of the agricultural soils increased total extractable P in the surface horizon (0-5 cm). A change in the distribution and amounts of P within inorganic (Pi) and organic P (Po) pools also occurred due conversion to agricultural uses. The quantity of Po followed a similar pattern to the organic C contents, confirming findings from previous work that the transformation of Po and organic C are linked. Fifty percent, or more, of the P in undisturbed soils was in organic form. Such results reflect the fact that within native ecosystems, the labile P pool is maintained primarily by the microbially-mediated mineralization of organic P. In agricultural systems, the release of organically bound P would be strongly influenced by factors such as residue management and tillage methods because they influence organic matter accumulation and decomposition. Total extractable Po increased under permanent kikuyu pasture and some forestry. Thus, cycling of P through the organic fractions may well have a substantial influence on P availability in these systems. By contrast, under arable systems much of the readily-mineralizable organic P has already been lost so P supply is presumably more dependent on adsorption/desorption of Pi and fertilizer Pi inputs. Indeed, comparison of soils under long-term maize monoculture to undisturbed soils shows an overall increase in total extractable Pi (Pi) compared to a decrease in the total extractable organic P (Po). This shows that successive applications of P fertilizer to these tilled soils accumulate mainly in inorganic forms while at the same time the Po fraction is being diminished. The results for arable soils also indicate a larger total extractable Po pool under maize (ZT) compared to maize (CT), indicating that soil P dynamics differ according to cropping practices. Accumulation of organic matter in the surface layer of soil under ZT results in an accumulation of Po in the soil. Much of this Po presumably arises from immobilization of fertilizer P during organic matter accumulation.

It is often reported that the NaOH-Po is the main sink and source of P in deeply weathered soils. The largest P fractions in both the fertilized soils and native grasslands are the NaOH-P, concentrated HCl-P and residual P. It appears that these fractions are the main sinks of added P in this soil. Al and Fe hydrous oxides, common in tropical and subtropical soils, bind the applied and native P, rendering it unavailable for plant uptake even though total levels may be high. Inorganic P fertilization of arable-cropped soils increased Pi fractions associated with Al and Fe oxide surfaces (i.e. $NaHCO_3$-Pi and NaOH-Pi). Not surprisingly the dilute HCl-Pi, which represents P associated with Ca minerals, constituted a small proportion of total P in most soils, with exception of the kikuyu pasture and pine forest sites.

The incubation experiment showed that applications of P from various organic and inorganic sources can have significant effects on P fractions in the short-term. In particular, the addition of organic and inorganic P sources increased soil levels of total P as would be expected. Incubation resulted in a change in dominance of organic P in original to inorganic P in incubated soils. Application of KH_2PO_4 at the rate of 30 kg P ha^{-1} and $Ca(OH)_2$ at 5 ton ha^{-1} rapidly increased labile P than animal manures and crop residues. These results are due to high solubility of KH_2PO_4 and the fact that residues have to first decompose before P is released. However, in the long-term, P sorption may decrease the availability of P derived from commercial fertilizers more than that of organic residue-derived P. This is because residue P is released more slowly so that a growing crop can potentially use it more effectively and also organic anions derived from the residues can become adsorbed to oxide surfaces thus blocking P adsorption sites. Thus, short-term incubation studies, such as that performed here, need to be followed up by long-term field investigations.

It will be important to extend this research and investigate the effects of the various land uses and management practices on P forms at deeper soil layers. Further, the impacts of soil amendments on P fractions can be evaluated under field conditions, in the presence of crops. Although increases in soil P fractions were noted in the incubation experiment, it was not certain whether the increases occurred due to transformation of native P between pools or whether they resulted from applied P. Isotopic labelling of added P has been applied to great effect in tracing the pathways of P in soils and this could be a fruitful area where this research could be extended in the future. The use of isotopic tracers in association with sequential fractionation procedures would enable the pathways of movement of applied P (from inorganic or organic sources) to be followed in detail both within and between Pi and Po fractions.

APPENDICES

Appendix 4.1 Concentration of soil P fractions (mg P/kg soil) in soil with various amendments after 1, 4, 8 weeks of incubation

Treatments	Resin Pi	$NaHCO_3$ -Pi	$NaHCO_3$-Po	NaOH-Pi	NaOH-Po	0.1M HCl-Pi	Conc. HCl -Pi	Conc. HCl –Po	Residual –P	[1]Labile P	[2]Total Po	[3]Total Pi	Total P (sum)
Week 1													
Control	12c	83ab	13c	86b	198ab	138	197b	17ab	1538	108d	227ab	470b	2280
Cattle	17a	95a	16c	83b	218a	129	220ab	22a	1490	127bc	256a	543ab	2289
Poultry	16ab	87ab	43a	102a	159bc	139	243ab	27a	1473	145a	228ab	586a	2287
Maize	12bc	89ab	11c	90ab	154bc	136	237ab	13bc	1545	112cd	179b	563ab	2287
Lime	11c	75b	42a	79b	145c	128	265a	5c	1531	129abc	192b	559ab	2282
PF	19a	83ab	27b	88ab	238a	131	237ab	7c	1457	129abc	272a	557ab	2286
Lime x PF	18a	83ab	35ab	87b	154bc	123	250ab	10bc	1527	136ab	199b	561ab	2287
LSD ($P \leq 0.05$)	4	15	8	14	45	NS	55	8	NS	17	50	93	NS
CV (%)	14	10	17	9	14	6	13	30	8	8	13	7	7
Week 4													
Control	11c	15a	35	94c	156abc	97	170bc	25c	1681	61c	216	387c	2283
Cattle	19b	17ab	35	95bc	177ab	104	160c	45a	1638	71bc	257	396abc	2290
Poultry	18bc	13ab	37	94c	203a	97	165bc	38ab	1627	68bc	278	386bc	2291
Maize	16bc	14ab	37	112ab	181a	94	173bc	38ab	1623	67bc	256	410abc	2289
Lime	12bc	11b	39	107bc	124bc	95	175bc	38ab	1667	74ab	201	400abc	2280
PF	28a	18a	39	103bc	147abc	97	182ab	33b	1643	85a	219	426abc	2288
Lime x PF	28a	18a	33	129a	120c	99	198a	45a	1621	78ab	198	472a	2290
LSD ($P \leq 0.05$)	7	6	NS	18	56	NS	19	8	NS	11	NS	82	NS
CV (%)	17	17	16	10	20	8	13	47	5	8	17	17	5
Week 8													
Control	5b	19a	47	82abc	183abcd	2	164	54	1727	71	284	271	2282
Cattle	8a	15bc	51	95ab	178bcd	2	158	48	1735	74	277	278	2290
Poultry	5b	18ab	47	70bc	207a	2	189	21	1731	70	275	283	2289
Maize	7ab	13c	52	59c	202ab	2	150	53	1749	72	307	230	2286
Lime	6ab	17ab	44	93ab	159d	3	197	38	1726	67	241	316	2283
PF	6ab	19a	47	96a	196ac	3	169	21	1731	71	263	293	2287
Lime x PF	5b	20a	47	102a	170cd	4	178	44	1720	71	260	309	2289
LSD ($P \leq 0.05$)	3	3	NS	26	28	NS	NS	NS	NS	NS	NS	NS	NS
CV (%)	24	11	17	18	9	13	18	29	17	13	12	14	8

[1] Labile P = resin P + $NaHCO_3$-Pi + $NaHCO_3$-Po

[2] Total organic P = $NaHCO_3$-Po + NaOH-Po + conc. HCl -Po

[3] Total inorganic P= resin P + $NaHCO_3$-Pi + NaOH-Pi + dilute HCl -Pi + conc. HCl -Pi

Means followed by the same letter in a column at the same incubation time are not significantly different at $P \leq 0.05$ level, NS: no significant difference ($P \leq 0.05$)

Appendix 4.2 Calculated P recovery (%) in P fractions for soil treated with various amendments and incubated for different times

Treatments	Resin P	NaHCO$_3$-Pi	NaHCO$_3$-Po	NaOH-P$_i$	NaOH-P$_o$	Dilute HCl-Pi	Conc. HCl -Pi	Conc. HCl -P$_o$	Residual –P
Week 1									
Cattle	42	12	25	-25	167	-75	192	42	-400
Poultry	33	4	250	133	-325	8	383	83	-542
Maize	0	6	-17	33	-367	-17	333	-33	58
Lime	-8	-8	242	-58	-442	-83	567	-100	-58
PF	58	0	117	17	333	-58	333	-83	-675
Lime x PF	50	0	183	8	-367	-125	442	-58	-92
Week 4									
Cattle	67	2	0	8	175	58	-83	167	-358
Poultry	58	-2	17	0	392	0	-42	108	-450
Maize	42	-1	17	150	208	-25	25	108	-483
Lime	108	-4	33	108	-267	-17	42	108	-117
PF	142	3	33	75	-75	0	100	67	-317
Lime x PF	142	3	-17	292	-300	17	233	167	-500
Week 8									
Cattle	25	-4	33	108	-42	0	-50	-50	67
Poultry	0	-1	0	-100	200	0	208	-275	33
Maize	17	-6	42	-192	158	0	-117	-8	183
Lime	8	-2	-25	92	-200	8	275	-133	-8
PF	8	0	0	117	108	8	42	-275	33
Lime x PF	0	1	0	167	-108	17	117	-83	-58

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