CHEMICAL NATURE AND PLANT AVAILABILITY OF PHOSPHORUS PRESENT IN SOILS UNDER LONG-TERM FERTILISED IRRIGATED PASTURES IN CANTERBURY, NEW ZEALAND.

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Soil P fractionation was used to examine changes in soil inorganic and organic P under grazed irrigated pasture in a long-term field trial at Winchmore in Mid-Canterbury. The soil P fractionation scheme used involved sequential extractions of soil with 0.5M $NaHCO_3$ @ pH 8.5 ($NaHCO_3$ P), 0.1M NaOH (NaOH I P), 1M HCl (HCl P) and 0.1M NaOH (NaOH II P). The Winchmore trial comprised 5 treatments: control (no P since 1952), 376R (376 kg superphosphate ha^{-1} yr^{-1} 1952-1957, none since), 564R (564 kg superphosphate ha^{-1} yr^{-1} 1952-1957, none since) 188PA (188 kg superphosphate ha^{-1} yr^{-1} since 1952) and 376PA (376 kg superphosphate ha^{-1} yr^{-1} since 1952 Topsoil (0-7.5cm) samples taken from the different treatments in 1958, 1961, 1965, 1968, 1971, 1974 and 1977 were used in this study.

Changes in soil P with time showed that significant increases in soil inorganic P occurred in the annually fertilised treatments (188PA, 376PA).

As expected, the overall increase in total soil inorganic P between 1958 and 1977 was greater in the 376PA treatment (159 μg P g^{-1}) than that in the 188PA treatment (37 μg P g^{-1}). However, the chemical forms of inorganic P which accumulated in the annually fertilised treatments changed with time. Between 1958 and 1971 most of the increases in soil inorganic P in these treatments occurred in the $NaHCO_3$ and NaOH I P fractions. On the other hand, increases in soil inorganic P in the annually fertilised treatments between 1971 and 1977 were found mainly in the HCl and NaOH II P fractions. These changes in soil P forms were attributed to the combined effects of lime addition in 1972 and increased amounts of sparingly soluble apatite P and iron-aluminium P in the single superphosphate applied during the 1970's.

In the residual fertiliser treatments (376R, 564R) significant decreases in all of the soil inorganic P fractions (i.e. $NaHCO_3$ P, NaOH I P, HCl P, NaOH II P) occurred between 1958 and 1977 following the cessation of P fertiliser inputs in 1957. This was attributed to continued plant uptake of P accumulated in the soil from earlier P fertiliser additions. However, levels of inorganic P in the different soil P fractions in the residual fertiliser treatments did not decline to those in the control which indicated that some of the inorganic P accumulated in the soil from P fertiliser applied between 1952 and 1957 was present in very stable forms.

In all treatments, significant increases in soil organic P occurred between 1958 and 1971. The overall increases in total soil organic P were greater in the annually fertilised treatments (70-86 μg P g^{-1}) than those in the residual fertiliser (55-64 μg P g^{-1}) and control (34 μg P g^{-1}) treatments which reflected the respective levels of pasture production in the different treatments. These increases in soil organic P were attributed to the biological conversion of native and fertiliser inorganic P to organic P in the soil via plant, animal and microbial residues. The results also showed that annual rates of soil organic P accumulation between 1958 and 1971 decreased with time which indicated that steady-state conditions with regard to net organic P accumulation were being reached.

In the residual fertiliser treatments, soil organic P continued to increase between 1958 and 1971 while levels of soil inorganic P and pasture production declined. This indicated that organic P which accumulated in soil from P fertiliser additions was more stable and less available to plants than inorganic forms of soil P.

Between 1971 and 1974 small (10-38 μg P g^{-1}) but significant decreases in total soil organic P occurred in all treatments. This was attributed to increased mineralisation of soil organic P as a result of lime (4 t ha^{-1}) applied to the trial in 1972 and also to the observed cessation of further net soil organic P accumulation after 1971. Liming also

appeared to affect the chemical nature of soil organic P as shown by the large decreases in NaOH I organic P (78-88 μg P g^{-1}) and concomitant smaller increases in NaOH II organic P (53-65 μg P g^{-1}) which occurred in all treatments between 1971 and 1974.

The chemical nature of soil organic P in the Winchmore long-term trial was also investigated using ^{31}P nuclear magnetic resonance (NMR) spectroscopy and gel filtration chromatography. This involved quantitative extraction of organic P from the soil by sequential extraction with 0.1M NaOH, 0.2M aqueous acetylacetone (pH 8.3) and 0.5M NaOH following which the extracts were concentrated by ultrafiltration. Soils (0-7.5cm) taken from the control and 376PA annually fertilised treatments in 1958, 1971 and 1983 were used in this study.

^{31}P NMR analysis showed that most (88-94%) of the organic P in the Winchmore soils was present as orthophosphate monoester P while the remainder was found as orthophosphate diester and pyrophosphate P. Orthophosphate monoester P also made up almost all of the soil organic P which accumulated in the 376PA treatment between 1958 and 1971. This indicated that soil organic P in the 376PA and control treatments was very stable.

The gel filtration studies using Sephadex G-100 showed that most (61-83%) of the soil organic P in the control and 376PA treatments was present in the low molecular weight forms (<100,000 MW), although the proportion of soil organic P in high molecular weight

forms (>100,000 MW) increased from 17-19% in 1958 to 38-39% in 1983. The latter was attributed to the microbial humification of organic P and indicated a shift toward more complex and possibly more stable forms of organic P in the soil with time.

Assuming that the difference in soil organic P between the control and 376PA soils sampled in 1971 and 1983 represented the organic P derived from P fertiliser additions, results showed that this soil organic P was evenly distributed between the high and low molecular weight fractions.

An exhaustive pot trial was used to examine the relative availability to plants of different forms of soil inorganic and organic P in long-term fertilised pasture soils. This involved growing 3 successive crops of perennial ryegrass (Lolium perenne) in 3 Lismore silt loam (Udic Ustochrept) soils which had received different amounts of P fertiliser for many years. Two of the soils were taken from the annually fertilised treatments in the Winchmore long-term trial (188PA, 376PA) and the third (Fairton) was taken from a pasture which had been irrigated with meatworks effluent for over 80 years (65 kg P ha^{-1} yr^{-1}). Each soil was subjected to 3 treatments, namely control, (no nutrients added), N100 and N200. The latter treatments involved adding complete nutrient solutions with different quantities of N at rates of 100kg N ha^{-1} (N100) and 200kg N ha^{-1} (N200) on an area basis. The soil P fractionation scheme used was the same as that

used in the Winchmore long-term trial study (i.e. $NaHCO_3$ P, NaOH I P, HCl P, NaOH II P).

Results obtained showed that the availability to plants of different extracted inorganic P fractions, as measured by decreases in P fractions before and after 3 successive crops, followed the order: $NaHCO_3$ P > NaOH I P > HCl P = NaOH II P. Overall decreases in the $NaHCO_3$ and NaOH I inorganic P fractions were 34% and 16% respectively, while corresponding decreases in the HCl and NaOH II inorganic P fractions were small (<10%) and not significant. However, a significant decrease in HCl P (16%) was observed in one soil (Fairton-N200 treatment) which was attributed to the significant decrease in soil pH (from 6.2 to 5.1) which occurred after successive cropping.

Successive cropping had little or no effect on the levels of P in the different soil organic P fractions. This indicated that net soil organic P mineralisation did not contribute significantly to plant P uptake over the short-term.

A short-term field experiment was also conducted to examine the effects of different soil management practices on the availability of different forms of P to plants in the long-term fertilised pasture soils. The trial was sited on selected plots of the existing annually fertilised treatments in the Winchmore long-term trial (188PA, 376PA) and comprised 5 treatments: control, 2 rates of lime (2 and 4 t ha^{-1}), urea fertiliser (400kg N ha^{-1}) and mechanical cultivation.

The above ground herbage in the uncultivated treatments was harvested on 11 occasions over a 2 year period and at each harvest topsoil (0-7.5cm) samples were taken from all of the treatments for P analysis. The soil P fractionation scheme used in this particular trial involved sequential extractions with 0.5M $NaHCO_3$ @ pH 8.5 ($NaHCO_3$ P), 0.1M NaOH (NaOH P), ultrasonification with 0.1M NaOH (sonicate-NaOH P) and 1M HCl (HCl P). In addition, amounts of microbial P in the soils were determined.

The results showed that liming resulted in small (10-21 μg P g^{-1}) though significant decreases in the NaOH soil organic P fraction in the 188PA and 376PA plots. Levels of soil microbial P were also found to be greater in the limed treatments compared with those in the controls. These results indicated that liming increased the microbial mineralisation of soil organic P in the Winchmore soils. However, pasture dry matter yields and P uptake were not significantly affected.

Although urea significantly increased dry matter yields and P uptake, it did not appear to significantly affect amounts of P in the different soil P fractions. Mechanical cultivation and the subsequent fallow period (18 months) resulted in significant increases in amounts of P in the $NaHCO_3$ and NaOH inorganic P fractions. This was attributed to P released from the microbial decomposition of plant residues, although the absence of plants

significantly reduced levels of microbial P in the cultivated soils.

Practical implications of the results obtained in the present study were presented and discussed.

KEYWORDS: soil inorganic P; soil organic P; P immobilisation; P mineralisation; soil P fractionation; single superphosphate; residual fertiliser P; Lime-P interactions; soil organic P molecular weight; ^{31}P NMR; perennial ryegrass; plant P uptake; soil pH; urea fertiliser; cultivation.

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CHAPTER 1

INTRODUCTION

The use of phosphatic fertilisers remains an important factor in the continued development and sustained high productivity of pastoral farming in New Zealand. Many New Zealand soils are low in native phosphorus and require additions of phosphatic fertiliser to enable the establishment and maintenance of productive pasture (During, 1984). Currently, around 1.5 million tonnes of phosphatic fertiliser (mainly single superphosphate) are applied to pastures in New Zealand annually at a total cost of approximately $250 million (New Zealand Fertiliser Statistics, 1984). In many areas of New Zealand, pasture growth is also restricted by inadequate or unevenly distributed rainfall such that drought conditions often prevail during summer months. The problem of summer drought can be overcome by irrigation which, in turn, increases pasture production and consequent phosphatic fertiliser requirements.

In a grazed pasture, only a small proportion of applied fertiliser phosphorus (P) is removed from the soil in the growing season following application. For example, in an irrigated pasture grazed by sheep, Quin and Rickard (1979) estimated that only 10-21% of the fertiliser P applied annually was lost from the soil by stock transfer and in off-farm produce.

Most of the applied fertiliser P remains in the soil as inorganic and organic forms of P. Thus, soluble fertiliser P is converted to various sparingly soluble forms of soil inorganic P by reaction with the soil mineral components (Sample et al., 1980). In addition, some of the applied P is converted to soil organic P via plant, animal and microbial residues (Dalal, 1977). Several studies in Australia and New Zealand have shown that a significant proportion (20-100%) of the fertiliser P applied following the establishment of legume-based pasture in P deficient soil is converted to soil organic P (Donald and Williams, 1954; Walker et al., 1954; Jackman, 1955; Rixon, 1966; Quin and Rickard, 1979).

The conversion of applied fertiliser P to soil inorganic and organic P reduces its immediate plant availability and therefore continued applications of P fertiliser are often required to maintain a given level of pasture production (Barrow, 1980). Thus, considerable accumulations of inorganic and organic P may occur in pasture soils which receive P fertiliser regularly for many years.

While several overseas studies have examined the agronomic effectiveness of residual fertiliser P in soils (e.g. Leamer, 1963; Bowman et al., 1978; Novias and Kamprath, 1978; Spratt et al., 1980), similar kind of studies has not been conducted extensively in New Zealand. Furthermore, the chemical nature of residual fertiliser P in soil and

the factors which may affect its utilisation by plants have not been examined in detail. In particular, the chemical nature of soil organic P remains largely unknown. In most soils studied, less than ½ of the total organic P has been identified as known P compounds (Dalal, 1977; Anderson, 1980).

The main objectives of the present study were:

(1) To develop and adapt a detailed P fractionation scheme to separate and identify different forms of inorganic and organic P in soils.

(2) To use the above P fractionation scheme for examining the amounts and relative availability to plants of different forms of inorganic and organic P which accumulated in irrigated pasture soils from long-term P fertiliser (single superphosphate) applications.

(3) To adapt and improve methods for determining the chemical nature of soil organic P and to use these methods for examining the specific forms of organic P which accumulated in a long-term fertilised pasture soil.

(4) To examine the effects of different soil management practices in the field on the availability to plants of inorganic and organic P forms present in long-term annually fertilised pasture soils.

CHAPTER 2

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LITERATURE REVIEW

2.1 FORMS OF PHOSPHORUS IN SOILS

2.1.1 Introduction

The amount of phosphorus (P) found in soils varies from 200 to 6000 $\mu g\ g^{-1}$ (mean 600 $\mu g\ g^{-1}$), almost all of which is present as various forms of phosphate (PO_4) (Lindsay, 1979). The characterisation of the different forms of P found in soil has been the subject of a great deal of research. For the purpose of this review, soil P is divided into 3 broad fractions:

(i) Abiotic inorganic P - this fraction includes soil solution inorganic P and compounds in which the P is associated with calcium (Ca), magnesium (Mg), iron (Fe), aluminium (Al) and mineral colloids (hydrous oxides, clays).

(ii) Abiotic organic P - P containing organic compounds associated with the soil organic matter.

(iii) Microbial P - inorganic and organic P associated with the soil microbial biomass.

2.1.2 Soil Inorganic and Organic Phosphorus

The relative proportions of inorganic and organic P in soils may vary widely. For example, reported topsoil values for organic P range from 4% of the total P in Spodosols (Podsols) to over 95% in Histosols (Peats) (Dalal, 1977).

In a native undisturbed ecosystem, the total amount of P in the soil is determined mainly by the P content of the parent material, which represents the immediate and principal source of P in these soils. The P in most parent materials is present mainly as hydroxy- and fluoro-apatite ($Ca_{10}(PO_4)_6(OH,F)_2$).

Studies of soil chronosequences have shown that total soil P decreases with increasing soil age as inorganic and organic P is removed from the soil by leaching (Walker and Syers, 1976; Dalal, 1977).However, during the initial stages of soil development, the amount of organic P in the soil increases as a result of the conversion of inorganic P to organic P via plant, animal and microbial residues and as such organic P makes up an increasing proportion of the total soil P with increasing soil age (Walker and Syers, 1976).

As a direct result of agricultural practices, man has greatly influenced the amounts and relative proportions of inorganic and organic P in many soils. For example, decreases in soil P may occur as a result of long-term arable cropping, while additions of P in fertilisers can increase the amounts of inorganic and organic P in soil.

However, problems are known to be associated with the accurate determination of the actual amounts of inorganic and organic P in soils. These involve the determination of total soil organic P in particular (Anderson, 1975; Stevenson, 1982).

The methods commonly used to determine total organic P in the soil were reviewed in detail by Anderson (1975). The amount of total organic P in the soil can be determined by extraction and ignition methods. In the extraction method, the soil is usually pretreated by shaking with mineral acid (e.g. hydrochloric acid - HCl) to remove binding cations such as calcium (Ca^{++}) prior to extraction of the organic P from the soil with a strong alkali such as sodium hydroxide (NaOH) (see section 2.1.5.2). The amount of organic P in the alkali extract, and hence the total organic P content of the soil, is determined colorimetrically as the difference between the amounts of total P and inorganic P in the extract.

The ignition method involves in-situ hydrolysis of the organic P to inorganic P by exposing the soil to high temperatures (350-700°). The P is then extracted from the ignited soil with mineral acid (0.1-1M sulphuric acid (H_2SO_4)) and determined as the total soil P. A similar extraction with acid is carried out on an unignited sample of the same soil to give the total inorganic P content of the soil. The difference between the total P and the total inorganic P thus determined gives the value for total organic P.

Errors are known to associated with both of the methods described above. For example, the total organic P content of the soil may be underestimated as a result of (i) incomplete recovery of the organic P from the soil using the extraction method and

(ii) hydrolysis of organic P by the mineral acids used in both the extraction and ignition methods. Also, with the ignition method some of the inorganic P forms present in the soil may only be extracted by acid following ignition which may, in turn, result in an overestimation of the actual amounts of organic P present. For example, Oniani et al. (1973) found that in a range of grassland soils the values obtained for total organic P were often greater when ignition method rather than extraction method was used. Anderson (1975) concluded that the actual amount of organic P (and hence inorganic P) in the soil is probably somewhere between that determined by the extraction and ignition methods.

Most determinations of the respective amounts of total inorganic and organic P in soil are carried out on air-dried samples of soil which are frequently ground very finely (< 150 μm) prior to analysis. This drying and grinding combined with the acid, alkali and high temperature treatments described above are likely to result in most of the microbial P being released. Therefore, in most cases the respective total inorganic and organic P values reported for soils do in fact include the inorganic and organic P originally held within the soil microbial biomass.

2.1.3 Soil Phosphorus Fractions

In most soils only a very small proportion (< 1%) of the total P is found in the soil solution (Ozanne,

Table 2.1.3.1 Chang and Jackson fractionation scheme for soil inorganic P (Williams et al., 1967).

Extractant	Soil P fraction
(i) 1M NH_4Cl	Non-occluded soluble P
(ii) 0.5M NH_4F (pH 8.2)	Non-occluded aluminium P
(iii) 0.1M NaOH I	Non-occluded iron P
(iv) 0.3M $Na_3C_6H_5O_7$-$Na_2S_2O_6$ (citrate dithionite)	Occluded P
(v) 0.1M NaOH II	
(vi) 0.5M H_2SO_4	Mineral P

1980). Most of the P in soil is present as various forms of inorganic and organic P associated with the soil mineral colloids (clay, hydrous oxides) and organic matter. These various inorganic and organic forms of P can be separated into discrete fractions by selective dissolution and extraction from the soil using different chemical reagents. The soil P fractionation scheme usually involves sequential extraction of the soil with a variety of neutral, acid and alkaline reagents.

Initial soil P fractionation methods were developed specifically to separate the various chemical forms of inorganic P in the soil. The original fractionation scheme for soil inorganic P as developed by Chang and Jackson (1957) has been modified by several workers (e.g. Petersen and Corey, 1966; Williams et al., 1967; Williams et al., 1971). A modified Chang and Jackson scheme as proposed by Williams et al. (1967) is shown in Table 2.1.3.1.

The non-occluded soluble P fraction includes the soil solution P and the P which is weakly adsorbed onto soil colloid surfaces. In most soils this fraction makes up only a very small proportion (< 2%) of the total inorganic P. The non-occluded iron and aluminium P fractions mainly represent the inorganic P adsorbed onto iron and aluminium oxides present on the surfaces of hydrous oxide and aluminosilicate (clay) minerals. These fractions may also include iron and aluminium P minerals such as strengite

($FePO_4.2H_2O$) and variscite ($AlPO_4.2H_2O$) which are present in the soil as discrete particles or as precipitates on colloid surfaces.

The ability to accurately distinguish between iron and aluminium bound forms of non-occluded P based on their respective solubilities in sodium hydroxide (NaOH) and ammonium fluoride (NH_4F) has been criticised by several workers (e.g. Williams et al., 1967). Consequently, in many studies these two extracts are often considered together as the non-occluded P fraction rather than as separate entities (Hartikainen, 1979). The occluded P fraction is assumed to be mainly P held within soil colloids (i.e. not adsorbed on the colloid surface). This includes P originally adsorbed on mineral surfaces which has slowly diffused into the mineral lattice (Barrow, 1983) and adsorbed and mineral forms of P enclosed by coatings of iron and aluminium oxides formed during soil development. Treatment with citrate-dithionite is primarily designed to dissolve these oxide coatings while ~~most~~ some of the occluded P is actually extracted from the soil by sodium hydroxide (Table 2.1.3.1). The fraction of the total inorganic P not extracted from the soil by NH_4Cl, NaOH, NH_4F, H_2SO_4 and citrate-dithionite is also considered as occluded P. This may include P minerals such as crandallite ($CaAl_3(PO_4)_2(OH)_5.H_2O$) which are insoluble in acid, alkali and citrate-dithionite (Williams et al., 1980).

The mineral P fraction is believed to be mainly basic calcium phosphate minerals such as octocalcium phosphate ($Ca_8H_2(PO_4)_6.5H_2O$) and hydroxy- and fluoro-apatites ($Ca_{10}(PO_4)_6(OH,F)_2$). However, this fraction may also include some iron and aluminium P minerals such as triplite ($Fe_2(PO_4)F$) which are insoluble in alkali and citrate-dithionite (Williams et al., 1980). Apatite is the predominant P mineral present in most soil parent materials and thereby constitutes the primary source of P from which all other forms of inorganic and organic P are derived under natural conditions (Walker and Syers, 1976).

Recent detailed studies of P cycling in temperate grasslands have indicated that soil organic P can contribute significantly to plant P nutrition (Blair et al., 1977; Cole et al., 1977). This has prompted more detailed investigations of the different forms of organic P in soils and their relative availability. To this end, Bowman and Cole (1978b) developed a sequential fractionation scheme for soil organic P which separated organic P into discrete fractions of differing relative stabilities based mainly on ease of extraction from the soil (Table 2.1.3.2). According to this fractionation scheme the labile and moderately-labile fractions were considered to be more readily plant-available than the moderately and highly resistant humic and fulvic acid forms of P. The designation of the sodium bicarbonate ($NaHCO_3$) extractable organic P as "labile" P may be appropriate (Halm et al.,

Table 2.1.3.2 Fractionation scheme for soil organic P (Bowman and Cole, 1978b).

Extractant	Soil P fraction
(i) 0.5M $NaHCO_3$ (pH 8.5)	Labile organic P
(ii) 1.0M H_2SO_4	Moderately labile organic P
(iii) 0.5M NaOH — acid soluble (fulvic acid)	Moderately resistant organic P
(iii) 0.5M NaOH — acid insoluble (humic acid)	Highly resistant organic P

1972). However, the relative stabilities assigned to the other organic P fractions appear to be rather arbitrary, which may partly explain why this particular scheme has not been used widely.

In a further development, Stewart et al., (1980) proposed a fractionation scheme for both inorganic and organic P. The fractionation scheme developed by these workers separated soil inorganic and organic P into pools according to their relative stabilities based on ease of extraction from the soil. Thus, the potentially more plant-available forms of P are extracted from the soil with mild reagents, while stronger reagents are used to extract the more stable P forms (Table 2.1.3.3).

The fractionation of inorganic P in this scheme is more detailed than in the Chang and Jackson scheme described earlier in that some attempt is made to differentiate between adsorbed and mineral forms of inorganic P on the basis of relative plant availability. Thus, the resin extractable P is considered to be more readily available to plants than the bicarbonate and sodium hydroxide extractable P. Ultrasonic dispersion is designed to break up soil aggregates and the sonicate-sodium hydroxide fraction therefore represents adsorbed and mineral forms of P present within soil aggregates which is less available to plants than the resin, bicarbonate and sodium hydroxide fractions.

As with the Chang and Jackson fractionation scheme, the acid extractable P is mainly calcium P

Table 2.1.3.3 Fractionation scheme for soil inorganic and organic P (Stewart et al., 1980).

Extractant	Soil P fraction
(i) Anion exchange resin - distilled water	Resin-P (IP)
(ii) 0.5M $NaHCO_3$ (pH 8.5)	$NaHCO_3$-P (IP/OP)
(iii) 0.1M NaOH	NaOH-P (IP/OP)
(iv) 0.1M NaOH (ultrasonic dispersion)	Sonicate NaOH-P (IP/OP)
(v) 1M HCl	HCl-P (IP)
(vi) H_2O_2 (oxidation)-H_2SO_4 (digestion)	Residual P[a]

IP = Inorganic P; OP = Organic P.

[a] = total P not extracted from soil.

minerals such as apatites, although it may include some occluded P in strongly weathered soils. However, most of the occluded P as described previously is not actually extracted from the soil in the Stewart et al. (1980) scheme and is included in the residual P fraction.

Organic P in the soil is also held in combination with mineral colloids. In the Stewart et al. (1980) fractionation scheme the organic P is separated into weakly and strongly held forms as the bicarbonate and sodium hydroxide fractions respectively. In terms of relative plant availability the sodium hydroxide extractable organic P may be more stable than that extracted by the bicarbonate. The sonicate-sodium hydroxide fraction represents organic P held within soil aggregates, while the more stable forms of organic P are probably included in the residual P fraction.

In the fractionation scheme of Stewart et al. (1980) the actual amounts of inorganic and organic P in the residual fraction were not determined. However, it has been shown that the residual P fraction makes

up a large proportion of the total P in many soils. For example, Hedley et al. (1982a) and Tiessen et al. (1983) found that on average the residual P fraction made up 42% of the total P in several chernozem soils. Consequently, Hedley et al. (1982b) modified the original Stewart et al. (1980) fractionation scheme to (i) extract a greater proportion of the total P from the soil, and (ii) determine the relative amounts of inorganic and organic P in the residual (i.e. non-extracted) fraction. Accordingly, these workers replaced the sonicate-NaOH extraction step with a stronger alkali extraction (1M sodium hydroxide), while the amounts of inorganic and organic P in the residual fraction were determined by ignition (see section 2.1.2). In one particular soil these modifications improved the actual proportion of the total P extracted from 45 to 85% (Hedley et al., 1982b; Hedley et al., 1983).

In recent years there has been a trend towards the development of soil P fractionation scheme which separate the soil P into discrete fractions of differing relative plant availability rather than solely on the basis of chemical solubility. However, while it may be valid to correlate ease of extraction from the soil with the relative plant availability of inorganic P, the assignment of relative availabilities to the different organic P fractions is more difficult. Chemical factors such as mineral solubility and the nature of P adsorption onto soil colloids determine the relative plant availability of inorganic P, whereas

the availability of organic P in the soil is likely to be influenced by a combination of chemical and biological factors. The relationships between the various soil organic P fractions and plant availability require further investigation.

2.1.4 Soil Microbial Phosphorus

Soil microbial P is the inorganic and organic P held within micro-organisms in the soil. Methods which enable the amounts of microbial P in the soil to be determined directly have been developed in recent years (Brookes et al., 1982; Hedley and Stewart, 1982). Prior to the development of these methods, the soil microbial P was estimated from the determined amounts of microbial carbon in the soil using the carbon:phosphorus ratio of culture-grown micro-organisms (Anderson and Domsch, 1980). However, this is only an approximate method of determining soil microbial P since it assumes that culture-grown micro-organisms are analagous to those present in the soil.

The procedures for determining soil microbial P directly developed by Brookes et al. (1982) and Hedley and Stewart (1982) are infact very similar. Both are based on the method for determining soil microbial carbon which involved fumigating the soil with chloroform ($CHCl_3$) to kill the micro-organism and determining the microbial carbon from the carbon dioxide (CO_2) produced during subsequent incubation with an added soil innoculum (Jenkinson and Powlson, 1976; Anderson and Domsch, 1978).

The determination of soil microbial P involves treating the soil with chloroform to kill the micro-organisms and thereby release their P content which is then extracted from the soil by shaking with alkaline sodium bicarbonate (0.5M $NaHCO_3$ @ pH 8.5).

The actual techniques used in the determination of soil microbial P are important. Firstly, the soil must be field moist and not air-dried since it has been shown that air-drying can result in significant quantities of microbial P being released (Sparling et al., 1985a). Secondly, soils should be stored moist for 7-21 days in the laboratory prior to microbial P determination to allow microbial respiration to stabilise after sampling (Brookes et al., 1982; Hedley and Stewart, 1982) and also to minimise interference from P in plant residues (Sparling et al., 1985b). McLaughlin and Alston (1985) also found that in soils containing large amounts of plant roots, a fraction of the apparent microbial P determined by the chloroform-bicarbonate technique did in fact originate from the plant roots. In order to minimise the erroneous contribution to microbial P from plant roots these workers recommended using a 16-hour bicarbonate extraction (Hedley and Stewart, 1982) rather than a 30 minute bicarbonate extraction (Brookes et al., 1982).

Hedley and Stewart (1982) and Brookes et al. (1982) determined the recovery of microbial P from the soil using culture-grown bacteria and fungi and found that on average about 40% of the added microbial P was

recovered from the soil using the chloroform-bicarbonate technique. This apparent recovery factor (Kp $\simeq$ 0.40) is included in determining the amount of microbial P in the soil from the increase in bicarbonate extractable P which occurs as a result of chloroform treatment:

$$\text{Soil Microbial P } (\mu g\ P\ g^{-1}) = \frac{NaHCO_3P\ (+CHCl_3) - NaHCO_3P\ (-CHCl_3)}{Kp}$$

However, it is not known how the culture-grown micro-organisms used to determine the value of Kp compare with the indigenous microbial population in the soil. This raises some doubts as to the precision of the chloroform-bicarbonate technique although this may be improved by using fresh soil micro-organisms to determine the value of Kp (Brookes et al., 1982).

It is possible that the value of Kp may be influenced by a number of other factors such as soil type (e.g. relative soil P retention capacity (Hedley and Stewart, 1982)) and as such Kp values should ideally be determined for each particular soil type under investigation. Due to the recent development of the chloroform-bicarbonate method for determining soil microbial P, few data are as yet available for the actual amounts of microbial P in soil. However, initial studies have found that a significant proportion of the total soil P (up to 16%) is held within the microbial biomass (Table 2.1.4.1).

Several factors may influence the actual amounts of microbial P in the soil at any particular time.

Table 2.1.4.1 Proportions of total P found as microbial P in a variety of topsoils.

Land Use	No. soils examined	Soil depth (cm)	Microbial P as % of total soil P	Reference
Arable	4	0-15	0.5-1.4 (0.9)*	Brookes et al. (1984)
Improved pasture	5	0-15	1.6-11.8 (5.3)	Brookes et al. (1984)
Native grassland	2	0-15	2.8-3.7 (3.2)	Hedley et al. (1982a)
Improved pasture	21	0-7.5	0.5-11.7 (4.7)	Sarathchandra et al. (1984)
Forest	2	ND	11.8-16.8 (14.4)	Williams & Sparling (1984)
Raised/blanket bog	3	0-15	6.8-16.1 (12.4)	Williams & Sparling (1984)

* figures in parenthesis are means

ND = not determined (F-H layer)

Thus microbial P is likely to be affected by the overall level of microbial activity in the soil which, in turn, is influenced by the climatic conditions and the availability of suitable carbonaceous substrates. For example, in temperate soils it is probable that the level of microbial P will be greater in spring than at any other time of the year as a result of the environmental conditions and substrate availability favouring optimum microbial activity in the soil (Hayman, 1975). Several workers have incorporated microbial P determination into conventional soil P fractionation schemes (Stewart et al., 1980; Hedley and Stewart, 1982). Such comprehensive fractionation schemes may be useful in examining the complex chemical and biological transformation of P which occur in the soil.

2.1.5 Chemical Nature of Soil Organic Phosphorus

2.1.5.1 Introduction. A large proportion of the total P in many soils is present in organic forms, particularly soils under pasture in which organic P often makes up over half of the total P (Dalal, 1977).

The chemical nature of soil organic P has been the subject of extensive study over many years. In order to determine its chemical nature the organic P must firstly be extracted from the soil using a suitable extractant such as strong alkali (e.g. NaOH). Most studies on soil organic P have concentrated on the identification of specific organic P compounds in

soil extracts using a variety of partition chromatography techniques, although nuclear magnetic resonance spectroscopy (^{31}P NMR) has also been used in recent years. Nevertheless, in most soils examined less than half of the total organic P present has been identified.

In addition to identifying specific organic P compounds present, the molecular weight characteristics of extracted soil organic P have also been determined using gel filtration chromatography.

2.1.5.2 Extraction of Organic Phosphorus from the Soil. In the soil negatively charged organic matter colloids (including organic P) are attached to minerals such as aluminosilicates (clays) and hydrous iron and aluminium oxides directly and via polyvalent bridging cations such as iron (Fe^{3+}) and calcium (Ca^{++}). It is possible to extract the organic matter from the soil by treatment with alkali. Alkaline solvents such as sodium hydroxide (NaOH) extract organic matter from the soil by (i) increasing the negative charge of both the organic and mineral colloids (i.e. electrostatic repulsion), and (ii) replacing the polyvalent bridging cations with markedly less effective monovalent cations (Na^{+}) (Russell, 1973). Pretreatment of the soil with mineral acid (e.g. hydrochloric acid - HCl) removes some of the polyvalent bridging cations (especially Ca^{++}) and thereby facilitates subsequent extraction of the organic matter from the soil with alkali.

Soil organic P is different from organic carbon and nitrogen in that it can be quantitatively extracted

Table 2.1.5.2.1 A selection of methods for quantitative extraction of organic P from the soil.

Extractants	Reference
Hot concentrated HCl/10 minutes; concentrated HCl at room temperature/1 hour; 0.5M NaOH at room temperature/1 hour; 0.5M NaOH at 90°C/8 hours.	Mehta et al. (1954)
0.1M HCl/1 hour; leached with hot HCl; 0.1M NaOH/16 hours (2x).	Saunders and Williams (1955)
0.3M NaOH/16 hours; hot concentrated HCl/ 10 minutes; concentrated HCl at room temperature/1 hour; 0.5M NaOH at room temperature/1 hour; 0.5M NaOH at 90°C/ 8 hours.	Anderson (1960)
0.05M HCl - 0.05M HF/2 hours; cation exchange resin (Na^+ form) in water/4 hours.	Thomas and Bowman (1966).
0.1M HCl; cation exchange resin (Na^+ form) in water/10 hours; 0.2M aqueous acetyl-acetone (pH 8.3)/12 hours (x4).	Hong and Yamane (1980).
0.1M HCl/30 minutes; ultrasonic dispersion in 0.2M aqueous acetylacetone (pH 8.0)/ 2 hours; 0.2M aqueous acetylacetone (pH 8.0)/16 hours; 0.2M aqueous acetyl-acetone (pH 8.0)/24 hours (x2); ultrasonic disperson in 0.2M aqueous acetylacetone (pH 8.0)/2 hours; 0.2M aqueous acetyl-acetone (pH 8.0)/24 hours (x2).	Halstead et al. (1966).

from the soil with alkali, whereas it is only possible to recover a fraction of the organic carbon and nitrogen by extraction (Russell, 1973).

The methods originally developed to quantitatively extract organic P from the soil were primarily those used to determine the actual total amount of organic P present. These methods generally involved pretreatment with a strong mineral acid (e.g. concentrated HCl - 12M) followed by extraction of the organic P with several sequential alkali extractions (Anderson, 1975). For example, the method developed by Mehta et al. (1954) involved two successive pretreatments with concentrated HCl followed by extraction with 0.5M NaOH at room temperature at 90° (see Table 2.1.5.2.1).

Some workers were concerned that the strong acid pretreatments used in many of the early extraction methods may cause some hydrolysis of organic P and consequently result in the amounts of total organic P in the soil being ~~over~~under estimated. For example, Saunders and Williams (1955) found that significant hydrolysis of soil organic P occurred as a result of pretreating the soil with concentrated HCl. These workers replaced the concentrated (12M) HCl with 0.1M HCl (Table 2.1.5.2.1) and found that this reduced the acid hydrolysis of soil organic P, as shown by the increased quantities of organic P extracted from the soil by alkali.

By the late 1950's there was increasing interest being shown in using quantitative extraction techniques to examine the chemical nature of soil organic P. Anderson (1960) showed that strong acid pretreatment (4M H_2SO_4, 12M HCl) hydrolysed 5-100% of several organic P esters (glucose-1-phosphate (G-1-P), ribonucleic acid (RNA) and deoxyribonucleic acid (DNA)) added to the soil. These organic P compounds were believed to be present in the soil as components of the soil organic P and would therefore be hydrolysed during such harsh acid pretreatment. Anderson (1960) showed that by including an alkali extraction (0.3M NaOH) prior to acid pretreatment any hydrolysis of the G-1-P, RNA and DNA (see Table 2.1.5.2.1) was eliminated. Since Anderson's method of extracting organic P from the soil reduced organic P hydrolysis it was therefore more suitable for determining the chemical nature of the organic P than the extraction schemes of Mehta et al. (1954) and Saunders and Williams (1955).

In addition to causing some hydrolysis of organic P, the use of strong acids and alkali to extract organic P from the soil may alter the chemical nature of the organic P. Consequently, several alternative methods for quantitatively extracting organic P from the soil have been developed. For example, Thomas and Bowman (1966) found that while the amounts of organic P extracted from a Spodosol were similar using a mild acid-cation exchange resin (CER) system (see Table 2.1.5.2.1) and a strong acid

(2M H_2SO_4)-alkali (0.5M NaOH) system, the proportion of high molecular weight organic P (> 50,000MW) was greater in the mild acid-CER extract than in the strong acid-alkali extract. Some breakdown of the high molecular weight organic P may have occurred during extraction with strong acid-alkali to account for the difference observed.

Similarly, Hong and Yamane (1980) showed that the method of extraction can affect the relative proportions of humic and fulvic acid organic P in the extract. These workers found that humic acid organic P (i.e. non acid-soluble) made up a greater proportion of the total organic P extracted from the soil by cation exchange resin - acetylacetone (CER-AA) (see Table 2.1.5.2.1) than by strong alkali (0.5M NaOH). Earlier, Halstead et al. (1966) proposed using alkaline aqueous acetylacetone as a reagent to quantitatively extract organic P from the soil with minimal alteration to its chemical nature. This, however, required several successive extractions with 0.2M aqueous acetylacetone (pH 8.0) and included ultrasonic dispersion to break up soil aggregates and facilitate quantitative extraction (Table 2.1.5.2.1).

It is very difficult to predict which of the many extraction methods is most suitable for examining the chemical nature of soil organic P. The ideal method should extract the organic P quantitatively from the soil with minimal alteration of its chemical nature. Consequently, it is often necessary to assess the

suitability of several different methods for use in a particular soil. Quantitative extraction of the organic P is difficult to assess since as yet there is no completely accurate method of determining the actual amount of organic P in the soil (see section 2.1.2). Nevertheless, minimal hydrolysis and alteration of the chemical nature of organic P during extraction from the soil is most likely when mild acid and alkali reagents are used.

2.1.5.3 Organic Phosphorus Compounds in Soils. Despite extensive investigations, in many soils only a small proportion of the total organic P has actually been identified. The characterisation of soil organic P generally involves detailed partition chromatography of the extracted organic P. This has enabled the identification and quantitative determination of several groups of organic P compounds including inositol phosphates, phospholipids and nucleic acids, although collectively these compounds have been found to make up less than half of the total organic P present in many of the soils examined (Table 2.1.5.3.1). Other organic compounds tentatively identified in soil extracts include sugar phosphates and ~~teeheic~~ teichoic acids (ribitol-glycerol phosphates) (Anderson, 1980).

2.1.5.3.1 Inositol Phosphates. Inositol phosphates are the predominant organic P species so far identified in most soils. The inositol phosphates are esters of cyclic inositol (a polyol) and inorganic orthophosphate; 6 different inositol phosphate esters

Table 2.1.5.3.1.1. Major organic P compounds identified in the soil and their relative proportions.

Organic P compound	% total soil organic P
Inositol phosphates	0.4-83 (more commonly 3-58)
Nucleic acids	0.2-2.4
Phospholipids	0.5-14

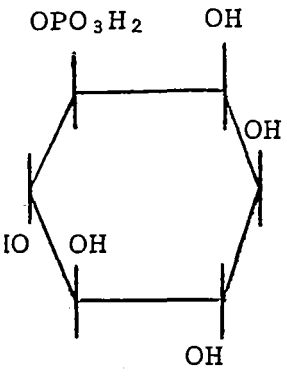
which may be present in one or other of 4 stereoisomer forms[+], have been identified in soil (Figure 2.1.5.3.1.1).

Inositol phosphates are recovered from alkali soil extracts by the precipitation of their sparingly soluble iron (Fe^{3+}) or barium (Ba^{3+}) salts (Cosgrove, 1963; Anderson, 1964). However, some of the inositol P in soil extracts is present in complex organic macromolecules which prevents quantitative precipitation with iron and barium. In order to hydrolyse the extraneous organic material and allow precipitation of the inositol P, it is often necessary to pretreat the soil extracts with strong alkali or hypobromite (Cosgrove, 1963; Anderson, 1964; Irving and Cosgrove, 1981).

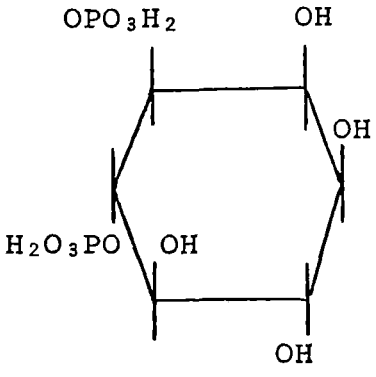
Following precipitation, the various inositol phosphates can be separated and identified using a combination of anion exchange, paper, thin layer and gas chromatography (Cosgrove, 1963; Anderson, 1964; Halstead and Anderson, 1970; Hong and Yamane, 1980).

Tinsley and Ozscavasci (1974) developed an alternative method of determining inositol P in soils. This method involved the precipitation of inositol phosphates directly from the soil as their titanium salts, following which the inositol P components were separated and characterised as described above. In several soils these workers (Tinsley and Ozscavasci, 1974) found that the amounts of inositol penta- and hexaphosphates determined by this method were similar to those determined using conventional alkali extraction.

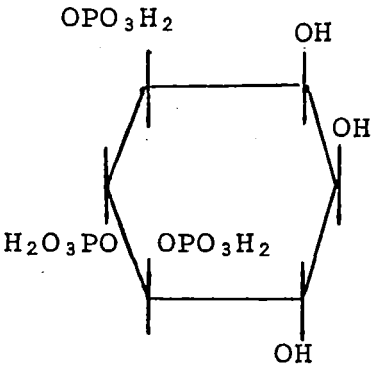
[+] myo-inositol, scyllo-inositol, neo-inositol, chiro-inositol.



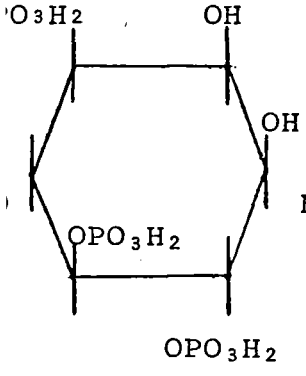
Inositol monophosphate



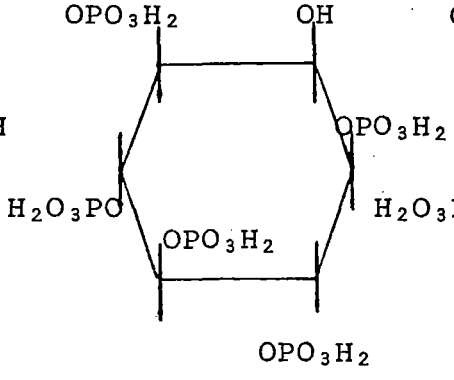
Inositol diphosphate



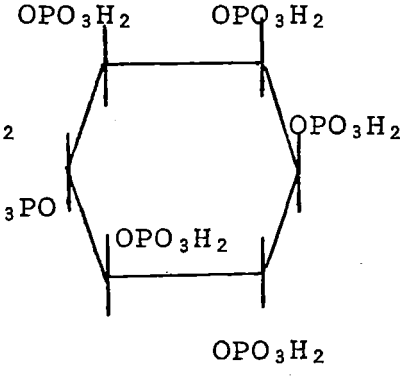
Inositol triphosphate



Inositol tetraphosphate



Inositol pentaphosphate



Inositol hexaphosphate

Figure 2.1.5.3.1.1 Different myo-inositol phosphate esters found in the soil (Baker, 1974).

Inositol P in soils originates from plant, animal and microbial residues. For example, Martin and Molloy (1971) found that pasture plants (ryegrass, clover) and sheep faeces contained mainly inositol penta- and hexaphosphate. Furthermore, Caldwell and Black (1958) found that the amount of inositol hexaphosphate in a soil increased following a laboratory incubation with added inorganic P, indicating that some in-situ microbial synthesis of inositol P had occurred.

Most studies have found that inositol penta- phosphate and hexaphosphate are the predominant forms of inositol P present in the soil, while only trace amounts of the monophosphate and diphosphate esters have been detected (Anderson, 1956; Omotoso and Wild, 1970b; Tinsley and Ozscavasci, 1974).

The relative proportion of the total soil organic P present as inositol P has been found to vary widely. For example, in a wide range of soils in Bangladesh inositol P made up between 9 and 83% (average 49.6%) of the total organic P present (Islam and Ahmed, 1973; Islam and Mandal, 1977); while Anderson et al. (1974) found that about half of the total organic P in several agricultural soils in Scotland was present as inositol hexa- and penta- phosphate. On the other hand, it has been shown that inositol phosphates made up less than 20% of the total organic P in soils from Africa, Australia and North America (Omotoso and Wild, 1970a; Anderson, 1980; Halm et al., 1982).

Several workers have observed a close relationship between the inositol P content of the soil and the P retention capacity (McKercher and Anderson, 1968; Anderson et al., 1974; Halm et al., 1982) which suggests that inositol P may be adsorbed onto soil colloids. Thus Anderson et al. (1974) found that added inositol hexaphosphate was readily adsorbed by several acid soils and that the mechanisms involved were similar to those for inorganic P adsorption. This adsorption onto soil colloids probably affects the rate of mineralisation of the inositol P in the soil. For example, Greaves and Webley (1969) demonstrated that added inositol hexaphosphate is rapidly hydrolysed in sand but not so in soils or sand-clay assemblages which suggests that the inositol P in soil is stabilised to some extent by adsorption onto soil colloids. Furthermore, Williams and Anderson (1968) found that as a result of long-term arable cropping a smaller proportion of the inositol P was lost by mineralisation than that of the soil organic P as a whole.

2.1.5.3.2 Nucleic Acids. Although large amounts of nucleic acid P (ribonucleic acid (RNA), deoxyribonucleic acid (DNA) are known to be added to the soil in plant, animal and microbial residues, only a very small proportion of the total soil organic P has been found in these forms (Anderson, 1967). This is undoubtedly due to the rapid mineralisation of nucleic acid P in the soil (see section 2.3.3.1.2). Nonetheless,

small amounts of nucleic acid P are present in the soil possibly as a result of being stabilised by adsorption onto soil colloids.

Ribonucleic acid and deoxyribonucleic acid polymers may exist in the soil but they are hydrolysed during the extraction process. While RNA is hydrolysed by alkali, DNA hydrolysis occurs as a result of the treatment with mineral acid necessary to separate it from humic acid (Anderson, 1967). Analysis of the hydrolysis products gives some approximate indication of the amounts of nucleic acid P present in the soil. This involves the chromatographic separation and determination of the nucleoside diphosphate monomers and purine and pyrimidine bases (adenine, guanine, cytosine, uracil and thymine) (Anderson, 1961; Anderson, 1970). For example, from the amounts of ~~purine and pyrimidine bases~~ nucleotides present in alkali extracts it has been estimated that between 0.5 and 2.3% of the total soil organic P is present as RNA (Adams et al., 1954; Islam and Ahmed, 1973). Similarly, analysis of the acid hydrolysis products of humic acid extracted from a wide range of soils suggest that DNA makes up a very small proportion (0.07-2.4%) of the soil organic P (Anderson, 1961; Islam and Mandal, 1977). Furthermore, the composition of the purine and pyrimidine bases suggests that most of the DNA in soils is of microbial origin (Anderson, 1961).

The methods currently used for the analysis of RNA and DNA permit only approximate values for their contents in the soil to be determined. In view of the large amounts of nucleic acid P present in the soil-plant system it is possible that the amounts of RNA and DNA in the soil are greater than have been determined thus far.

2.1.5.3.3 Phospholipids. Only small amounts of phospholipid P have been found in soils despite the large amounts added to soil under field conditions, particularly in plant residues (Dalal, 1977). Phospholipids are similar to nucleic acids in that they have been found to be mineralised very rapidly in the soil (see section 2.3.3.1.2) which probably accounts for the small amounts of phospholipid P found in soils.

The determination and characterisation of phospholipid P in soil involves extraction of the soil with a variety of organic solvents (e.g. hexane-acetone, ethanol-benzene, methanol-chloroform) followed by detailed partition chromatography (mainly thin layer - TLC) (Hance and Anderson, 1963; Kowalenko and McKercher, 1970; Baker, 1975).

The relative amounts of phospholipid P found in topsoils range from 0.06 to 7.0% of the total organic P (Hance and Anderson, 1963a; Kowalenko and McKercher, 1970; Islam and Ahmed, 1973; Islam and Mandal, 1977; Borie and Barea, 1984), although in one particular Canadian soil, Dormaar (1970) found that 14% of the total organic P was present as phospholipid P.

The principal phospholipid compounds which have been identified in soil are phosphatidyl choline, phosphatidyl ethanolamine, glycerophosphate and choline phosphate (Islam and Mandal, 1977; Anderson, 1980; Tate and Newman, 1982). Phospholipids found in soil are believed to be mainly of microbial origin (Kowalenko and McKercher, 1971) and may persist in the soil as a result of adsorption onto clay minerals which affords some protection from immediate mineralisation (Hance and Anderson, 1983b).

2.1.5.4 ^{31}P Nuclear Magnetic Resonance Analysis of Soil Organic Phosphorus. The application of ^{31}P nuclear magnetic resonance (NMR) spectroscopy to soil P studies is a recent development. NMR analysis enables direct quantitative determination of various inorganic and organic P species present in alkali extracts of soils (Tate and Newman, 1982; Hawkes et al., 1984). Thus NMR analysis eliminates the elaborate and often time consuming chromatographic preparation and separation involved in identifying specific organic P species in these extracts.

In alkali extracts (0.5M NaOH) of a wide range of soils under native and improved pasture, Newman and Tate (1980) were able to resolve 6 different peaks on the ^{31}P NMR spectra corresponding to inorganic orthophosphate, orthophosphate monoester P, orthophosphate diester P, phosphonate P, inorganic pyrophosphate and polyphosphate P (Figure 2.1.5.4.1). In a similar study, Hawkes et al. (1984) detected several additional

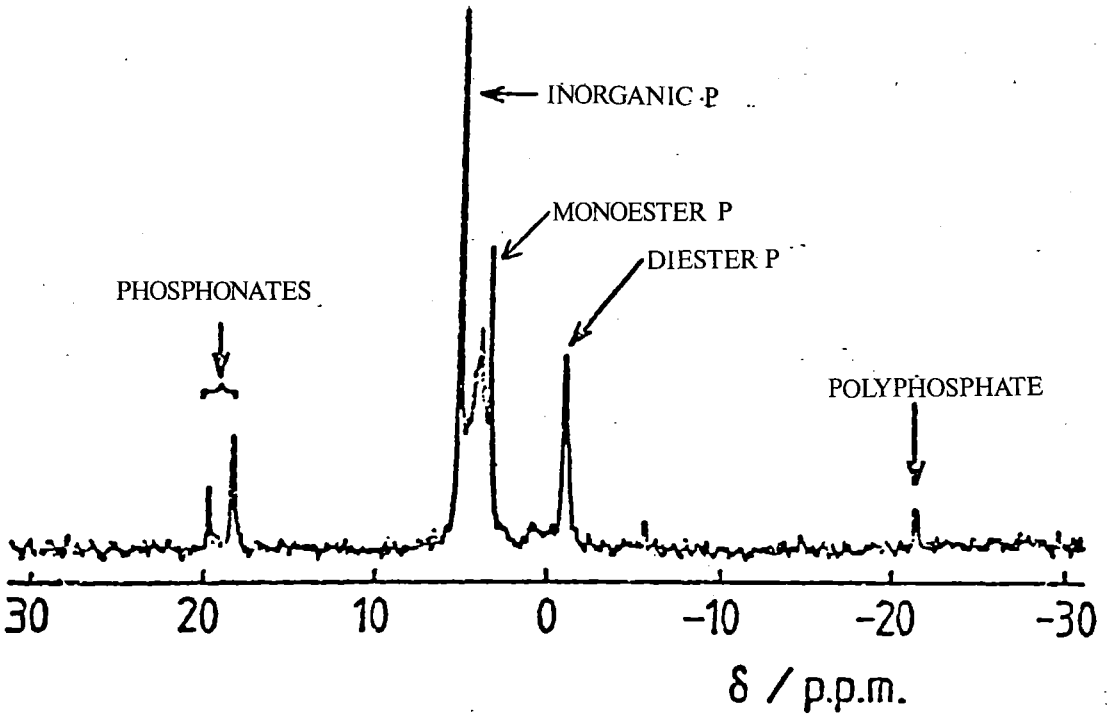


Figure 2.1.5.4.1 Different forms of phosphorus detected in alkali soil extract using ^{31}P NMR (Newman and Tate, 1980).

peaks in the spectra of soil extracts which they suggested may have been sugar phosphates.

The relative amounts of the different P species resolved by ^{31}P NMR analysis of soil extracts can be determined from their respective peak areas. Thus it has been found that, in pasture soils at least, orthophosphate monoester P is the predominant form of organic P present (Table 2.1.5.4.1), while some of the inorganic pyrophosphate P detected by Hawkes et al. (1984) may have been originally present in organic P esters which may have been hydrolysed during alkali extraction (Anderson and Russell, 1969).

Orthophosphate monoester P includes the inositol phosphates, while orthophosphate diesters include nucleic acid and phospholipid forms of P (Newman and Tate, 1980). Thus, the predominance of orthophosphate monoester P found in the soils examined by ^{31}P NMR appears to confirm the results of other studies on the chemical nature of soil organic P (see section 2.1.5.3). Nonetheless, Hawkes et al. (1984) found that in an unimproved (unfertilised) grassland soils, the orthophosphate diesters made up a greater proportion of the total organic P than the orthophosphate monoesters.

The detection of phosphonate P in 2 high altitude native grassland soils in New Zealand by Newman and Tate (1980) was the first time this form of P had been found in soils. Phosphonates in the soil are believed to be mainly of microbial origin and are readily mineralised under most soil conditions. Consequently, phosphonate

Table 2.1.5.4.1 Relative proportions of different organic P species detected by ^{31}P NMR analysis of alkali extracts of soils under pasture.

No. soils examined	% total organic P extracted			Reference
	Orthophosphate monoesters	Orthophosphate diesters	Phosphonates	
8	69-100	0-14	0-11	Tate & Newman (1982)
4	45-74	7-39	0-3	Hawkes *et al.* (1984)

P has been detected only in soils where organic P mineralisation is restricted by low pH and/or cool moist environmental conditions (Tate and Newman, 1982; Hawkes et al., 1984).

In general, ^{31}P NMR analysis of soil extracts has enabled the quantitative determination of broad groups of organic P species rather than specific organic P compounds. The problems involved in determining specific organic P compounds in the ^{31}P NMR spectra were demonstrated by Newman and Tate (1980) who found that inositol hexaphosphate gave four different peak maxima within the orthophosphate monoester region of the spectra (5.26, 4.37, 4.02 and 3.85 ppm). Nonetheless, Newman and Tate (1980) found that choline phosphate (a phospholipid) was clearly resolved as a single peak at 3.56 ppm within the orthophosphate monoester region of the spectra, and later showed that this choline phosphate constituted up to 7% of the total P (10% organic P) extracted from a range of pasture soils (Tate and Newman, 1982).

In all of the studies mentioned above the same method was used to extract the P from the soil for ^{31}P NMR analysis and involved ultrasonic dispersion of 6.7g soil in 20mls of 0.5M NaOH for 3 minutes (Steward and ~~Tate~~ Oades, 1972). This rapid extraction method was chosen to minimise denaturation of the organic P during its extraction from the soil, while the low soil:solution ratio used (1:3) ensured that sufficient concentrations

of P for quantitative ^{31}P NMR (> 100 μg P ml^{-1}) were easily obtained. However, using this extraction technique Tate and Newman (1982) and Hawkes et al. (1984) extracted less than half of the total organic P from most of the soils examined. Further development of the soil extraction and extract preparation techniques is necessary if a larger proportion of the total soil organic P is to be examined using ^{31}P NMR.

2.1.5.5 Fractionation of Soil Organic P According to Molecular Weight Groupings. Over the past 20 years, methods have been developed to determine the molecular weight characteristics of the macromolecules which make up the soil organic matter. The most widely used technique has been gel filtration, largely because of its effectiveness and relative simplicity of operation (Swift and Posner, 1971).

2.1.5.5.1 Gel Filtration of Soil Organic Matter. Gel filtration is a method of partition chromatography which enables the separation of macromolecules in solution on the basis of differential size and molecular weight. This separation is effected by partition between two distinct physical phases:

(i) Stationary Phase (gel) - porous particles packed in an upright column.

(ii) Mobile Phase - unbound solvent flowing between the porous particles.

The behaviour of a particular solute molecule passing through a column of gel is determined by how easily it can enter the gel matrix. This is expressed in

terms of a distribution coefficient (Kd):

$$Kd = \frac{Ve - Vo}{Vi}$$

where Ve = elution volume of a particular solute molecule (elution volume); Vo = volume of solvent outside the gel matrix (void volume); Vi = volume of solvent held within the gel matrix (internal volume).

The entry of a given solute molecule into the gel is affected mainly by the size of the molecule in relation to the pore size of the gel matrix. Thus, if a particular molecule is too large to enter the gel then it is eluted from the column in the void volume (Vo) and its Kd value will be zero. However, if a molecule is small enough to enter the gel then its elution volume from the column will be somewhere betwen the void volume (Vo) and the total column volume (Vo + Vi). Consequently, its Kd value will be between zero and 1 depending on the relative size of the molecule.

Several theories have been advanced to explain the partition of different sized molecules within the gel matrix. For example, Ackers (1964) suggested that the partition was due to a combination of thermodynamic and hydrodynamic effects. The thermodynamic effect was due to the exclusion of the molecule from the gel based on the probability of entry being greater for smaller than for larger molecules (i.e. steric hindrance). The hydrodynamic effect is due to the fact that molecules within the gel particles are subject to capillary forces such that larger molecules moved faster through the gel

than smaller molecules as the latter experienced greater hydrodynamic resistance.

Molecular size can be used to calculate the corresponding molecular weight only if the molecular species being examined are shaped similarly and fall within a narrow molecular weight range. However, soil organic matter macromolecules are known to be present in a wide variety of sizes and shapes (polydisperse) and as such only nominal molecular weights can be assigned using the gel filtration technique (Cameron et al., 1972).

Several matrix materials for gel filtration are available, although Sephadex is the most widely used in soil organic matter studies. Sephadex is manufactured by cross-linking dextran (40,000 MW; 95% α 1:6, 5% β 1:3) with epichlorohidrin. By varying the degree of cross-linkage a range of Sephadex gels with different porosities is produced which are capable of fractionating a wide range of different molecular weight species (Table 2.1.5.5.1.1).

Gel filtration is carried out on organic matter extracted from the soil with alkali (e.g. NaOH) or neutral (e.g. pyrophosphate) reagents. To enable meaningful determination of the molecular weight characteristics of the soil organic matter there should be no chemical interaction between the organic molecules and the gel matrix. However, several workers have found that soil organic matter, especially humic acids, can be adsorbed onto Sephadex gel (Posner, 1963; Dubach et al., 1964). Swift and Posner (1971) found that this

Table 2.1.5.5.1.1 The fractionation properties of different Sephadex gels.

Sephadex gel type	Fractionation range (molecular weight)	
	Peptides-globular proteins	Dextrans
G-10	700	700
G-15	1500	1500
G-25	1,000-5,000	100-5,000
G-50	1,500-30,000	500-10,000
G-75	3,000-70,000	1,000-50,000
G-100	4,000-100,000	1,000-100,000
G-150	5,000-150,000	1,000-150,000
G-200	5,000-250,000	1,000-200,000

adsorption onto gel was mainly caused by phenolic, heterocylic and aromatic groupings on the organic molecules and demonstrated that this could be overcome by using an alkaline amino buffer as eluent.

In contrast to the situation with humic acid, several studies have shown that no significant adsorption onto Sephadex occurred when fulvic acid or unfractionated organic matter extracts were fractionated using distilled water as the eluent (Schnitzer and Skinner, 1968; Goh and Reid, 1975; Goh and Williams, 1979). In fact, Goh and Williams (1979) suggested that the use of organic matter fractions such as humic acid and its preparation (i.e. acid precipitation, freeze drying) prior to gel filtration may have partly caused the observed adsorption onto Sephadex gels.

Based on gel filtration, a wide range of molecular weights of soil organic matter have been found by several workers. For example, Orlov et al. (1975) found that the nominal molecular weights of organic matter in some Russian soils ranged from 500 to 340,000 MW. In other studies Dell' Agnolla and Ferrari (1971) showed that soil aggregate stability was directly related to the proportion of high molecular weight organic matter (>100,000MW) present, while Goh and Williams (1979) observed that during soil development there was a shift from high (>200,000MW) to lower (10,000-200,000MW) molecular weight organic matter with increasing soil age.

2.1.5.5.2 Gel Filtration of Soil Organic Phosphorus. Very few studies have examined the particular molecular weight characteristics of organic P in the soil using the gel filtration technique.

It appears that a significant proportion of the soil organic P is present in high molecular weight forms. Moyer and Thomas (1970) found that 36% of the total organic P extracted from a Spodosol was excluded from a Sephadex G75 gel (i.e. >50,000MW). This same high molecular weight organic P fraction was examined in greater detail by Veinot and Thomas (1972) who found that the molecular weight of most (82%) of this organic P exceeded 200,000MW. In a wide range of Australian soils, Steward and Tate (1972) also found that a large proportion of the total organic P was present in high molecular weight forms (>200,000MW).

The findings of Swift and Posner (1972) suggest that high molecular weight forms of organic P in the soil may be more stable than lower molecular weight organic P forms. These workers found that in two Australian soils the amount of P in the humic acid fraction increased with increasing molecular weight, which they attributed to long-term preferential mineralisation of lower molecular weight humic acid organic P. This may also partly explain the results of Goh and Williams (1982) who found that the proportion of the soil organic P present in high molecular weight forms (>50,000MW) increased with increasing soil age.

Goh and Williams (1982) found that the organic P which accumulated in soil as a result of P fertiliser addition was mainly present in low molecular weight forms. Thus, they observed the P:carbon ratio of the low molecular weight organic matter (<50,000 MW) was greater in soil under pasture which had received P fertiliser than in the corresponding soil under native vegetation. However, the change to pasture had

no effect on the P:carbon ratio of the higher molecular weight soil organic matter fraction (> 50,000 MW).

The chemical form of soil organic P has also been shown to vary with molecular weight. Omotoso and Wild (1970b) determined the amounts of different inositol phosphates in various molecular weight fractions of fulvic acid extracted from a calcareous soil and found that while inositol tetra- and hexaphosphate were present only in the high molecular weight fraction (> 10,000 MW), inositol mono-, di- and triphosphates were present only in the lower molecular weight fraction (< 10,000 MW).

In view of the limited data available it is difficult to make any definite conclusions regarding the molecular weight characteristics of soil organic P, particularly in relation to its chemical form and relative stability.

2.2 SOURCES OF PHOSPHORUS FOR PLANTS IN THE SOIL

2.2.1 Introduction

Phosphorus is an essential plant nutrient mainly because of its role in genetic and metabolic processes via ribonucleic acid (RNA) and adenosine triphosphate (ATP) respectively. Plants obtain all

of their P requirements from the soil and the major sources of P for plants in the soil are shown in Figure 2.2.1.1.

2.2.2 Soil Solution Inorganic Phosphorus

The immediate source of P for plant roots in the soil is the inorganic P in the soil solution. The concentration of inorganic P in the soil solution ranges from 0.05 to 0.30 μg P ml^{-1} (Ozanne, 1980), which on its own is insufficient to meet plant P requirements. For example, it has been calculated that a soil solution P concentration of 0.30 μg P ml^{-1} is equivalent to 0.04 kg P ha^{-1} in the top 30cm of a typical agricultural soil (Russell, 1973). If this was the only source of P for plants in the soil then the total plant P uptake over a growing season would be 0.2 kg P ha^{-1}, whereas the actual amount of P taken up by plants over this period generally exceeds 10 kg P ha^{-1}. Thus the bulk of plant P requirements are derived from other sources in the soil via the soil solution.

2.2.3 Labile Inorganic Phosphorus

In most soils the concentration of inorganic P in the soil solution is determined by the amounts and forms of chemically fixed inorganic P on the mineral colloids with which it is in equilibrium. The relationship between the solution and solid phase

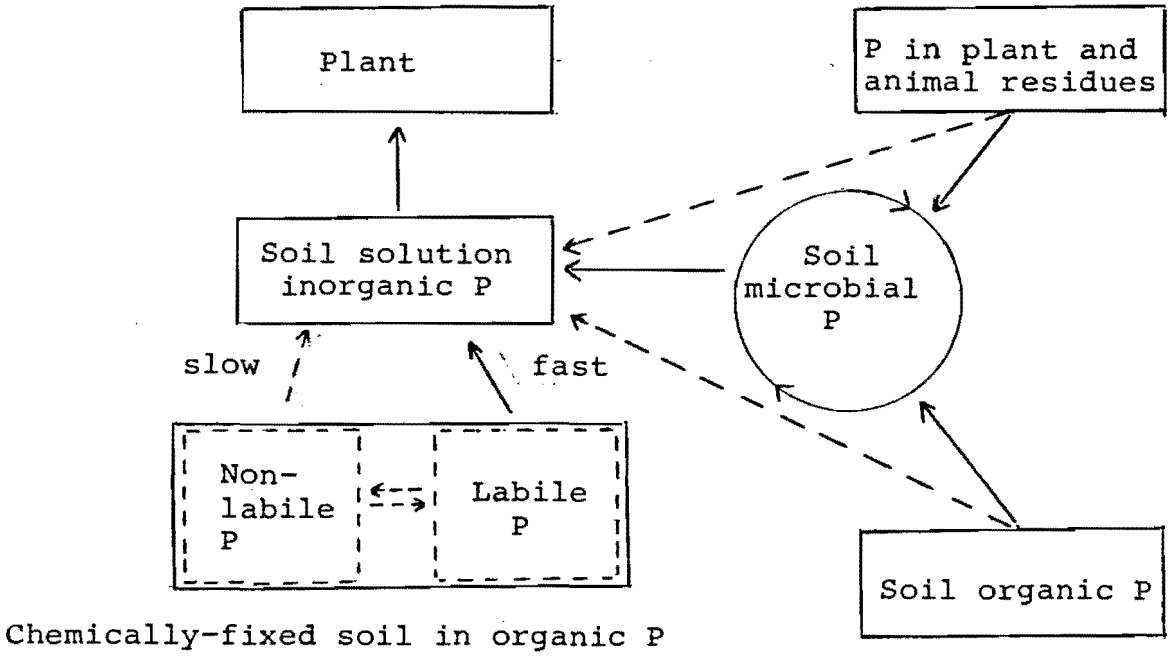


Figure 2.2.1.1 Sources of phosphorus for plants in the soil.

inorganic P in any particular soil is affected by the phosphate buffering capacity which is defined as "the ability of a soil to resist changes in the concentration of the equilibrium solution as P is added or removed" (Ozanne, 1980). Thus inorganic P removed from the soil solution by plant uptake will be replenished mainly by the desorption of adsorbed P held in the soil mineral colloids. On the other hand, if there is an increase in the level of inorganic P in the soil solution as a result of soluble P fertiliser addition, organic residue decomposition or soil organic P mineralisation then equilibrium will be restored mainly by adsorption of P onto the mineral colloids.

In addition to the desorption-adsorption reactions described above the equilibrium between the solution and solid phase inorganic P may involve the formation-dissolution of sparingly soluble P minerals, particularly in soils treated with soluble P fertilisers (see section 2.4.3).

The adsorbed and mineral forms of inorganic P which are in direct equilibrium with the soil solution represent the major sources of P for plants. This fraction of the solid phase inorganic P in soil is known variously as the "labile", "exchangeable" or "available" P.

Several methods have been developed to determine the actual amounts of labile inorganic P

in the soil. For example, labile inorganic P can be determined using the isotope exchange technique (Mattingly and Talibudeen, 1960). According to this method, the observed equilibration of added ^{32}P isotope between the soil and solution in a soil-water suspension and the amount of ^{31}P in the solution are used to determine the amount of labile P in the soil:

$$\text{Labile P}(\mu g\ P\ g^{-1}) = {}^{32}P \text{ added } (\mu Ci) \times \frac{{}^{31}P \text{ solution}(\mu g\ P\ g^{-1})}{{}^{32}P \text{ solution}(\mu Ci)}$$

Labile P in the soil can also be determined using an anion exchange resin (Amer, 1955; Sibbesen, 1977). This method involves extracting the labile P from the soil by equilibrating a soil suspension with a strong anion exchange resin (e.g. Dowex 1 x 8 - HCO_3^- form). This extraction involves initial displacement of the adsorbed inorganic P ($H_2PO_4^-$, $HPO_4^=$) by bicarbonate (HCO_3^-) from the resin, and subsequent "transfer" of the released P onto the resin (i.e. anion exchange). Extraction of inorganic P from the soil by anion exchange resin is assumed to simulate prolonged uptake of P from the soil by plants and is therefore considered to give a good estimate of labile P.

A wide range of methods for determining labile P which involve physical extraction of the P from the soil with mild acid and alkali reagents have also been developed. These methods are commonly known as "soil tests", several of which are shown in Table 2.2.3.1.

The reagents used in these soil tests were designed primarily to dissolve the various forms of

Table 2.2.3.1 Some examples of "soil tests" used to determine the amounts of labile P in the soil.

Extractant	Soil:solution ratio	Soil test name	Reference
0.025M HCl + 0.03M NH_4F	1:10	Bray I	Kamprath and Watson (1980)
0.05M HCl + 0.01M H_2SO_4	1:4	North Carolina	
0.001M H_2SO_4 (pH 3)	1:100	Truog	
0.54M HoAc + 0.7M NaOH (pH 4.8)	1:10	Morgan	
0.5M $NaHCO_3$ (pH 8.5)	1:20 ($\frac{1}{2}$hr)	Olsen	
0.5M $NaHCO_3$ (pH 8.5)	1:100 (16hrs)	Colwell	Colwell (1963)

labile P from the soil. For example, the mild acid extractants (pH 2-3) used in the Bray, North Carolina and Truog tests dissolve iron, aluminium and calcium forms of labile inorganic P, although there is some preferential extraction of calcium P. On the other hand, alkaline sodium bicarbonate (Olsen, Colwell tests) extracts mainly iron and aluminium bound forms of P from the soil. In addition to dissolving labile inorganic P forms, anions in extractants such as bicarbonate (HCO_3^-) and acetate (CH_3COO^-) extract labile P by displacing adsorbed inorganic P (i.e. anion exchange).

The relationship between labile inorganic P determined by the methods described above and plant response has been studied extensively. For example, Bowman et al. (1978) found that in a wide range of calcareous soils the labile P determined by the isotope exchange and anion exchange resin methods accounted for 89-93% of the total P taken up by plants over a 3 year period in the glasshouse. On the other hand, the proportion of plant P accounted for by the 30 minute Olsen and 16 hour Colwell bicarbonate tests was only 52-79%. Thus it appeared from this study that the isotope exchange and anion exchange resin methods were better than bicarbonate extraction in predicting the amounts of labile P in calcareous soils. Nonetheless, other studies have shown a satisfactory

relationship between plant response and labile P determined by a variety of soil tests (Kamprath and Watson, 1980).

Soil tests such as those described in Table 2.2.2.1 are used widely by farm advisory services to assist in determining P fertiliser requirements (e.g. Cornforth and Sinclair, 1982).

2.2.4 Non-Labile Inorganic Phosphorus

While labile inorganic P represents the major source of P for plants in most soils, the more stable less readily exchangeable forms of inorganic P in the soil can also contribute to plant P nutrition. This "non-labile" P includes strongly adsorbed forms of inorganic P, P held within mineral lattices and various sparingly soluble P minerals such as apatites.

Recent studies using soil P fractionation have shown that non-labile P can contribute significantly to plant P requirements. For example, Tiessen et al. (1983) found that the sodium hydroxide (NaOH) extractable fraction of inorganic P in a soil originally under native grassland declined by 58% as a result of 65 years of arable cropping. This NaOH soluble fraction mainly represents the strongly adsorbed forms of inorganic P (see section 2.1.3). In a similar study Hedley et al. (1982a) observed that a significant decrease (12%) occurred in the acid soluble apatite P fraction of a calcareous soil as

a result of 65 years of rotational cropping.

Non-labile forms of inorganic P in the soil contribute to plant P nutrition directly via the solution and indirectly by conversion to more labile P forms (Larsen, 1977).

2.2.5 Biological Sources of Phosphorus for Plants

Several biological processes in the soil result in the release of inorganic P to the soil solution and therefore can contribute to plant P nutrition. These processes are controlled mainly by micro-organisms in the soil and include the decomposition of plant and animal residues and soil organic P mineralisation. The mineralisation of indigenous soil organic P is discussed in detail in section 2.3.3 of this review and the following mainly concerns residue decomposition and the turnover of P through the microbial biomass.

In grazed pasture a large proportion (60-95%) of the total P taken up by plants is returned to the soil in various plant and animal residues (Quin and Rickard, 1979; Karlovsky, 1982; Cornforth and Sinclair, 1982), whereas in cropped soil a smaller proportion of the total plant P (18-38%) is recycled in plant residues (Hanway and Olsen, 1980). In general, the actual amounts of P added to the soil in plant and/or animal residues is influenced by many

factors including fertiliser inputs (including P), plant genus (e.g. crop variety), grazing management (e.g. stocking rate) and environmental conditions.

Plant residues are mainly composed of dead top (litter) and root material while the major animal residues are urine and faeces (dung). In pasture grazed by sheep or cattle the distribution of plant and animal residue addition to the soil is often not uniform. The distribution of plant residues within a particular area of pasture is determined mainly by how much of the pasture is actually ingested by the grazing animals which, in turn, is influenced by several factors such as the type of grazing animal, grazing management, pasture composition and palatability. Thus, some stock (e.g. sheep) tend to selectively graze certain species in the sward such as clover (Lancashire and Keogh, 1966) while grazing animals in general tend to avoid pasture fouled by either urine or dung (Sears and Newbolt, 1942; Marsh and Campling, 1970; MacDiarmid and Watkin, 1972).

The behaviour of grazing animals greatly influences the distribution of urine and dung within an area of pasture. For example, sheep tend to select small areas of pasture in sheltered or elevated locations as permanent campsites. Consequently, disproportionately large amounts of urine and dung tend to be deposited at these sites.

For example, in New Zealand hill country sheep campsites make up between 5 and 20% of the total pasture area yet about 90% of the total P returned to the pasture in dung annually is deposited in these areas (Gillingham and During, 1973; Gillingham, 1980; Gillingham et al., 1980). As a result of the selective deposition of dung and urine, pasture growth is often greater in the campsite areas. This, in turn, means that on an area basis greater amounts of P will be returned to the soil in plant residues within the campsite then in the remainder of the pasture area (Gillingham, 1980; Gillingham et al., 1980).

Plant and animal residues contain both inorganic and organic forms of P, although there tends to be more inorganic than organic P present. The relative proportion of inorganic P found in plants varies, although it is often greater in young tissue (78-87% of total P) than in older more mature material (47-62% of total P) (Birch, 1961). The latter is probably more typical of the kind of plant material added to the soil as residues. Thus Jones and Bromfield (1969) found that inorganic P made up 58% of the total P in pasture litter.

Almost all of the P excreted from grazing animals is contained in the faeces (Sears and Newbolt, 1942; Peterson et al.,1956). Floate (1970) found that the proportion of total P present as

inorganic P in sheep faeces (c.75%) was greater than in undigested pasture (c.62%). This finding suggests that some mineralisation of the organic P in plants may occur during passage through the digestive tract. Similar proportions of inorganic P (70-80%) in the faeces of grazing animals has been reported by other workers (Bromfield, 1961; Gunary, 1968).

Plant and animal residues undergo decomposition in the soil by the actions of micro-organisms (Hayman, 1975). As a result of this microbial decomposition inorganic P is released from the residues directly and as a result of mineralisation of residue organic P.

During the initial stages of residue decomposition there are probably sufficient quantities of inorganic P present to satisfy microbial P demands and as such there is likely to be very little mineralisation of the organic P in the residues. Accordingly, several studies have found that no significant mineralisation of residue organic P occurs during the early stages of decomposition (1-3 months) (Birch, 1961; Bromfield, 1961; Gunary, 1968; Jones and Bromfield, 1969; White and Ayoub, 1983). Nonetheless, Floate (1970) showed that some mineralisation of organic P in sheep faeces occurred after only seven days and continued throughout the three month period of decomposition studied. Floate (1970) also

observed that some mineralisation of organic P in pasture plant residues occurred, although this was only apparent after 9 weeks. These findings suggest that the organic P in faeces is in some way more readily mineralised by micro-organisms than that present in the herbage from which the faeces was derived. In the longer term Van Diest (1968b) found that between 58 and 87% of the organic P added to soil in various plant residues (grass, peach leaves) had been mineralised after 18 months.

The inorganic P released from plant and animal residues as described above can be taken up directly by plants. For example, Till and Blair (1978) found that 22-28% of the total P added to a soil in ^{32}P-labelled clover plant residues was taken up by plants after only 56 days. Over a slightly longer period of decomposition (70 days) Dalal (1982b) found that 38-42% of the P added to a soil in ^{32}P-labelled clover shoot and root material was taken up by oats (Avena sativa). It is likely that most of the inorganic P released from plant and animal residue decomposition and soil organic P mineralisation will be removed from the soil solution by adsorption onto soil colloids to form part of the labile and non-labile pools and thereby contribute to plant P nutrition indirectly (see Figure 2.2.1).

In the course of the microbial decomposition of plant and animal residues and mineralisation of

indigenous soil organic P, some of the inorganic P released may be taken up by the micro-organisms involved for use in various genetic and metabolic processes. This microbial immobilisation is believed to occur particularly during the early stages of residue decomposition when microbial activity is greatest due to the abundance of carbonaceous substrate material (Birch, 1961; Van Diest, 1968b; Floate, 1970). In addition, micro-organisms involved in the decomposition process may obtain some inorganic P from the soil, especially during the initial stages of decomposition of residues which have high carbon:phosphorus ratios (>300:1) (Alexander, 1977).

It is likely that most of the P taken up by micro-organisms in the early stages of residue decomposition is released again as microbial activity declines in response to the exhaustion of available carbon substrates. However, some of the inorganic P taken up by micro-organisms may be converted to organic P forms and released as such in microbial residues (see section 2.3.2).

It is clear from the above that there is an active turnover or flux of P through the soil microbial biomass. The concept of the microbial biomass as a biological reservoir of labile inorganic and organic P in the soil has only been investigated in recent years. This has been prompted by the recent development of methods which

enable direct determination of the amounts of P in the soil microbial biomass (see section 2.1.4). Initial studies indicate that there is a large annual turnover of P through the soil microbial biomass. In a wide range of agricultural soils, Brookes et al. (1984) calculated that the annual flux of P through the microbial biomass ranged from 2.7 to 40.4 kg P ha^{-1} (average 15.9 kg P ha^{-1}). In many of the soils examined this microbial P flux was greater than the amount of P taken up by plants annually (8-28.8 kg P ha^{-1}) and therefore suggests that the microbial biomass may be an important indirect and direct source of P for plants.

In temperate soils the importance of the various biological sources of inorganic P described above will be determined primarily by the level of microbial activity in the soil. Thus the release of inorganic P from residue decomposition and soil organic P mineralisation is likely to be greatest in spring when the availability of carbon substrates and environmental conditions favour high microbial activity in the soil.+

2.3 TURNOVER OF SOIL ORGANIC PHOSPHORUS

2.3.1 Introduction

The turnover of organic P in the soil involves the concurrent biological processes of immobilisation

+ Earthworms may also play an important role in P cycling in pasture soils via their effects on biological activity, the availability of P to plants and the redistribution of organic residues in the soil (Syers and Springett, 1984).

and mineralisation. Immobilisation refers to the processes by which inorganic P is converted to organic P in the soil via plant, animal and microbial residues, while mineralisation involves the hydrolysis of organic P to release inorganic P. It is often difficult to distinguish between these processes since steady-state conditions prevail in many soils whereby the respective overall rates of immobilisation and mineralisation are approximately equal. In general net immobilisation or mineralisation occurs seasonally or when the balance of P inputs and outputs to and from the soil are disturbed (e.g. by fertiliser P addition or rotational cropping).

2.3.2 Immobilisation

Phosphorus immobilisation involves the biological conversion of inorganic P to organic P in the soil. Two major pathways are involved in this immobilisation. Firstly, inorganic P in the soil may be taken up by micro-organisms and some of this may be released again as organic P in microbial detritus. Secondly, some of the inorganic P removed from the soil by plants may be returned as organic P in plant and animal residues (see sections 2.2.4 and 2.4.4.3).

Direct microbial immobilisation of P in the soil was demonstrated by Chauhan et al. (1979; 1981). During a nine month incubation period these workers found that in soils amended with a carbon substrate

(cellulose) there was an increase in the amounts of organic P present (Table 2.3.2.1). The addition of cellulose enhanced microbial activity in the soil which, in turn, increased the conversion of inorganic P to organic P in microbial residues.

In addition to direct immobilisation of soil inorganic P some of the inorganic P in plant and animal residues can be converted to organic P by the actions of micro-organisms involved in their decomposition in the soil (see section 2.2.4).

2.3.3 Mineralisation of Soil Organic Phosphorus

Soil organic P cannot be utilised by plants until it is mineralised by the actions of soil micro-organisms and enzymes to inorganic P.

The mechanisms involved in the mineralisation of soil organic P are not yet fully understood. Appiah and Thompson (1974) proposed that the mineralisation of soil organic P by micro-organisms involves two stages: (i) the breakdown of polymeric P-containing organic material by a variety of microbial enzymes, and (ii) the mineralisation of the organic P esters by phosphohydrolase enzymes (phosphatases). Similarly, Dalal (1977) concluded that micro-organisms were responsible for the mineralisation of organic P in the soil, both directly and indirectly via extracellular phosphatase enzymes. More recently, McGill and Cole (1981) suggested that mineralisation of soil organic P was principally

Table 2.3.2.1 Changes in soil organic P during extended laboratory incubation (Chauhan et al., 1979; 1981).

Soil	Total soil organic P (μg P g^{-1})		
	Prior to incubation	After 9 months incubation	
		Control	Cellulose amended*
Oxbow Ap	479	474	500
Oxbow Bm	313	311	327
Bradwell Ap	348	345	360

* = cellulose added every 30 days

caused by extracellular phosphatase enzymes produced by plant roots, mycorrhizae and soil micro-organisms.

In view of the large amounts of organic P present in many soils the role of organic P mineralisation in plant P nutrition has been the subject of extensive study.

Net mineralisation of soil organic P is usually determined by a decrease in the amount of organic P present in the soil over a period of time. Monitoring changes in total soil organic P have been shown to be satisfactory for determining organic P mineralisation over a long period of time. For example, several workers have found that large decreases in total soil organic P occurred as a result of long term (>50 years) arable cropping (e.g. Chater and Mattingly, 1980; Tiessen et al., 1982). However, it is less satisfactory for determining soil organic P mineralisation over a short time period (e.g. <1 year) which often involves very small changes in total soil organic P, particularly under field conditions (Harrison, 1982b).

In order to determine organic P mineralisation over the short-term, several alternatives to measuring changes in total organic P have been developed. For example, soil organic P fractionation can be useful in determining changes in organic P in the soil over the short-term. Thus Hedley et al.(1982b) were able to detect that significant mineralisation of a particular organic P fraction had occurred after

only 41 days in a simulated rhizosphere soil under laboratory conditions. In the field, Sharpley (1985), using the Bowman and Cole (1978b) fractionation scheme for soil organic P (see section 2.1.3), found that significant mineralisation of the moderately labile organic P fraction occurred during the growing season (March - September) in several native grassland and cropped soils in North America.

Bowman and Cole (1978a) considered that organic P extracted from the soil with sodium bicarbonate (0.5 M $NaHCO_3$ @ pH 8.5) represented the most readily available or labile fraction of the soil organic P. Consequently, Harrison (1982a) developed an assay technique designed to determine the rate of mineralisation of organic P as an indicator of overall soil organic P mineralisation. This mineralisation assay involved incubating the soil with ^{32}P labelled ribonucleic acid (RNA) and determining the amount of ^{32}P released as inorganic P. Using this technique Harrison (1982b) found that significant organic P mineralisation occurred in a wide range of temperate woodland soils.

Integration of the various transformations of P which occur in the soil-plant system over a period of time has enabled several workers to construct detailed models of P cycling in the soil (Cole et al., 1977; Blair et al., 1977; Jones et al., 1984a,b; Sharpley et al., 1984). In some cases these modelling

studies have shown that under temperate conditions soil organic P mineralisation plays an important role in plant P nutrition. Thus Cole et al. (1977) found that in a native grassland soil most of the inorganic P taken from the soil by plant uptake was replenished by the mineralisation of organic P. In a grazed fertilised pasture soil, Blair et al. (1977) showed that the inorganic P released by organic P mineralisation was equivalent to 26-34% of the P taken from the soil by plants annually.

2.3.3.1 Factors Affecting Soil Organic Phosphorus Mineralisation. In general the factors which affect biological activity in the soil also influence the mineralisation of organic P. The major environmental, chemical and soil factors which are known to affect soil organic P mineralisation and are relevant to the present study are discussed below.

2.3.3.1.1 Phosphatase Enzymes. As mentioned previously (section 2.3.3) phosphatase enzymes are widely believed to be involved in the mineralisation of soil organic P. These enzymes mainly function extracellularly in the soil and are thought to be held on mineral colloids (Kiss et al., 1975; Speir and Ross, 1978).

Soil phosphatase activity is commonly assayed by incubating the soil with an artificial substrate such as 4-nitrophenylphosphate or phenylphosphate

and determining the quantity of the organic moiety released as 4-nitrophenol or phenol (Tabatabai and Bremner, 1969; Gerritse and Van Dijk, 1978). However, there is a general lack of uniformity in the assay methods currently used (Malcolm, 1983) which has probably affected progress in understanding the exact role of phosphatase enzymes in soil organic P mineralisation.

Several kinds of phosphatase enzyme capable of mineralising different organic P esters under various soil conditions have been identified. For example, Eivasi and Tabatabai (1977) examined a wide range of soils and identified three different phosphatase enzymes viz. phosphomonoesterase, phosphodiesterase and phosphotriesterase which specifically hydrolysed organic P monoesters, diesters and triesters respectively. The phosphomonoesterase was found to be the predominant enzyme present in most of the soils examined and further determination of its optimum pH revealed that in fact two different monoesterases operated at low and high soil pH. The phosphodiesterase and phosphotriesterase enzymes were less important than the monoesterase and each was present in only one form with the same high pH optimum of 10.

Extracellular phosphatase enzymes in the soil are believed to originate mainly from micro-organisms and plant roots, although earthworms

may also be involved (Speir and Ross, 1978). A wide range of soil micro-organisms have been shown to produce phosphatase enzymes capable of mineralising most organic P compounds known to be present in the soil (Cosgrove, 1967). Evidence that plant roots produce phosphatase enzymes comes from the observed ability of plants growing in solution culture to utilise organic P compounds (Martin, 1973). The respective proportions of the total soil phosphatase activity which originate from the micro-organisms and plant roots are not known, although it is believed that plant phosphatases dominate in the rhizosphere soil (Ridge and Rovira, 1971; Martin, 1973, Hayman, 1975).

Soil phosphatase activity is affected by the level of biological activity in the soil which, in turn, is influenced by seasonal environmental conditions. It is, however, very difficult to distinguish a seasonal pattern of soil phosphatase activity due to the large subsample heterogeneity associated with its determination under field conditions (Harrison, 1979; Harrison and Pearce, 1979). Nonetheless, Harrison (1982b) found that the average phosphatase activity in 50 woodland soils was significantly greater in spring (May) than in autumn (October). Likewise, Bolton et al. (1985) observed that phosphatase activity in soils under arable cropping was greater in summer (July) than in winter (November). Harrison (1982b) showed

that the observed seasonal variation in phosphatase activity was directly related to similar fluctuations in other biological activity indices (labile organic P mineralisation, respiration rate). The increased level of microbial and phosphatase activity in the soil in spring is particularly attributable to the decomposition of accumulated plant residues (Dalal, 1982).

The relationship between phosphatase enzymes and soil organic P mineralisation is not clearly understood. Soil phosphatase activity has been shown to be reduced by the addition of inorganic P (Juma and Tabatabai, 1978; Appiah et al., 1982), while the removal of inorganic P from the soil by plant uptake increases the soil phosphatase activity (Neilsen and Eiland, 1980; Hedley et al., 1982b, 1983). However, the enhanced phosphatase activity observed by Neilsen and Eiland (1980) and Hedley et al. (1982b; 1983) appeared to have little effect on the mineralisation of soil organic P. Furthermore, Halstead and his co-workers (Halstead et al., 1963; Halstead, 1964) found that adding lime to acid soils increased organic P mineralisation but resulted in decreased levels of phosphatase activity. On the other hand, some studies appear to suggest that organic P mineralisation and phosphatase enzyme activity in the soil are closely related (Harrison, 1982b; Dalal, 1982a). Soil P cycling studies conducted by Cole et al. (1977) confirmed

that the relationship between phosphatase activity and organic P mineralisation requires further investigation.

2.3.3.1.2 Chemical Nature of Soil Organic Phosphorus. The mineralisation of soil organic P is believed to be affected by its chemical form. This has been investigated by comparing the mineralisation of various organic P esters added to the soil. The organic P compounds used in these studies have generally been those which are thought to exist in the soil such as inositol phosphates, phospholipids and nucleic acids (see section 2.1.5.3).

In general it has been found that phospholipid and nucleic acid forms of P are more readily mineralised in the soil than inositol phosphates. Bowman and Cole (1978a) showed that after an 18 day incubation period in the soil 60-75% of the added phospholipid and nucleic acid P was mineralised compared with only 3% of the added inositol hexa-phosphate. The observation that the growth and P uptake of barley plants (Hordeum vulgare) were similar in soils supplied with P as inorganic orthophosphate and nucleic acid P (DNA) also suggests that significant mineralisation of nucleic acid P occurs (McKercher and Tollefeson, 1978).

The reduced mineralisation of inositol phosphate compared with phospholipid and nucleic acid P in the soil is believed to be mainly due to

its adsorption onto soil colloids. Greaves and Webley (1969) found that the microbial mineralisation of inositol hexaphosphate in sand was markedly reduced by the inclusion of soil or clay minerals. These workers concluded that this effect was mainly due to adsorption of the inositol hexaphosphate and/or phosphatase enzymes onto the clay minerals. Greaves and Webley (1969) also found that the greatest inhibitory effect on mineralisation was observed with aluminium-saturated clays which suggests that the reduced mineralisation may have been partly due to the formation of the sparingly-soluble aluminium salts of inositol hexaphosphate. Anderson et al. (1974) also attributed the relative stability of inositol hexaphosphate in soil to a combination of colloid adsorption and the formation of sparingly-soluble iron and aluminium salts. Their results indicated that in the acid agricultural soils examined (pH 5.8-6.4) the capacity to adsorb inositol phosphate far exceeded the amounts added to the soil under field conditions and therefore there was a tendency for inositol P to accumulate in the soil.

Since less than half of the total organic P in many soils has actually been identified (see section 2.1.5.3) determining the relative mineralisation of organic P esters such as inositol P, phospholipids and nucleic acids is of somewhat limited value. However, a clearer understanding

of the relationships between the chemical nature of soil organic P and their relative stability may result from the continued use of techniques such as ^{31}P NMR in soil P studies (see section 2.1.5.4). For example, the results of Hawkes et al. (1984) suggest that orthophosphate diester forms of organic P in the soil may be more readily mineralised than orthophosphate monoester organic P compounds. These workers compared a soil maintained under permanent pasture with the same soil which had been continuously cultivated and left fallow for 20 years and found that while there was no difference between the amounts of orthophosphate monoester P in both soils, 87% of the orthophosphate diester P fraction had been lost from the cultivated soil. Orthophosphate monoester P includes the inositol phosphates while diester P includes nucleic acid and phospholipid P and as such the findings of Hawkes et al. (1984) appeared to confirm the relative stabilities of the different organic P compounds as described previously in this section.

2.3.3.1.3 Temperature and Seasonal Effects. Microbial activity in the soil is enhanced by increased temperature, which in turn, can result in increased mineralisation of organic P (Dalal, 1977). Thompson and Black (1949) found that an increase in the temperature from 30° to 35°C increased the mineralisation of soil organic P by 140%.

Not surprisingly several studies have shown that a greater proportion of plant P requirements is derived from organic P mineralisation in soils under tropical than temperate conditions (Anderson, 1980). Nonetheless, significant organic P mineralisation has been shown to occur in soils under temperate conditions. In temperate soils, organic P mineralisation tends to be seasonal and occurs particularly in spring when biological activity in the soil is greatest. Thus Saunders and Metson (1971) found that the increased uptake of P by pastures in spring had no significant effect on the level of available inorganic P in the soil. Although these workers did not determine actual changes in the soil organic P, their results suggested that the additional P taken up by plants in spring was derived from mineralisation of organic P in the soil. On the other hand, both Dormaar (1972) and Sharpley (1985) monitored seasonal variation in the levels of organic P in soils under pasture and rotational cropping and found that there was a marked decrease in organic P in spring which indicated that significant mineralisation had occurred. These workers also found that organic P levels in soil were greater in winter than in spring, indicating that low soil temperatures in winter restricts biological activity and consequent organic P mineralisation (Harrison, 1979).

Harrison (1982b) also showed that the average rate of labile organic P mineralisation in a range of woodland soils was greater in spring (46 ng P day^{-1} cm^{-3}) than in autumn (9 ng P $day^{-1}cm^{-3}$) which agrees with the higher levels of respiration and phosphatase enzyme activity observed in these soils in spring.

2.3.3.1.4 Cultivation and Cropping. Disturbance of the soil by mechanical cultivation can result in an increase in the rate of organic P mineralisation. For example, Hawkes et al. (1984) found that 40% of the total organic P originally present in a soil was mineralised as a result of 20 years continuous fallow maintained by regular mechanical cultivation.

Enhanced mineralisation of soil organic P as a result of mechanical cultivation is mainly due to (i) increased exposure of the soil organic P to microbial attack, and (ii) improved soil aeration increasing overall microbial activity (Anderson, 1980). The increased exposure of organic P to microbial attack under cultivated conditions involves the breakdown of soil aggregates by repeated wetting and drying (Russell, 1973). This was demonstrated by Birch and Friend (1961), who subjected a soil to 204 successive wetting-incubation-drying cycles and found that in addition to all of the soil organic P being mineralised there was a complete breakdown in the soil structure.

Several studies have shown that significant organic P mineralisation occurs as a result of long-term arable cropping. For example, Hass et al. (1961) found that organic P mineralisation accounted for almost all of the P removed from a range of North American soils during 30-48 years of continuous and rotation cropping. These workers found that on average the proportion of total soil organic P mineralised as a result of long-term cropping was greater in the warmer southern states (42%) than in the colder northern states (27%).

In a similar study Tiessen et al. (1982) found that 18-29% of the total soil organic P was mineralised as a result of 60 years of cropping. Organic P mineralisation was greater in a sandy loam soil (27%) than in two heavier textured clay loam soils (17-18%) which may reflect the lower stability of the soil aggregates and hence the organic matter in the sandy soil.

Significant mineralisation of soil organic P has also been shown to occur in several long-term cropping experiments at Rothamsted in England. Using determined decreases in the amounts of total soil organic P over the duration of these trials (20-147 years), Chater and Mattingly (1980) calculated that the annual rate of organic P mineralisation ranged from 0.5 to 8.5 kg P ha^{-1}. In the warmer climates of Oklahoma and Texas in North America, Sharpley (1985) determined much

higher rates of annual organic P mineralisation (27-42 kg P ha^{-1} yr^{-1}) in fertilised and unfertilised soils under continuous and rotation cropping.

2.3.3.1.5 Soil pH and Liming. Several workers have found that organic P mineralisation in the soil can be affected by soil pH. Thompson et al. (1954) used a laboratory incubation technique (40°C for 25 days) to determine organic P mineralisation in a wide range of soils (pH 5.2-8.1) and found that the amount of organic P mineralised was directly related to soil pH. Similarly, Harrison (1982b) observed a close relationship between the rate of labile organic P mineralisation and soil pH (r^2 = .54, $p > .01$) in 28 temperate woodland soils.

As a result of enhanced mineralisation, the total amount of organic P in a particular soil tends to be greater under low pH conditions (Oniani et al., 1973; Harrison, 1982b). For example, Oniani et al. (1973) found that in a pasture soil which had received single superphosphate fertiliser annually for 100 years the amount of organic P which had accumulated was greater in soil maintained at pH 4.5 (134 μg P g^{-1}) than at pH 6.5 (19 μg g^{-1}).

Soil pH is known to affect the levels of microbial activity in the soil. Thus it has been shown that raising the pH of acid soils (pH <6) by the addition of liming materials ($Ca(OH)_2$; $CaCO_3$) can result in increased carbon dioxide (CO_2) evolution

from the soil indicative of increased microbial activity (Halstead et al., 1963; Sarathchandra and Upsdell, 1981). Halstead et al. (1963) also found that the enhanced microbial activity following the addition of lime to an acid soil resulted in an increase in organic P mineralisation. Several other laboratory incubation studies have shown that the addition of liming materials to acid soils increases organic P mineralisation (Islam and Ahmed, 1973; Islam and Mandal, 1977).

The enhancement of soil organic P mineralisation by liming may also be partly due to an increase in the solubility of certain organic P compounds in the soil. Thus Pearson et al. (1941) found that the addition of lime to an acid soil (pH 5.8) increased the proportion of added inositol hexaphosphate mineralised during a laboratory incubation from 5% to 45%. These workers suggest that this might have been due to the predominance of the soluble calcium (Ca) salt of inositol hexaphosphate in the limed soil, whereas the inositol hexaphosphate may have been present mainly as the insoluble iron (Fe) and aluminium (Al) salts in the unlimed soil.

In spite of the importance of liming in pasture management and the large amounts of organic P present in many pasture soils, very few studies have determined the effects of liming on organic P mineralisation in pasture soils.

Quin and Rickard (1979; 1981) found that a large application of lime (4 tonnes ha^{-1}) to slightly acid (pH c. 5.8) pasture soils resulted in some mineralisation of organic P as indicated by a decrease in the total soil organic P. These workers also found that the addition of lime effectively stopped the continued accumulation of organic P in these soils which had occurred during the previous 25 years, particularly in those soils which received annual topdressings of phosphatic fertiliser.

2.3.4 Relationships Between Organic Phosphorus and Other Soil Organic Matter Components

Nitrogen (N), sulphur (S) and phosphorus (P) are the major plant nutrients associated with carbon (C) in the soil organic matter. Their relative proportions vary widely although the greatest variability is associated with the organic P. Thus the organic C:P ratios found in soils range from 46 to 648 compared with ranges of 10-12 and 60-120 for organic C:N and C:S ratios respectively (Stevenson, 1982). This suggests that the level of organic P in the soil is determined and controlled by different mechanisms to those which control the levels of organic C, N and S. Thus it has been found that the relative ratios of soil organic P mineralisation differ

from those of organic C and N. Thompson et al. (1954) and Tiessen et al. (1982) found that during long-term arable cropping (> 20 years) greater proportions of the total organic C and N were mineralised (32-46%) than organic P (18-29%). On the other hand, it has been shown that during laboratory incubation relatively more organic P is mineralised than organic C and N (Thompson et al., 1954; Birch and Friend, 1961).

McGill and Cole (1981) reviewed the available data on the turnover of soil organic C, N, S and P and concluded that the mechanisms involved in organic P turnover are different from those of the other organic matter components. These workers proposed that two separate mechanisms are involved in the cycling of plant nutrients through the soil organic matter:

(i) Organic N and a proportion of the organic S are directly bonded to C in the soil organic matter (i.e. C-N, C-S). These nutrients are released (mineralised) during the microbial oxidation of the organic C to provide energy (biological mineralisation).

(ii) Organic P and the remainder of the organic S are present in the soil organic matter as esters (i.e. P-O-C, S-O-C). These esters are mineralised by the actions of extra-cellular phosphatase and sulphatase enzymes produced by plant roots, mycorrhizae and soil micro-organisms in response to their P and S requirements (biochemical mineralisation).

2.4 FERTILISER PHOSPHORUS IN THE SOIL

2.4.1 Introduction

The use of phosphatic fertiliser has remained an important factor in the development and expansion of pastoral farming in New Zealand. The following review examines the fate of fertiliser P in the soil-plant system and the chemical and biological processes which influence its utilisation.

2.4.2 Single Superphosphate

Ordinary or single superphosphate (SSP) makes up over 90% of the total phosphatic fertiliser used in New Zealand annually (N.Z. Fertiliser Statistics, 1984).

Superphosphate is manufactured by the addition of sulphuric acid (H_2SO_4) to ground phosphate rock to convert the sparingly soluble apatite P ($Ca_{10}(PO_4)_6(OH,F)_2$) to water soluble monocalcium phosphate monohydrate (MCP - $Ca(H_2PO_4)_2.H_2O$) This acidulation of phosphate rock involves two concurrent reactions:

$$18H_2SO_4 + 2Ca_{10}(PO_4)_6(F)_2 \rightarrow 18CaSO_4 + 12H_3PO_4 + 2CaF_2$$

$$12H_3PO_4 + Ca_{10}(PO_4)_6(F)_2 + 9H_2O \rightarrow 9Ca(H_2PO_4)_2.H_2O + CaF_2$$

The main products of the full stoichiometric acidulation or rock phosphate therefore are MCP, calcium sulphate ($CaSO_4$) and calcium fluoride (CaF_2). However, under large scale production conditions full stoichiometric

acidulation of the rock phosphate is not always achieved and thus commercial SSP often contains some low solubility unreacted apatite as well as some free phosphoric acid (H_3PO_4). Incomplete acidulation of rock phosphate during SSP manufacture is a particular problem with rocks containing large quantities of carbonate minerals which neutralise a fraction of the added H_2SO_4. Geochemically, calcium minerals such as calcite ($CaCO_3$) and dolomite ($CaMg(CO_3)_2$) are closely associated with ~~apaite~~ apatite and consequently many phosphate rocks (e.g. Gafsa, Moroccan and Israeli) contain significant quantities of these minerals (McClellan and Gremillion, 1980).

In addition to incomplete acidulation the presence of significant quantities of iron (Fe) and aluminium (Al) oxides (collectively referred to as R_2O_3 minerals) in rock phosphate can result in the formation of sparingly soluble Fe and Al phosphates during the acidulation process. Sedimentary rock phosphates, which are the major forms used in fertiliser manufacture, are known to contain acid soluble Fe and Al oxides such as ge~~e~~oethite and bauxite (McClellan and Gremillion, 1980). During the acidulation process R_2O_3 minerals dissolve and the Fe and Al released can react with the Ca, P and F present to form a wide range of Fe/Al phosphates. The initial products of these reactions are believed to be mainly metastable colloidal gels (e.g. Al/Fe $PO_4 . xH_2O$). In the later stages of the acidulation

process these amorphous Fe/Al-P gels are transformed into more stable crystalline compounds. Several broad groups of Fe/Al phosphates have been identified in commercial superphosphate: $CRP_2F(Ca(Fe,Al)(HPO_4)_2F.4H_2O)$; $CRP_2F_2(Ca(Fe,Al)H(HPO_4)_2F_2.4H_2O)$; $RPF((Fe,Al)(HPO_4)F.2H_2O)$; $R_3P_6(Fe_3(H_3O)H_8(PO_4)_6.6H_2O)$ (White, 1976).

The presence of large amounts of R_2O_3 in phosphate rock and the subsequent formation of various Fe/Al phosphates during the acidulation process also affects the physical characteristics of the SSP produced. Thus SSP made from high R_2O_3 containing phosphate rocks (> 5% R_2O_3) tends to be "sticky" and is difficult to granulate, store and apply (McClellan and Gremillion,1980).

Currently in New Zealand the major phosphate rocks used in SSP manufacture are Nauru (48%) and Christmas Island (43%), while small quantities of North Carolina (6%) and Florida (3%) phosphate rocks are also used (N.Z. Fertiliser Statistics, 1984). The Nauru, Florida and North Carolina phosphate rocks contain low levels of R_2O_3 (0.92-2.12% w/w) compared with Christmas Island phosphate rock which contains about 5% R_2O_3 (McClellan and Gremillion, 1980; Charleston, 1984). Consequently, the SSP manufactured in New Zealand generally contains significant quantities of Fe/Al phosphates (up to 20% of the total P), which may influence the agronomic effectiveness of the fertiliser.

Thus in commercially produced SSP, most of the P is present as water-soluble MCP while the remainder is mainly present as Fe/Al phosphates and unreacted apatite.

2.4.3 Chemical Reactions of Soluble Fertiliser Phosphorus in the Soil

When a mainly water-soluble P source such as the MCP in SSP is added to the soil there is an immediate and large increase in the concentration of inorganic P in the soil solution. The dissolution of added MCP in the soil and the subsequent reactions which occur between the soluble P released and the soil mineral colloids have been studied extensively and are discussed below.

2.4.3.1 Initial Reactions of Soluble Fertiliser Phosphorus in the Soil. The initial dissolution of the MCP in granular SSP results from the diffusion of water into the fertiliser granule from the surrounding soil. Thus the MCP is hydrolysed by water to form an acid (pH 1.48) meta-stable triple point solution (MTPS) of MCP, dicalcium phosphate (DCP-$CaHPO_4$) and phosphoric acid (H_3PO_4) which diffuses rapidly from the granule, although 20-34% of the original MCP-P is precipitated as the dihydrate form of DCP (DCPD-$CaHPO_4.2H_2O$) within the granule (Lawton and Vomocil, 1954; Lehr et al., 1959; Lindsay and Stephenson, 1959; Sample et al, 1980).

The MTPS diffuses into the soil surrounding the granule and causes a dramatic decrease in the pH of the soil solution (pH 1-3) which, in turn, results in the dissolution of various metal cations (Al^{3+}, Fe^{3+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, K^{+}) from the mineral colloids and organic matter. Under these conditions of high P and metal cation concentrations and low pH, various sparingly soluble inorganic P salts may be precipitated (Sample et al., 1980).

The chemical nature of the precipitates formed by reaction of the MTPS with soil colloids are largely unknown although they are likely to be influenced by the dominant cations present in the soil. Thus, in neutral and acid soils amorphous iron and aluminium phosphates such as metastrengite ($FePO_4.2H_2O$) and metavariscite ($AlPO_4.2H_2O$) are likely to be the dominant P species formed, while calcium phosphates such as dicalcium phosphate (DCPD - $CaHPO_4.2H_2O$) and octocalcium phosphate ($Ca_8H_2(PO_4)_6.5H_2O$) are most likely to be formed in calcareous soils (Sample et al., 1980).

The combined effects of the precipitation reactions described above and the slow diffusion of the soluble fertiliser P into a greater soil volume, decreases the concentration of P in the soil solution. As the concentration of P in the soil solution decreases and the pH of the soil solution increases, conditions become less favourable for precipitation and more favourable for the adsorption of the

inorganic P onto soil colloids (Rajan, 1977; Sample et al., 1980).

The principal sites for P adsorption in most soils are the variably charged surfaces of hydrous iron and aluminium oxides and aluminosilicate minerals (clays), while some adsorption onto calcium carbonate (calcite) can occur in calcareous soils (White, 1980).

It is generally believed (Barrow, 1980; White, 1980) that inorganic P is specifically adsorbed onto the reactive surfaces of hydrous iron and aluminium oxides by the displacement of bonded hydroxyl ions (OH^-) and water molecules (Figure 2.4.3.1.1).

The initial stages of P adsorption onto hydrous iron and aluminium oxides are believed to involve electrostatic attraction between the negatively charged phosphate ($H_2PO_4^-$, HPO_4^{2-}) and the positively charged oxide surface. However, the adsorption of P can result in a decrease in the overall positive charge of the surface (see Figure 2.4.3.1.1) which may, in turn, make further adsorption of negatively charged P more difficult.

The adsorption of P onto aluminosilicates is thought to mainly occur at the amorphous edges of these minerals. The mechanisms involved are believed to be similar to those for adsorption of P onto hydrous iron and aluminium oxides described above, although it may involve the displacement of

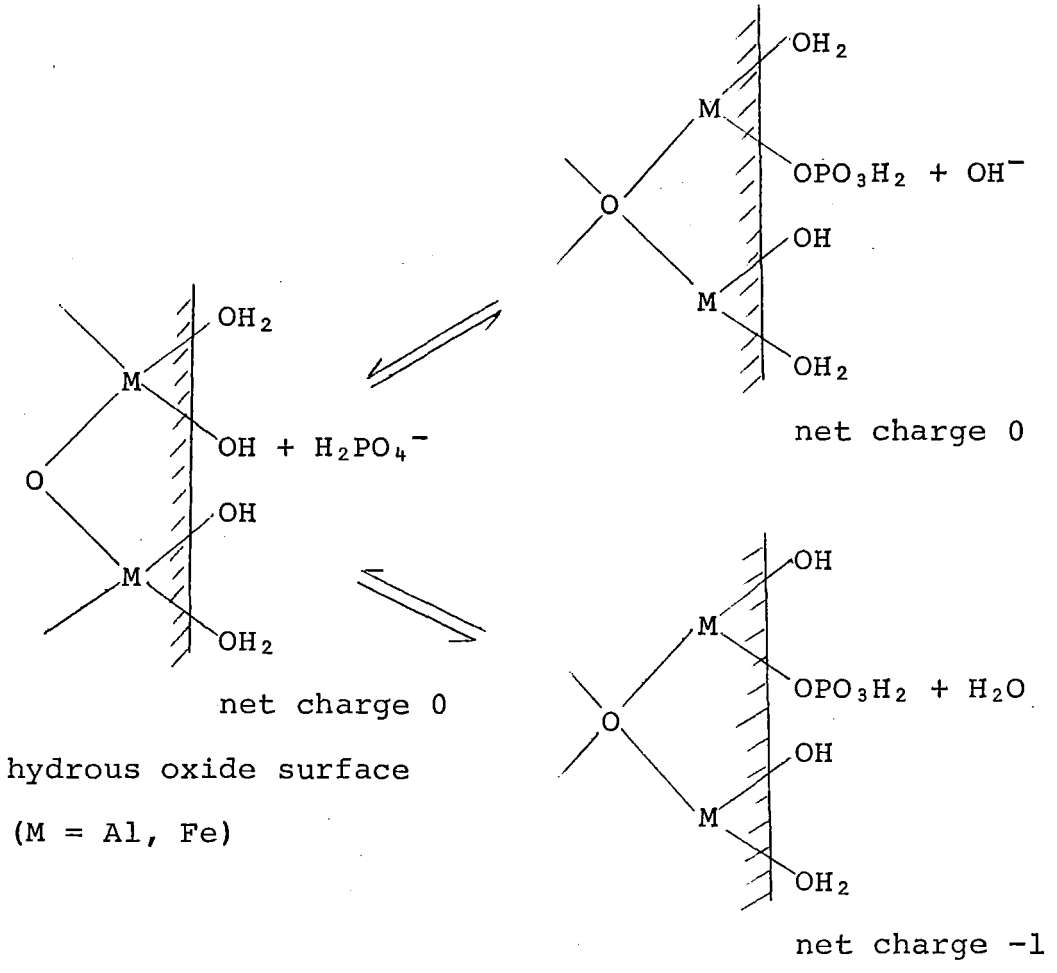


Figure 2.4.3.1.1 The adsorption of inorganic P onto hydrous iron and aluminium oxide surfaces in the soil (Barrow, 1980).

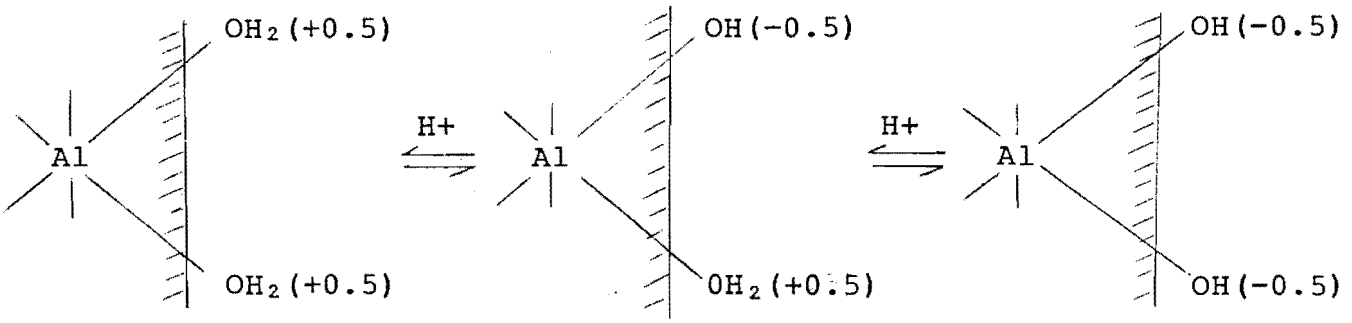
bonded silicates in addition to hydroxyl ions and water molecules (White, 1980).

In some situations saturation of the adsorption sites in hydrous oxide and aluminosilicate surfaces with P may lead to some breakdown of their mineral structure and result in the formation of new amorphous iron and aluminium phosphates which, in turn, may adsorb more P (Rajan, 1977).

The mechanisms involved in the adsorption of P onto calcium carbonate (calcite) surfaces are not fully understood. It is believed to involve adsorption of P onto the mineral surface which then acts as a "nucleus" for the formation of calcium phosphates such as octocalcium phosphate ($Ca_8H_2(PO_4)_6.5H_2O$) and hydroxy-apatite ($Ca_{10}(PO_4)_6(OH_2)$) (White, 1980). Thus, the "adsorption" of P onto calcium carbonate in soil may involve both adsorption and precipitation reactions.

Soil organic colloids may also adsorb P via their associated iron and aluminium complexes. However, very little is known about the mechanisms involved and the importance of P adsorption on organic matter relative to mineral colloids in the soil (White, 1980).

Soil pH may be an important factor in determining the nature and strength of P adsorption onto soil colloids. Thus pH is known to affect the net charge on the surface of hydrous iron and aluminium oxides as shown in Figure 2.4.3.1.2.



Net charge:+1 Net charge:0 Net charge:-1

PZC (pH 8.5)

Figure 2.4.3.1.2 The nature of the pH dependent charge on a hydrous aluminium oxide surface (White, 1980).

The point of zero charge (Pzc) of pure hydrous iron and aluminium oxide surfaces occurs at pH 8.5-9.0, which means that at most soil pHs (4.0-6.0) the surfaces of these minerals are likely to be positively charged (White, 1980). This net positive charge facilitates the attraction and subsequent adsorption of negatively charged P (HPO_4^{2-}, $H_2PO_4^-$) from the soil solution. However, increasing the pH of a soil by the addition of liming materials ($CaCO_3$, $Ca(OH)_2$) may decrease the positive charge ~~as~~ of the hydrous iron and aluminium oxides thereby making them less favourable sites for P adsorption (Figure 2.4.3.1.2). On the other hand, the addition of calcium in these liming materials may increase the positive charge on these colloid surfaces which may, in turn, partly offset the effects of increased pH (Haynes, 1984).

While it is known that approximately 25% of the added MCP is precipitated as dicalcium phosphate (DCP) within the fertiliser granule, the relative importance of the precipitation and adsorption reactions of the remaining soluble P with the soil colloids described above has not been determined (Sample et al., 1980).

2.4.3.2 Long-term Reactions. The precipitation and adsorption reactions described above (Section 2.4.3.1) converts the water-soluble fertiliser P (MCP) to a variety of sparingly soluble mineral (precipitated) and adsorbed forms of P in the soil. This mineral

and adsorbed fertiliser P in the soil can contribute to plant P nutrition via equilibration with the soil solution. Thus, inorganic P removed from the soil solution by plant uptake may be replenished by dissolution of the precipitated fertiliser P and desorption of the adsorbed fertiliser P. These reactions therefore provide a continued supply of fertiliser P to plants over a period of time. However, with time there is tendency for the precipitated and adsorbed fertiliser P in the soil to slowly become more stable, less exchangeable with the soil solution and thereby less plant available. These so called "slow reactions" of fertiliser P in the soil affect the overall long-term utilisation of the fertiliser P (Barrow, 1980).

The precipitates formed initially in the soil from added soluble P are believed to be metastable (amorphous) and in time change to more stable, crystalline mineral forms which are less soluble and therefore less plant available. For example, Lindsay et al. (1959) found that aluminium phosphates formed from MCP during its initial reactions in the soil had declined in solubility by 10-30 fold after 18 months. Similarly, amorphous dicalcium phosphate (DCPD) formed during the initial reactions of MCP in the soil may gradually change to more stable octocalcium phosphate (OCP) and eventually hydroxyapatite (HAP):

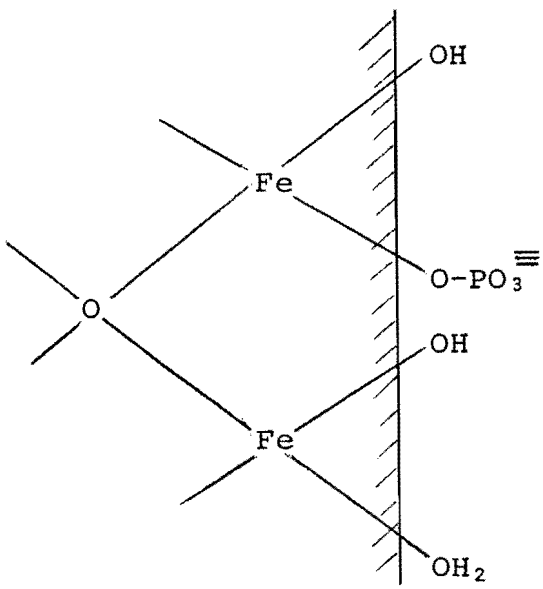
$$CaHPO_4.2H_2O \rightarrow Ca_8H_2(PO_4)_6 \rightarrow Ca_{10}(PO_4)_6(OH)_2$$

DCPD OCP HAP

Initially fertiliser P adsorbed ~~in~~ on soil colloid surfaces is readily released again (desorbed) in response to a decrease in the amount of inorganic P in the soil solution (e.g. by plant uptake). However, in time some of the adsorbed P on the colloid surfaces is converted to forms which are less readily desorbed.

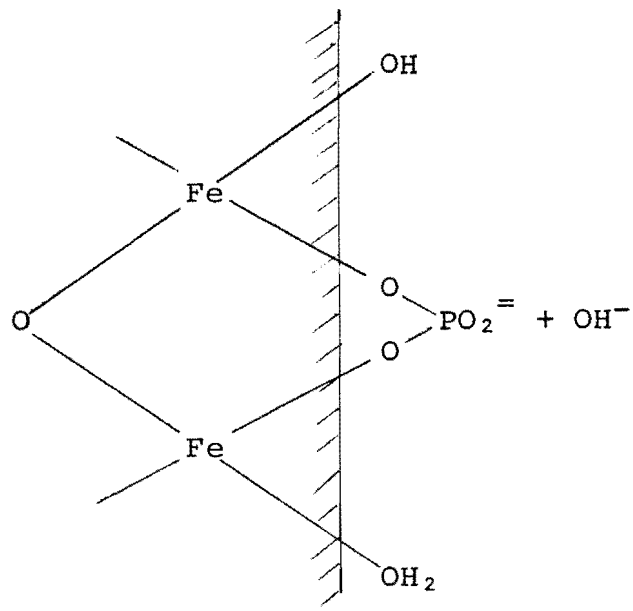
Two mechanisms have been proposed to explain the conversion of readily desorbable P to more stable forms. Firstly, the chemical nature of the adsorbed P is slowly changed from a monodentate ligand form to a bidentate ligand form which is more stable (Mattingly, 1975 - see Figure 2.4.3.2.1). Secondly, some of the adsorbed P initially on the surface of the mineral colloids is slowly transferred into the interior of the mineral by solid-state diffusion (Barrow, 1983). This "penetrated P" is less exchangeable with the soil solution and therefore less available to plants than surface adsorbed P.

The overall effect of the slow reactions of the adsorbed and precipitated forms of soluble fertiliser P on its plant availability in the soil ~~were~~ was demonstrated by Devine et al. (1968). These workers found that incubating single superphosphate in the soil for 1,2 and 3 years reduced its subsequent plant availability by 41, 62 and 80% respectively when compared with freshly applied fertiliser.



Monodentate

("Reversibly adsorbed P")



Bidentate

("Irreversibly adsorbed P")

Figure 2.4.3.2.1 The change in the chemical nature of P adsorbed on an hydrous iron oxide surface with time (Mattingly, 1975).

It is thought that the conversion of initially precipitated and adsorbed fertiliser P in the soil to increasingly stable forms may be reversed only slowly (Barrow, 1980). Consequently, these reactions may effectively represent a permanent reduction in the plant availability of added fertiliser P in the soil.

2.4.4 Biological Reactions of Fertiliser Phosphorus

2.4.4.1 Direct Microbial Immobilisation.

In addition to the removal of soluble fertiliser P from the soil by reaction with soil colloids as described above (section 2.4.3) some of the soluble P may be taken up by soil micro-organisms (i.e. microbial immobilisation).

The significance of the microbial immobilisation of soluble fertiliser P following its addition to the soil is not yet known, although it has been shown to occur in laboratory incubation studies. The demand for P by soil micro-organisms is influenced by their overall metabolic activity which is affected mainly by the availability of suitable carbon substrate material as energy sources. Thus, both Ghoshal (1975) and Ghoshal and Jansson (1975) found that the inclusion of glucose increased the microbial immobilisation of added soluble P during a laboratory incubation. Ghoshal (1975) also showed that as a result of enhancing microbial immobilisation, the inclusion of glucose reduced the immediate plant

availability of the added P. In his experiments conducted over 17 days, ryegrass seedlings recovered only 37% of the added P from glucose amended soils compared with 48% from unamended soils.

Most of the added soluble P taken up by micro-organisms in the soil is likely to eventually be released again as inorganic and organic P in microbial residues. Thus several workers have observed an increase in the amount of organic P in soil during incubation with added soluble P, particularly when a carbon substrate was included (Goring, 1955; Chauhan et al., 1979; 1981; Hedley et al., 1982a). For example, Chauhan et al. (1979; 1981) found that in cellulose amended soils microbial immobilisation of added soluble inorganic P increased the amounts of organic P present (Table 2.4.4.1.1).

It is possible that some microbial immobilisation of soluble fertiliser P occurs in the field since P fertiliser is generally applied in spring when the environmental conditions and substrate availability favour high microbial activity (Hayman, 1975; Harrison, 1979). The microbial immobilisation of fertiliser P may also contribute to the accumulation of organic P observed in many fertilised soils (see section 2.4.4.3).

Table 2.4.4.1.1 Total organic P in differently amended soils after a 9 month laboratory incubation period (Chauhan et al., 1979; 1981).

Soil	Total soil organic P (μg P g^{-1})		
	Control[a]	+ inorganic P[b]	+ inorganic P and cellulose[b]
Oxbow Ap	474	483	503
Oxbow Bm	311	317	335
Bradwell Ap	345	348	380

[a] = no amendments

[b] = added every 30 days

2.4.4.2 Recycling of Fertiliser Phosphorus in Plant and Animal Residues. Additions of soluble P in fertiliser generally increases plant growth which results in an increase in the amounts of P returned to the soil in plant and animal residues. This recycling of fertiliser P is particularly important in soils under grazed pasture. For example, in an intensively grazed irrigated pasture Quin and Rickard (1979) estimated that the combined annual returns of P to the soil in plant and animal residues actually exceeded the amounts of P added in fertiliser. These workers calculated that in pastures receiving 19 and 38 kg P ha^{-1} yr^{-1} as SSP the corresponding returns of P in plant litter and animal dung were 31 and 46 kg P ha^{-1} yr^{-1} respectively.

As discussed previously, the inorganic and organic P present in plant and animal residues is released in the soil mainly as a result of microbial decomposition (see section 2.2.4). The inorganic P released directly from plant and animal residues and as a result of the mineralisation of organic P present in the residues may be taken up directly by plants, although most of it is probably adsorbed onto soil colloids to form part of the labile and non-labile pools (see sections 2.2.2 and 2.2.3).

2.4.4.3 Accumulation of Organic Phosphorus in Soil Resulting from the Addition of Phosphatic Fertiliser. It is possible that some of the soluble fertiliser P recycled as organic P in plant, animal and microbial residues may be mineralised slowly in the soil and may therefore accumulate. For example, it is known that nucleic acids and phospholipids are mineralised more rapidly in the soil than inositol phosphates (see section 2.3.3.1.2).

Thus a fraction of the soluble fertiliser P added to the soil may be converted to relatively stable forms of organic P which may, in turn, accumulate in the soil as soil organic P. The accumulation of organic P in the soil as a result of P fertiliser application has been found to occur particularly in soils under grazed pasture. In Australia and New Zealand many of the soils are low in native P and require regular additions of phosphatic fertiliser to enable the establishment and maintenance of productive pasture. Several studies have shown that a large proportion of the fertiliser P applied following the establishment of legume based pasture on P deficient land is converted to organic P in the soil (Donald and Williams, 1954; Walker et al., 1954; Jackman, 1955; Rixon, 1966; Quin and Rickard, 1979 - see Table 2.4.4.3.1).

The rate of accumulation of organic P in improved pasture soils decreases with time following pasture establishment. In New Zealand, Jackman (1964)

Table 2.4.4.3.1 The accumulation of fertiliser P as organic P in soils under recently established pasture.

Years under pasture	Total fertiliser P added (kg P ha^{-1})	% total fertiliser P in soil organic P	Reference
4	170	82-100	Rixon (1966)
4	150	20-53	Jackman (1955)
25	160	$\underline{c}$ 50	Donald & Williams (1954)
25	475-950	41-68	Quin & Rickard (1979)

found that during the first 10 years following pasture establishment around 30% of the fertiliser P applied annually was converted to organic P in the soil. However, the rate of organic P accumulation decreased to about 17% during the following 20 years (i.e. 10-30 years under pasture) indicating that a steady-state was slowly being attained. Under steady-state conditions the respective rates of organic P addition to the soil and soil organic P mineralisation are approximately equal. Thus, Oniani et al. (1973) found that in soils which had been under pasture for 100 years and received annual topdressings of phosphatic fertiliser only 1-6% of the total P added was present as organic P in the soil.

The data presented by Jackman (1964) indicated that in most cases there was a close relationship between the accumulation of organic P and other organic matter compounds (carbon and nitrogen) in fertilised pasture soils. However, Quin and Rickard (1981) found that while the accumulations of organic carbon and nitrogen in the soil ceased 10-15 years after pasture establishment, soil organic P was still increasing after 25 years. This is consistent with the model of nutrient cycling through the organic matter proposed by McGill and Cole (1981) in which the turnover of organic P in the soil is controlled separately from that of the organic carbon and

nitrogen (see section 2.3.4).

The accumulation of organic P in the soil from P fertiliser addition is not restricted to pasture soils. Van Diest (1968a) found that large increases in the amounts of organic P occurred in a wide range of cultivated soils. This occurred as a result of large inputs of P fertiliser after 30 years.

2.4.5 Residual Effects of Fertiliser Phosphorus in the Soil

In most agricultural systems, less than half of the applied fertiliser P is actually lost from the soil in the growing season immediately following application.

Fertiliser P is lost from the soil mainly in off-farm produce (e.g. grain, meat, wool, milk) and by stock transfers of P in urine and dung to stock campsites or other parts of the farm. The extent of this loss of P from the soil is influenced mainly by the type and intensity of the land use practised. Thus the actual loss of added fertiliser P from the soil is likely to be greater with intensive cropping and dairying than with meat and wool production (Holford, 1977). For example, in New Zealand the annual losses of P in off-farm produce and stock transfers from soils under dairy and sheep farming have been estimated at 47% and 10-21% of the applied fertiliser P respectively

(Quin and Rickard, 1979; Parfitt, 1980). On a national basis it has been estimated that between 10 and 30% of the fertiliser P applied annually is lost from the soil in farm produce (Holford, 1977; Barrow, 1980).

In addition to the removal of P in farm produce and by stock transfer, some fertiliser P may be lost from the soil by surface erosion (run-off) and leaching. The latter is important in light textured sandy soils where between 20 and 80% of the fertiliser P applied annually may be leached from the topsoil (Barrow, 1980).

The fraction of the original applied fertiliser P not lost from the soil in the growing season immediately following application can continue to supply P to plants in the following seasons. Thus a particular application of fertiliser P can have a residual effect on plant P nutrition and production for several years (Read et al., 1973; 1977; Quin and Rickard, 1979). For example, Quin and Rickard (1979) found that previous applications of fertiliser P (SSP) to an irrigated pasture continued to have a significant effect on pasture yield for 20 years. Thus in 1978/79 dry matter yields from plots which had received large dressings of SSP (38-56 kg P ha^{-1} yr^{-1}, 1952-57) were about 50% greater than that from adjacent plots which had not received P fertiliser since 1952. Similarly, several studies have shown that a single large application of P fertiliser can be sufficient for several maximum yielding crops before further

fertiliser addition is required (Leamer, 1963; Spratt et al., 1980; Tonello et al., 1981).

The actual amount of residual fertiliser P in the soil and its effects on plant P nutrition are compounded by continued annual applications of P fertiliser. Several workers have found that in soils which have received regular applications of P fertiliser for many years, the levels of plant-available or labile inorganic P far exceed those required for maximum crop yields (Bowman et al., 1978; Novais and Kamprath, 1978; Adepoju et al., 1982) as shown in Table 2.4.5.1. The high levels of labile inorganic P in these soils suggest that they may be capable of supporting several crops without any further additions of P fertiliser. Thus Bowman et al. (1978) demonstrated that in the greenhouse between 5 and 7 successive crops could be grown in previously heavy fertilised soils before any symptoms of P deficiency were apparent. Similarly, Novais and Kamprath (1978) estimated that the residual fertiliser P in 3 heavily fertilised soils could provide sufficient P (21-131 kg P ha^{-1}) for up to 6 maximum yielding crops of corn (Zea mays).

Obviously, the accumulation of such excessive quantities of plant-available residual fertiliser P in the soil is unnecessary and indicative of poor management and/or inaccurate calculation of the P fertiliser requirements. The problem of overestimating (and underestimating) immediate and long-term P

Table 2.4.5.1 Levels of plant-available P in some heavily fertilised soils in relation to plant P requirements.

Soil test[a]	No. soils	Soil test value (µg P g^{-1})		Reference
		Mean	Critical[b]	
Olsen P	23	74	22	Bowman et al. (1978)
Olsen P	12	44	10-15	Adepoju et al. (1982)
North Carolina P	3	114	25	Novais & Kamprath (1978)

[a] = see Table 2.2.2.1

[b] = Critical soil test value indicates the level of available P in the soil above which further addition of fertiliser P makes no difference to crop yield.

fertiliser requirements can be partly overcome by the use of computer models such as those recently developed in Australia and New Zealand (Bowden and Bennett,1975; Cornforth and Sinclair, 1982).

When a soluble P source such as MCP is applied to soils under steady-state conditions (i.e. soils in which there is no significant net conversion of added fertiliser P to soil organic P), most of the residual fertiliser P present in the soil at the end of the growing season following application will be present as adsorbed and mineral forms of inorganic P on the soil colloids (Chang and Chu, 1961; Barrow, 1980). These mineral forms of P result from the initial dissolution, adsorption and precipitation reactions ~~methods~~ of the soluble fertiliser P in the soil described in section 2.4.3.1.

The nature and plant availability of the residual fertiliser P in the soil will depend on the nature of the initial reactions of the soluble P with the soil colloids which, in turn, will be influenced by various soil properties. In an extensive study of 23 heavily fertilised calcareous soils (pH 6.8-7.8), Olsen et al. (1983) found that octocalcium phosphate (OCP - $Ca_8H_2(PO_4)_6.5H_2O$) was the major form of residual fertiliser P present, and showed that this OCP was readily utilised by plants during exhaustive greenhouse cropping. In a similar study Adepoju et al. (1982) examined several heavily fertilised neutral and calcareous soils

(pH 6.4-8.2) and found that the relative plant availability of the residual fertiliser P present was greater in the calcareous soils than in the more acid soils. Accordingly, 2 broad groups of soils were identified on the basis of the relative plant availability of the residual fertiliser P (Table 2.4.5.2). The findings of this particular study tentatively suggest that the predominant mechanisms of soluble fertiliser P fixation in acid and neutral soils (precipitation of iron/aluminium phosphates and P adsorption onto clays and hydrous oxides) reduce the long-term agronomic effectiveness of the residual P compared with calcareous soils in which the soluble P is mainly fixed by the precipitation of calcium phosphates and adsorption onto free calcite (see section 2.4.3.1).

The initial products of the various reactions of soluble fertiliser P in the soil are known to become more stable and consequently less plant available with time (see Section 2.4.3.2). These "slow reactions" of fertiliser P in the soil will undoubtedly affect the long-term utilisation of added fertiliser P although their importance is difficult to determine, particularly in soils where P fertiliser is added annually over a number of years.

Clearly, further studies are necessary to identify the specific forms of residual fertiliser P and determine their relative short and long-term plant availabilities.

Table 2.4.5.2 Relationship between selected soil properties and the plant availability of residual fertiliser P in neutral and calcareous soils (Adepoju et al., 1982).

Soil Property	Residual P availability	
	High	Low
pH	7.8	6.9
Calcium carbonate (%)	6.6	2.2
Soluble calcium (meq l^{-1})	20.6	3.7
Iron and aluminium oxides (%)	1.8	2.5

CHAPTER 3

EFFECTS OF LONG-TERM PHOSPHATIC FERTILISER APPLICATIONS ON AMOUNTS AND FORMS OF INORGANIC AND ORGANIC PHOSPHORUS IN SOILS UNDER IRRIGATED PASTURE

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CHAPTER 3

EFFECTS OF LONG-TERM PHOSPHATIC FERTILISER APPLICATIONS ON AMOUNTS AND FORMS OF INORGANIC AND ORGANIC PHOSPHORUS IN SOILS UNDER IRRIGATED PASTURE.

3.1 INTRODUCTION

In 1952 a comprehensive field trial was initiated at Winchmore Irrigation Research Station in Mid-Canterbury to determine the long-term phosphatic fertiliser requirements of intensively grazed irrigated pasture.

Soil samples taken regularly from the long-term trial were used mainly to determine the general fertility status of the soils, although changes in total inorganic and organic P contents of the soils were also determined. The latter analyses have shown that total soil organic P increased during the first 20 years of the trial, particularly in soils under pasture which received P fertiliser (Quin and Rickard, 1981).

Topsoil (0-7.5cm) samples taken from the various treatments in the long-term trial since 1958 have been retained and were made available for the present study. The objective of this study was to determine the different forms of inorganic and organic P in the soil and the changes which occurred as a result of the different treatments included in the trial. This involved using a sequential fractionation method to separate and identify different forms of inorganic and organic P present.

3.2 MATERIALS AND METHODS

3.2.1 Trial Site and Treatments

Winchmore Irrigation Research Station, situated 13km north-east of Ashburton in Mid-Canterbury (Figure 3.2.1), is administered by the Research Division of the New Zealand Ministry of Agriculture and Fisheries. The station was established in 1946 primarily to determine the effects of border-dyke irrigation on pasture and crop production in the Canterbury region.

The Winchmore site is typical of large areas of Canterbury which are prone to drought conditions during the summer months. The average annual rainfall at Winchmore is 750mm and the mean monthly temperature ranges from 4.9°C in July to 15.9° in January. However, during December and January the temperature often exceeds 25°C and warm, dry north-westerly winds are common during spring and summer. Consequently, in the absence of irrigation, drought conditions are commonly experienced in summer in this area.

Another contributing factor to drought susceptibility in Canterbury is the predominance of shallow, free-draining soils. The Lismore stoney silt loam (Udic Ustochrept) at Winchmore is the typical soil type. It is a shallow (30-45cm), moderately weathered, free-draining soil derived from accumulations of Greywacke alluvium and loess on alluvial gravels. Some of the major chemical and

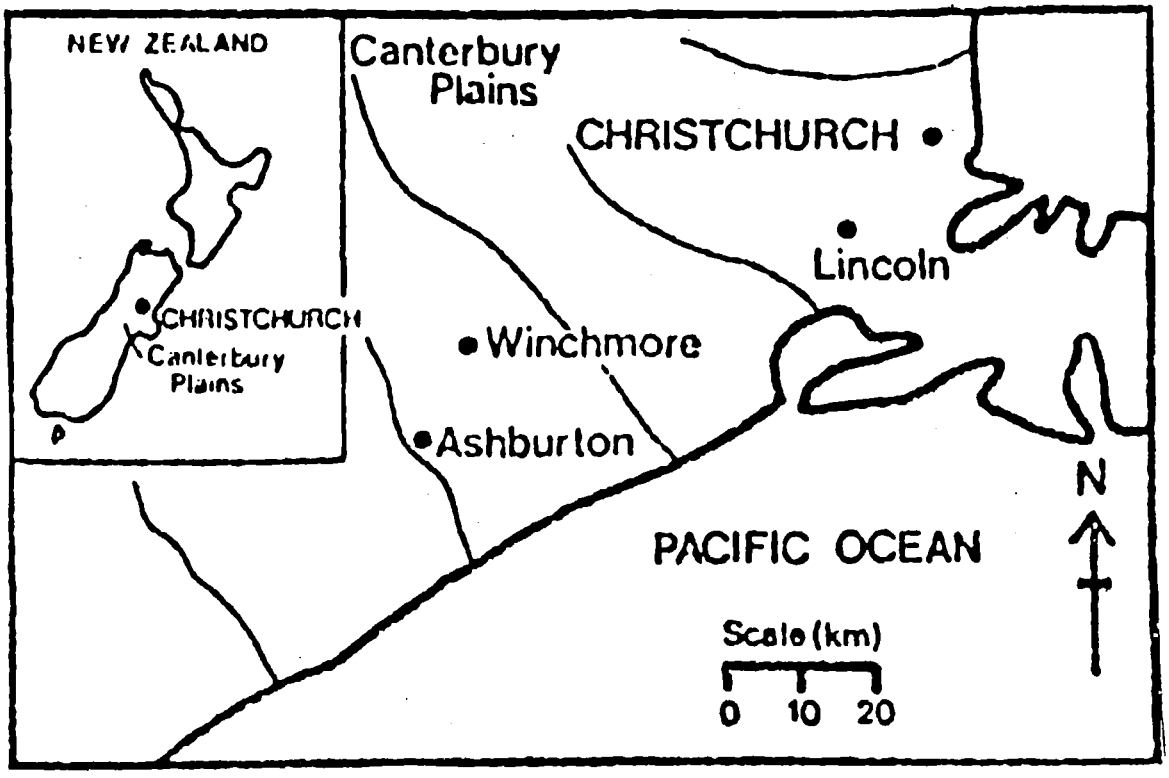


Figure 3.2.1. Location of the Winchmore Irrigation Research Station in New Zealand.

Table 3.2.1.1 Major chemical and textural properties of Lismore stoney silt loam soil (Udic Ustochrept)[a].

Soil property	Soil depth (cm)	
	0-7.5	7.5-15.0
pH (H_2O)	5.6	5.8
organic carbon (%)	3.6	2.8
CEC (meq %)	13.6	12.0
P retention (%)	27	30
sand (%)	45	41
silt (%)	30	29
clay (%)	24	24

[a] From Soils of New Zealand (part 3). N.Z. Soil Bureau Bulletin 1968. 26(3) pp.28-29.

textural properties of the Lismore silt loam soil are shown in Table 3.2.1.1.

The site on which the long-term superphosphate trial is situated was originally under unfertilised browntop (Agrostis tenuis) pasture. In 1948 the browntop pasture was ploughed up and the site prepared for border-dyke irrigation. This involved the subdivision of the trial area into 20 separately fenced separately irrigated 0.08 ha. borders. In 1950, a new perennial ryegrass (Lolium perenne)-white clover (Trifolium repens) pasture was sown and allowed to establish for 2 years prior to the commencement of the long-term trial in 1952. During this preparatory period (1948-1952), the whole trial area received 420 kg ha^{-1} of superphosphate (42 kg P ha^{-1}) and 4t ha^{-1} of lime ($CaCO_3$).

The long-term trial incorporated 5 different treatments with 4 replicates each arranged in a randomised complete block design. Details of the various treatments are shown in Table 3.2.1.2.

Each treatment was grazed rotationally with a separate flock of dry ewes at an appropriate stocking rate, and the trial was irrigated as required (4-5 times $year^{-1}$). In 1972 the whole trial received lime (4 t ha^{-1}) to maintain soil pH above 6.

3.2.2 Soils Used

Composite topsoil (0-7.5cm) samples taken from each of the 4 replicates of each treatment in autumn (April) of the following years were selected for the

Table 3.2.1.2 Treatments included in the Winchmore long-term superphosphate fertiliser trial.

Treatment	Superphosphate applied[a]
Control (CON)	None since 1952
188PA	188kg ha^{-1} yr^{-1} since 1952
376PA	376kg ha^{-1} yr^{-1} since 1952
376R	376kg ha^{-1} yr^{-1} 1952-57, none 1958-80[b]
564R	564kg ha^{-1} yr^{-1} 1952-57, none 1958-80[b]

[a] = superphosphate - c.10% P: applied in spring (September).

[b] = treatments discontinued in 1980.

present study: 1958 1961 1965 1968 1971 1974 1977. These samples were already air dried and were uniformly ground (< 150 μm) prior to analysis.

3.2.3 Soil Phosphorus Fractionation

The fractionation scheme used was based on that of Stewart *et al*. (1980) (see section 2.1.3). This involved sequential extraction of P from the soil with anion exchange resin, 0.5M $NaHCO_3$ (pH 8.5), 0.1M NaOH, ultrasonification-0.1M NaOH, and 1M HCl. Several modifications were made to the original fractionation scheme before it was adopted for use in the present study. These include:

(i) The elimination of the anion exchange resin extraction procedure because it was found that it was difficult to quantitatively recover the soil following extraction.

(ii) The elimination of the ultrasonic dispersion treatment to break up soil aggregates which was unnecessary since the soil was already finely ground (< 150 μm).

(iii) A second 0.1M NaOH extraction was included after the 1M HCl step to improve the recovery of total P from the soil by sequential extraction.

(iv) Residual P (non-extracted P) was determined by the difference between the total soil P and the sum of the total P in the various fractions extracted from the soil as:

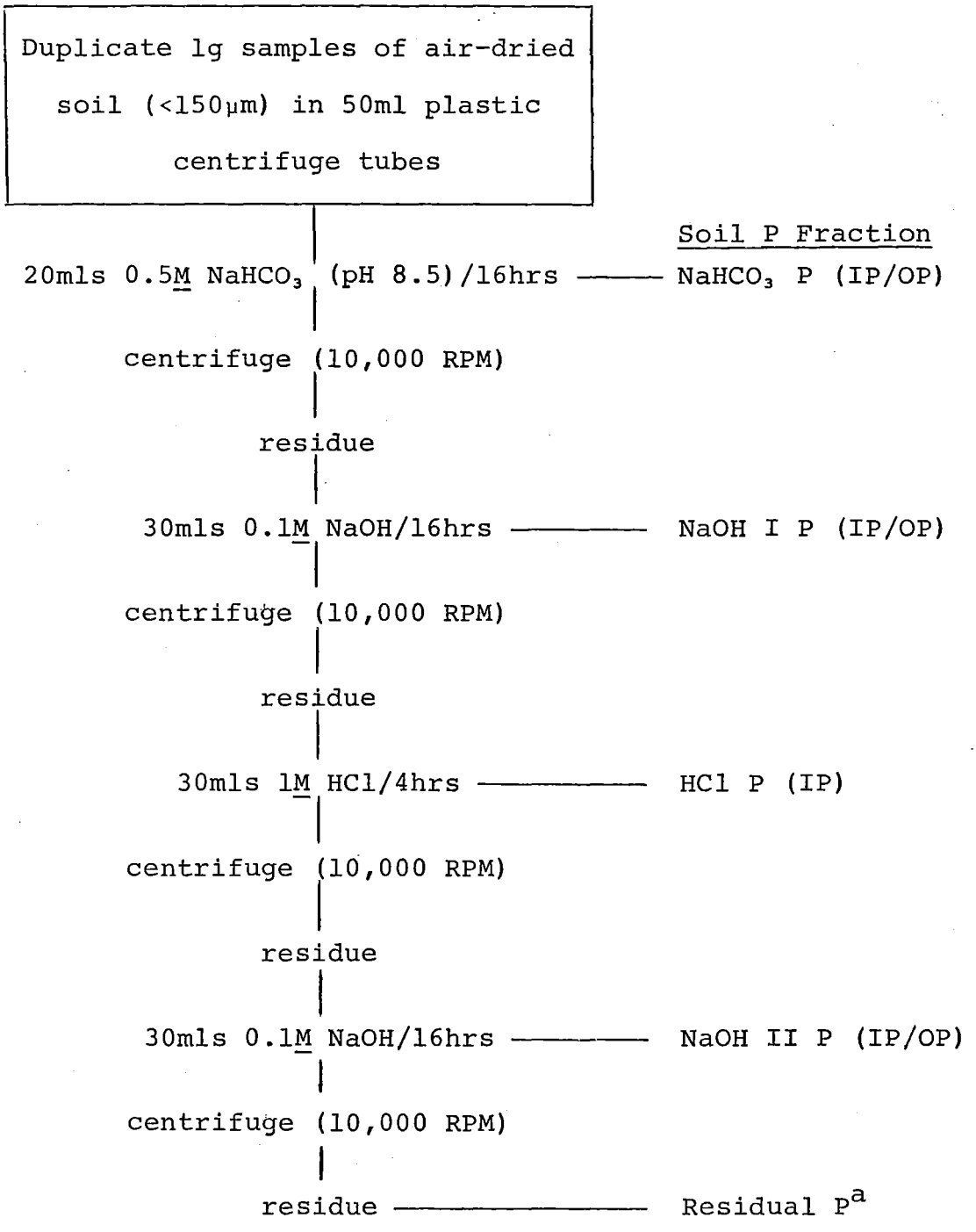
Residual P = total soil P - ($NaHCO_3$ P + NaOH I P + HCl P + NaOH II P).

The soil P fractionation scheme developed for use in the present study is shown in Figure 3.2.3.1. All of the reagents used were prepared from analytical grade (Anlar) reagents and distilled water and all analyses were performed in duplicate.

3.2.4 Determination of Phosphorus in Soil Extracts

Amounts of inorganic, total and organic P in the various extracts obtained from the soil P fractionation scheme (i.e. $NaHCO_3$, NaOH I, HCl and NaOH II) were determined colorimetrically. Most of the reagents used in the determination of P in soil extracts were analytical grade (Anlar), although laboratory grade (LR) reagents were used where appropriate.

3.2.4.1 Inorganic Phosphorus. Inorganic P in aliquots (1-5mls) of soil extracts was determined according to the method of Dick and Tabatabai (1977) which overcomes any possible overestimation of inorganic P by the acid hydrolysis of organic P present in the soil extracts. The absorbance of the sample at 700nm was determined using a Shimadzu 210-A double beam spectrophotometer fitted with a 1cm automatic flow-cell. The results obtained were corrected for any absorbance due to organic matter in the soil extracts using blanks containing the appropriate aliquots of soil extract and distilled



IP = inorganic P; OP = organic P

[a] = Total soil P - ($NaHCO_3$ P + NaOH I P + HCl P + NaOH II P).

Figure 3.2.3.1 Fractionation scheme for soil inorganic and organic phosphorus.

water only.

The results of inorganic P analysis of soil extracts ($\mu g\ P\ ml^{-1}$) were converted to $\mu g\ P\ g^{-1}$ soil using the appropriate soil:solution ratio of the particular extract (see Figure 3.2.3.1).

3.2.4.2 Total Phosphorus. In order to determine the total P content of the soil extracts it is necessary to hydrolyse all of the organic P present to inorganic P for colorimetric analysis. This involves vigorous digestion of the soil extract with strong mineral acids at elevated temperatures followed by colorimetric determination of the inorganic P in the digest.

3.2.4.2.1 Acid Digestion of Soil Extracts. Acid digestion of soil extracts involved:

(i) 1-5 mls soil extract placed in 50ml capacity borosilicate digestion tubes (20 x 150mm).

(ii) 0.5ml saturated magnesium chloride ($MgCl_2$) was added to minimise evaporative loss of P during subsequent digestion (Brookes and Powlson, 1981).

(iii) 2mls concentrated nitric acid (16M HNO_3) and 1ml 70% perchloric acid ($HClO_4$) were added and the digestion mix was left standing overnight in a fume cupboard.

(iv) Batches of 16 samples were digested in an aluminium heating block (30cm diameter x 5cm depth) mounted on a thermostatically controlled hotplate. This involved initial digestion of the samples for 20-25 minutes at 170-180°C,

following which the temperature was increased to 220-230°C. The digestion was continued at 220-230°C until white fumes of ~~chlorine gas (Cl_2)~~ perchloric acid were evolved which indicated that digestion was completed (this latter step generally took 25-30 minutes). Care was taken to avoid digesting the samples to dryness since this adversely affects quantitative recovery of P from the digest tubes and can become explosive.

(v) After digestion was completed the tubes were removed from the heating block and allowed to cool to room temperature. The digested extracts were then diluted with 20-25mls of distilled water and transferred quantitatively to 50ml volumetric flasks using a vortex shaker for inorganic P determination.

3.2.4.2.2 Determination of Inorganic Phosphorus in Soil Extract Digests. Prior to determining the amount of inorganic P present it was necessary to neutralise the soil extract digests since it has been shown that very acid conditions detrimentally affect the stability of the blue colour forming phosphomolybdate complex (John, 1970). This involved adding a few drops of p-nitrophenol indicator (0.3% w/v $NO_2 \cdot C_6H_4OH$ in 50% aqueous ethanol) followed by a sufficient quantity of 2M NaOH until the solution turned to a permanent yellow colour (i.e. alkaline). Acid (2M HCl) was then added dropwise until the

solution went clear again indicating neutrality (i.e. pH 6-7). Some precipitation of magnesium hydroxide ($Mg(OH)_2$) occurred when the 2M NaOH was added, although this redissolved upon the addition of acid (2M HCl).

The colorimetric method used to determine the amount of inorganic P in the neutralised soil extract digest was the modified Murphy-Riley procedure described by Blackmore et al.(1977). The results obtained for inorganic P in soil extract digests ($\mu g\ P\ ml^{-1}$) represented the total P and were converted to $\mu g\ P\ g^{-1}$ soil using the appropriate soil: solution ratio of the particular extract (see Figure 3.2.3.1).

3.2.4.3 Organic Phosphorus. The amount of organic P in the soil extracts was determined by the difference between the respective amounts of total and inorganic P present:

Organic P ($\mu g\ P\ g^{-1}$) = Total P ($\mu g\ P\ g^{-1}$) - Inorganic P ($\mu g\ P\ g^{-1}$).

3.2.5 Determination of Total Soil Phosphorus by Ignition

An ignition method based on that proposed by Saunders and Williams (1955) was used. Duplicate 2g samples of soil were ignited in silica crucibles for 1 hour at 550°C to ~~hydrolyse~~ convert the organic P to inorganic P. The P was then extracted from the ignited soil with 100mls of 1M H_2SO_4 for 16 hours.

The soil suspension was centrifuged at 10,000 RPM and duplicate aliquots of the supernatant were neutralised prior to inorganic P determination (see section 3.2.4.2.2).

3.2.6 Statistical Analysis

Analysis of variance (anova) of the soil P data for each of the selected years was performed using the Genstat V Statistical Package (Lawes Agricultural Trust, Rothamsted Experimental Station, 1982) in accordance with the experimental layout of the Winchmore long-term trial (i.e. randomised complete block design). The significance of the observed differences between the means of the various treatments within each year sampled was determined using Tukey's honestly significant difference (HSD) procedure (Steele and Torrie, 1980).

Regression analysis was used to determine the nature and significance of changes in the different soil P fractions with time (1958-77) in response to the various treatments included in the trial. This involved fitting separate linear and quadratic functions for each treatment and, since it was evident that in most cases the relative effect of lime addition in 1972 was similar in all treatments, a uniform step function adjustment for lime was included. According to this analysis, the regression coefficients of the best fitting polynomial functions were used to determine the nature (i.e. linear or

quadratic) and associated significance of trends with time. Thus, the significance of the linear and quadratic trends of the control treatment were determined using the students' t-test at the 5, 1 and 0.1% significance levels ($p \leq .05$, .01, .001). In addition to determining the overall trends with time, the inclusion of a step function adjustment in this regression analysis enabled the significance of any apparent liming effect to be determined.

3.3 RESULTS AND DISCUSSION

3.3.1 Soil Phosphorus Fractionation

3.3.1.1 Recovery of Phosphorus from Soil. The P fractionation scheme used in the present study extracted 89-93% of the total P from the Winchmore Soils examined (Table 3.3.1.1.1). This recovery of P from the soil by sequential extraction is markedly greater than that found by several other workers using similar soil P fractionation schemes. For example, Hedley *et al.* (1982a, b) and Tiessen *et al.* (1983) used fractionation schemes which involved sequential extraction with anion exchange resin, 0.5M $NaHCO_3$ (pH 8.5), 0.1M NaOH and 1M HCl/H_2SO_4 and found that in a wide range of soils only 46-62% of the total P was recovered by extraction. Even with the inclusion of an additional extraction using 1M NaOH immediately prior to the acid extraction step Hedley *et al.* (1982b) found that only between 53 and

Table 3.3.1.1.1 Total phosphorus (µg P g^{-1}), and phosphorus (µg P g^{-1}) recovered by sequential extraction in soils (0-7.5cm) from the Winchmore long-term trial (1958-1977).

Treatment		Year						
		1958	1961	1965	1968	1971	1974	1977
Control	Total soil P[a]	665	662	672	658	670	654	648
	Total P extracted[b]	592(89)[c]	590(89)	610(91)	596(91)	598(89)	591(90)	586(90)
188PA	Total soil P	731	743	760	795	799	803	830
	Total P extracted	653(89)	669(90)	692(91)	722(91)	735(92)	736(92)	758(91)
376PA	Total soil P	736	812	867	902	956	931	984
	Total P extracted	664(90)	736(91)	791(91)	823(91)	881(92)	859(92)	911(93)
376R	Total soil P	753	734	747	738	729	725	728
	Total P extracted	679(90)	664(90)	676(90)	670(91)	660(90)	661(91)	660(90)
564R	Total soil P	811	790	792	774	763	725	734
	Total P extracted	733(90)	716(91)	720(91)	704(91)	689(90)	656(90)	680(93)

[a] = Total soil P determined by ignition (µg P g^{-1})

[b] = Sum of total P (µg P g^{-1}) in component extracts of soil P fractionation scheme ($NaHCO_3$-P + NaOH I-P + HCl-P + NaOH II-P).

[c] = Figures in parentheses are the percentages of total soil P recovered by sequential extraction.

70% of the total P was extracted from various soils. The high proportion of total P extracted from the Winchmore soils in the present fractionation study tends to suggest that the inclusion of a second extraction with 0.1M NaOH (NaOH II) after the acid step was beneficial. This, therefore, enabled most of the stable forms of P to be extracted from the soil and thereby increased the accuracy of determining the relative amounts of inorganic and organic P in the residual fraction. In the past methods commonly used to determine the relative proportions of inorganic and organic P were somewhat crude and gave only approximate results. For example, Hedley et al. (1982b) used ignition (380°C) and extraction with concentrated HCl ($10\frac{2}{\emptyset}$M) to determine non-extracted inorganic and organic P, while Tiessen et al. (1983) used hydrogen peroxide (H_2O_2) and sulphuric acid (1M H_2SO_4) to selectively hydrolyse and extract the organic P not recovered from the soil by previous alkali and acid extractions. It is generally assumed that the amount of residual P (i.e. non-extracted P in the Stewart et al. (1980) fractionation scheme) does not change very rapidly. However, recent studies (Hedley et al., 1982a; Tiessen et al., 1983) showed that large decreases in residual P occurred as a result of long-term (60 years) arable cropping. In addition, Hedley et al. (1982b) found that a significant decrease in residual P occurred after

only 41 days in a rhizosphere soil. Thus, it is important that as much of the residual P should be extracted if changes in soil P fractions are to be followed over time.

Proportions of total P in non-extracted forms in the Winchmore soils were generally small (7-11%) and were similar in all treatments over the time period examined (1958-1977) (Table 3.3.1.1.1). It was therefore not considered necessary to determine the relative amounts of inorganic and organic P in the non-extracted or residual fraction.

3.3.1.2 Distribution of Soil Phosphorus Fractions. As the distribution of soil P fractions was generally similar between years (Appendices 3.1 to 3.6) only the results for 3 years (1958, 1971, 1977) are presented in Table 3.3.1.2.1. The largest proportion of the total P was found in the extracted organic P fractions. For example, the 3 organic P fractions (i.e. $NaHCO_3$, NaOH I and NaOH II) made up 45-57% of the total P, while the 4 extracted inorganic P fractions (i.e. $NaHCO_3$, NaOH I, HCl and NaOH II) collectively accounted for 34-46% of the total P. The remainder of the total soil P (7-11%) was present in the residual or non-extracted fraction and probably included inorganic and organic forms of P.

The inorganic P fractions can be divided into alkali-extractable ($NaHCO_3$, NaOH I and NaOH II) and acid-extractable (HCl) forms which made up 30-38% and

Table 3.3.1.2.1 Fractionation of P in soils from the long-term trial at Winchmore in 1958, 1971 and 1977.

Year	Treatment	Total soil P ($\mu g\ P\ g^{-1}$)[a]	Soil P fractions ($\mu g\ P\ g^{-1}$)							
			Inorganic P				Organic P			Residual[c]
			$NaHCO_3$	NaOH I	HCl	NaOH II	$NaHCO_3$	NaOH I	NaOH II	
1958	CONTROL	665	17(3)[b]	111(17)	30(4)	93(14)	42(6)	208(31)	98(15)	66(10)
	188PA	731	20(3)	128(18)	38(5)	98(13)	48(7)	224(31)	99(14)	76(9)
	376PA	736	25(3)	140(19)	41(6)	96(13)	50(7)	210(28)	102(14)	72(10)
	376R	758	26(3)	142(19)	45(6)	105(14)	48(6)	209(28)	111(15)	67(9)
	564R	812	36(4)	172(21)	54(7)	106(13)	52(6)	212(26)	103(13)	77(10)
1971	CONTROL	667	17(3)	95(14)	23(4)	80(12)	42(6)	237(36)	101(15)	72(11)
	188PA	818	28(3)	129(16)	42(5)	94(11)	56(7)	262(32)	123(15)	84(10)
	376PA	943	49(5)	195(21)	63(7)	110(12)	56(6)	263(28)	128(14)	79(8)
	376R	736	20(3)	108(15)	30(4)	86(12)	50(7)	257(35)	116(16)	69(9)
	564R	765	23(3)	114(15)	34(4)	89(12)	54(7)	264(34)	113(15)	74(10)
1977	CONTROL	648	20(3)	89(14)	31(5)	84(13)	41(6)	160(25)	159(25)	64(10
	188PA	830	26(3)	129(16)	58(7)	107(13)	59(7)	202(24)	178(21)	71(9)
	376PA	984	48(5)	189(19)	103(10)	122(12)	71(7)	198(20)	180(18)	73(7)
	376R	728	23(3)	105(14)	36(5)	97(13)	51(7)	182(25)	168(23)	66(9)
	564R	744	21(3)	113(15)	40(5)	95(13)	55(7)	188(26)	168(23)	54(7)

[a] = Total soil P determined by ignition

[b] = Figures in parentheses are percentages of total soil P.

[c] = Residual P = total soil P - ($NaHCO_3$-P + NaOH I-P + HCl-P + NaOHII-P).

4-10% of the total P in the Winchmore soils respectively (Table 3.3.1.2.1).

The alkali-extractable inorganic P generally comprises the various adsorbed and mineral forms of P (i.e. secondary P minerals) associated mainly with iron and aluminium in the soil (see section 2.1.3). In the fractionation scheme used (Figure 3.2.3.1) 2 broad categories of this alkali-extractable inorganic P are present, namely P extracted from the soil with alkali alone ($NaHCO_3$, NaOH I) and P extracted with alkali following acid treatment (NaOH II).

The $NaHCO_3$ and NaOH I inorganic P fractions made up 3-5% and 14-21% of the total P in the Winchmore soils (Table 3.3.1.2.1). Generally speaking, this fractionation of alkali-soluble inorganic P in the soil reflects the different strengths and chemical natures of the interactions between the P and the mineral colloids (Stewart and McKercher, 1980). The inorganic P extracted by weak alkali (i.e. $NaHCO_3$ P) is usually assumed to represent the less strongly fixed forms of inorganic P in the soil than that subsequently extracted by stronger alkali (i.e. NaOH I P). This implies that the $NaHCO_3$ P is more readily exchangeable with the soil solution and therefore potentially more readily available to plants than NaOH I P.

The NaOH II inorganic P fraction made up 12-14% of the total P in the Winchmore soils (Table 3.3.1.2.1).

The fact that some forms of inorganic P are extracted by alkali only following acid treatment may be due to the removal of polyvalent bridging cations (e.g. Ca^{++}) and/or protective iron-aluminium oxide coatings by the acid (i.e. occluded P - see section 2.1.3). The NaOH II inorganic P fraction in the soil represents different, and possibly more complex, forms of adsorbed and mineral P than that extracted from the soil in the $NaHCO_3$ and NaOH I fractions.

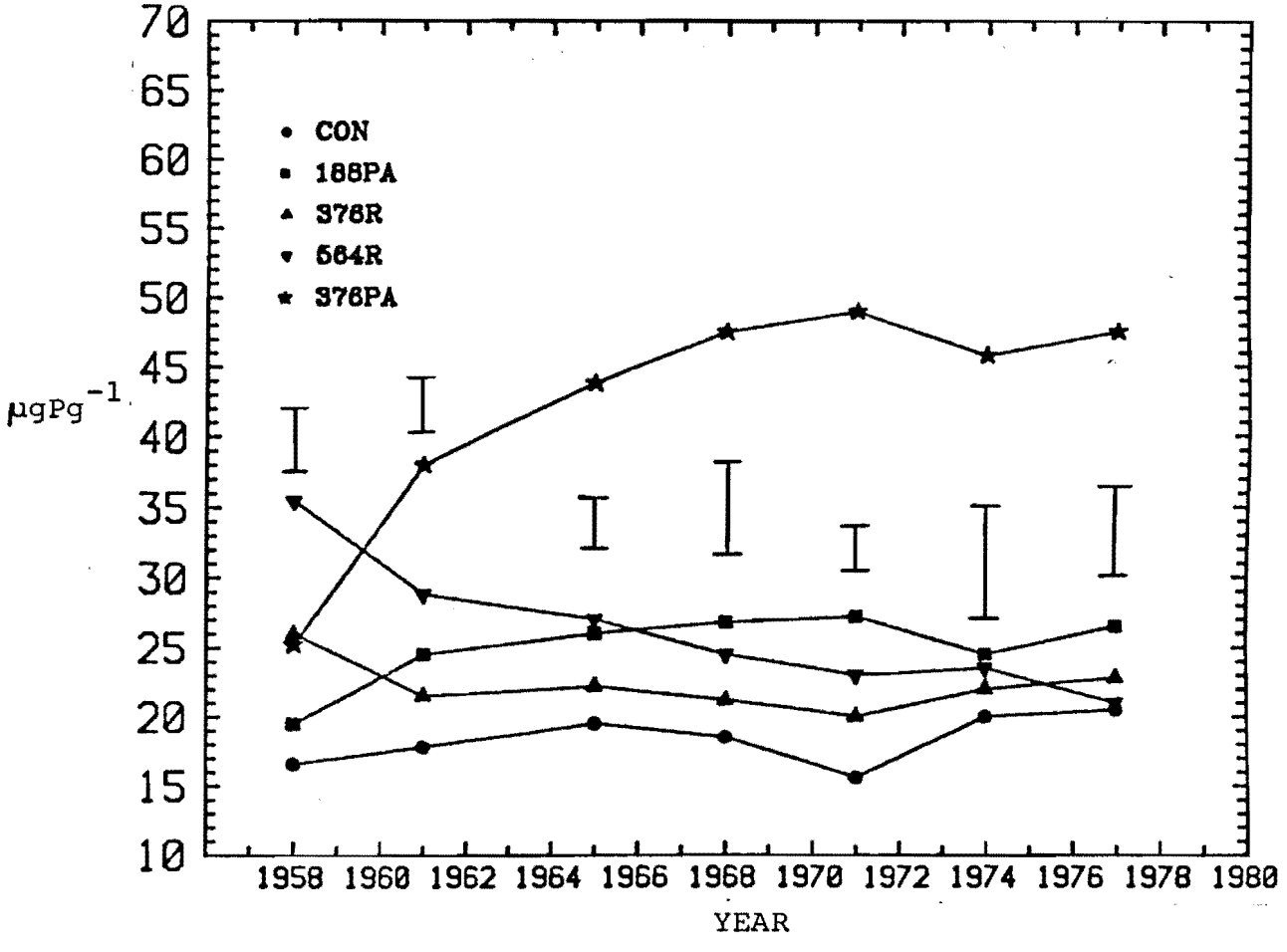
The HCl fraction, which made up 4-10% of the total P in the Winchmore soils (Table 3.3.1.2.1), is generally assumed to be predominantly calcium phosphate minerals such as apatite ($Ca_{10}(PO_4)_6(OH,F)_2$), although it may also include some complex calcium-iron-aluminium P minerals which are not soluble in alkali (see section 2.1.3). As expected no organic P was found in the HCl fraction of the Winchmore soils.

In the soils examined in this fractionation study, 6-7% of the total P was found in the $NaHCO_3$ organic P fraction, while the NaOH I and NaOH II organic P fractions made up 20-36% and 13-25% of the total P respectively (Table 3.3.1.2.1). The fractionation of soil organic P by sequential extractions is in many ways similar to that described above for inorganic P. Organic P in the soil is believed to be mainly held in association with mineral colloids as part of complex organic polymers

or as discrete organic P compounds such as inositol hexaphosphate (Russell, 1973; Anderson, 1980). The strength and nature of the interactions between the organic P and mineral colloids are likely to influence the fractionation of soil organic P by sequential extraction. Treatment with weak alkali was sufficient to extract weakly held forms of organic P from the soil (i.e. $NaHCO_3$ organic P), while stronger alkali was required to extract the more strongly held forms of organic P (i.e. NaOH I organic P) (Stewart and McKercher, 1980). In addition, treatment of the soil with acid was necessary to dissolve polyvalent cations (e.g. Ca^{++}) and hydrated silicate minerals involved in organic matter-mineral complexes, thereby enabling extraction of the organic P with alkali (i.e. NaOH II organic P).

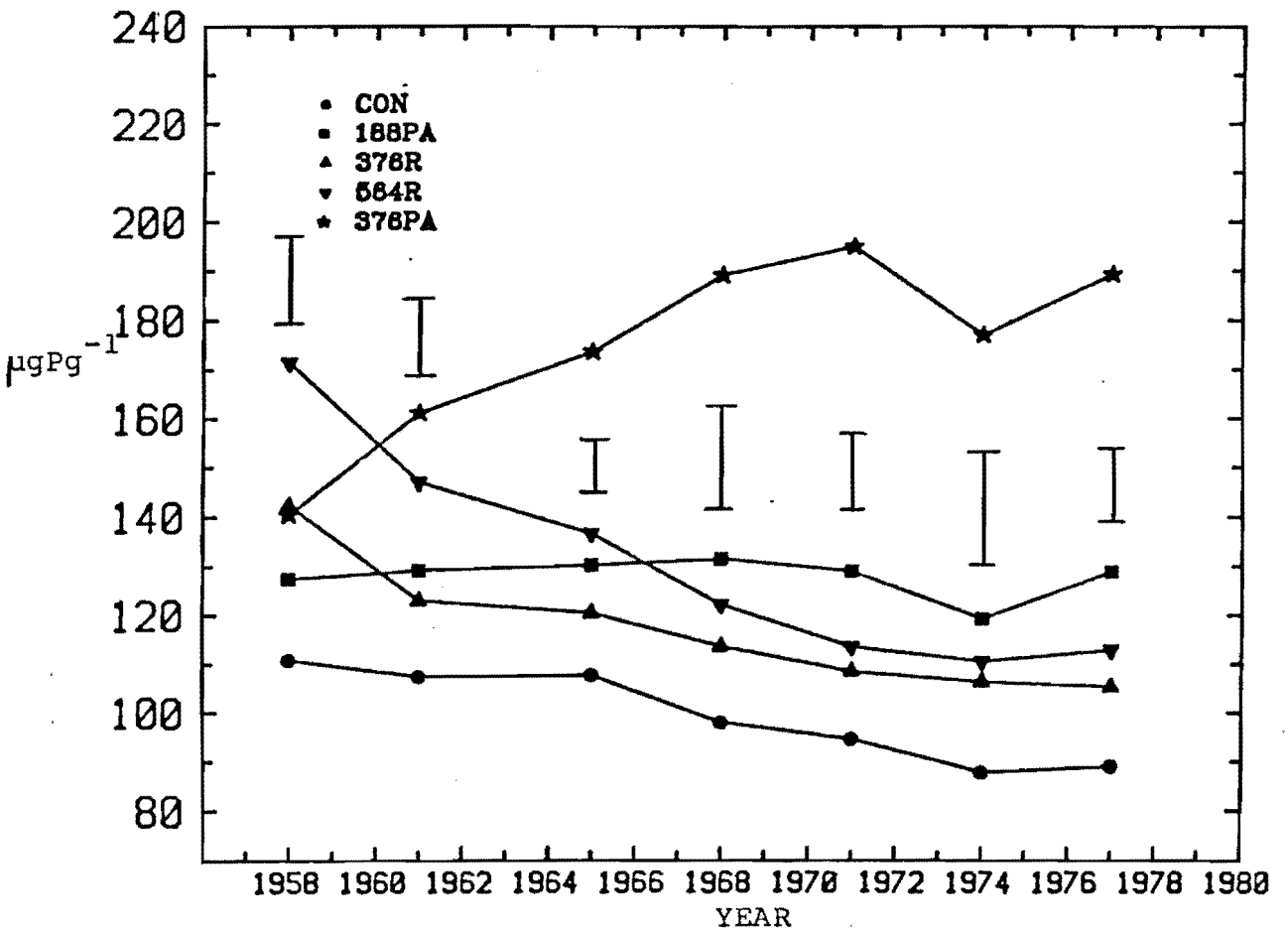
3.3.2 Soil Phosphorus Fractions in the Long-Term Trial at Winchmore

3.3.2.1 Inorganic Phosphorus. The amounts of inorganic P in the various soil P fractions ($NaHCO_3$, NaOH I, HCl, NaOH II and the total extracted inorganic P (i.e. $NaHCO_3$ + NaOHI + HCl + NaOHII) in soils from the different treatments in the long-term field trial at Winchmore between 1958 and 1977 are shown in Figures 3.3.2.1.1 to 3.3.2.1.5. In addition, results of the regression analysis of changes in the various soil P fractions with time (1958-1977) and as a result of the addition of lime in 1972 are



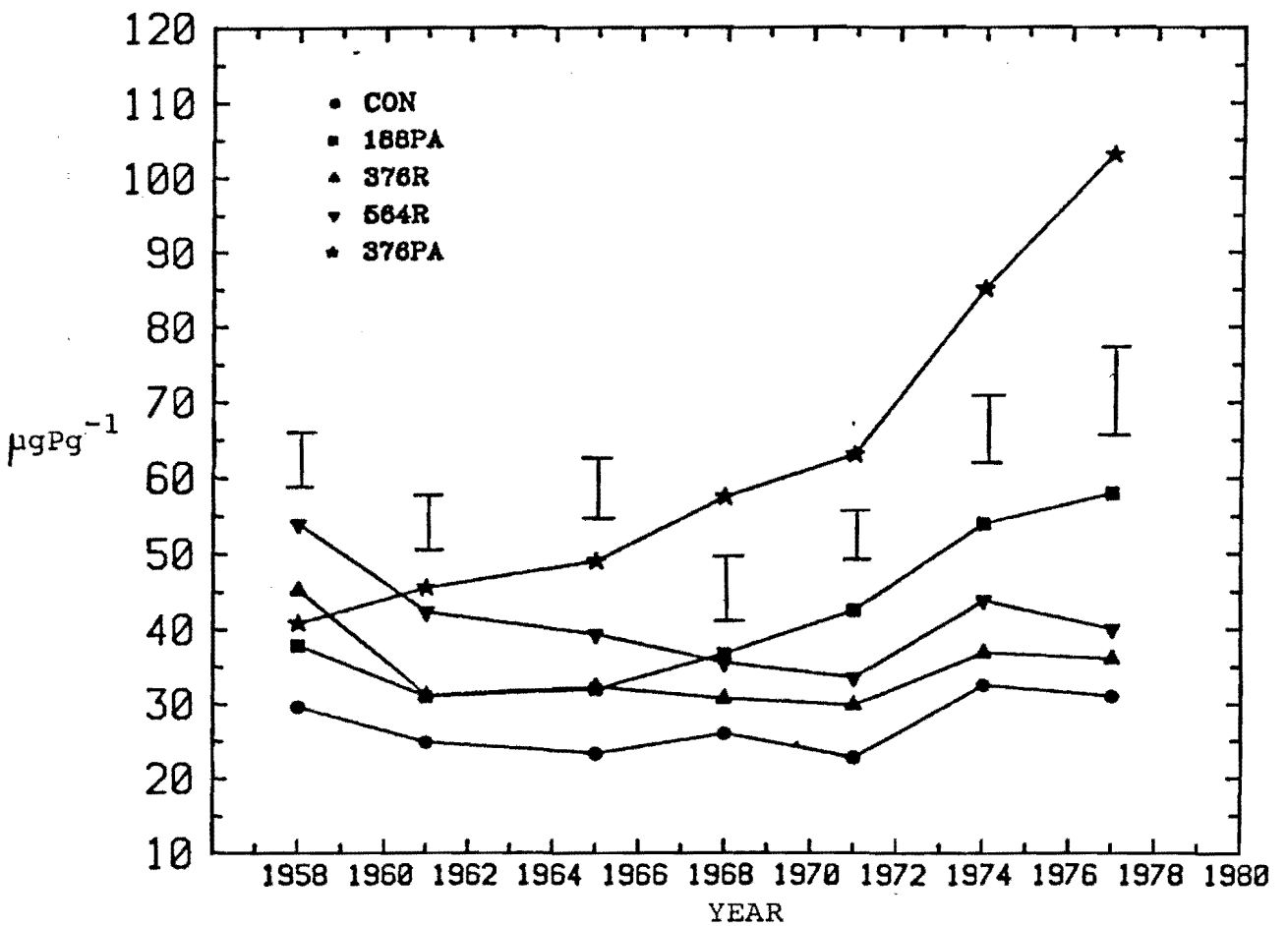
I=Tukey's honestly significant difference(HSD)at 5% level.

Figure 3.3.2.1.1. Amounts (μgPg^{-1}) of $NaHCO_3$ inorganic P in soils (0-7.5cm) from the different treatments in the Winchmore long-term trial between 1958 and 1977.



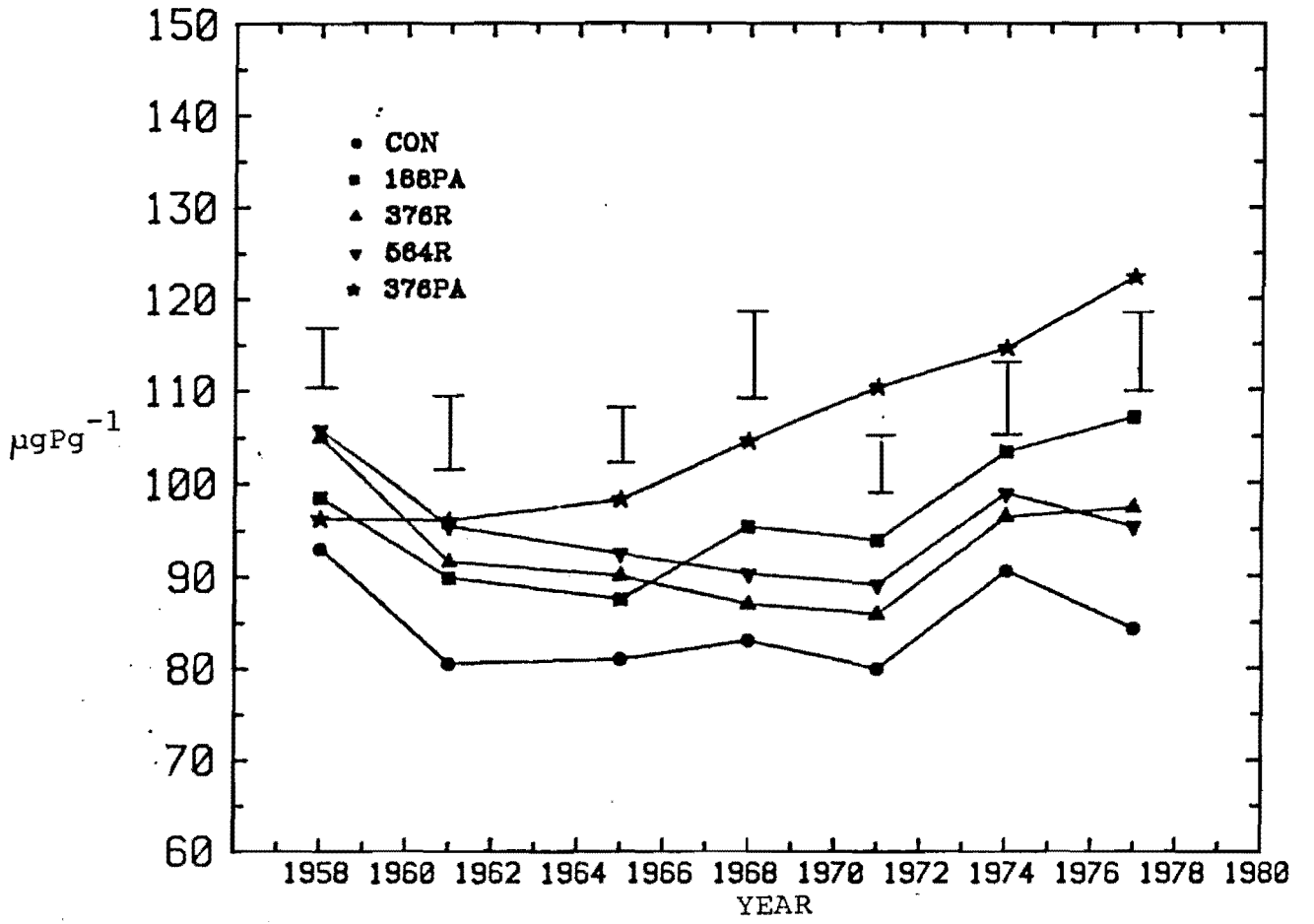
I=Tukey's honestly significant difference(HSD) at 5% level.

Figure 3.3.2.1.2. Amounts (μgPg^{-1}) of NaOH I inorganic P in soils (0-7.5cm) from the different treatments in the Winchmore long-term trial between 1958 and 1977.



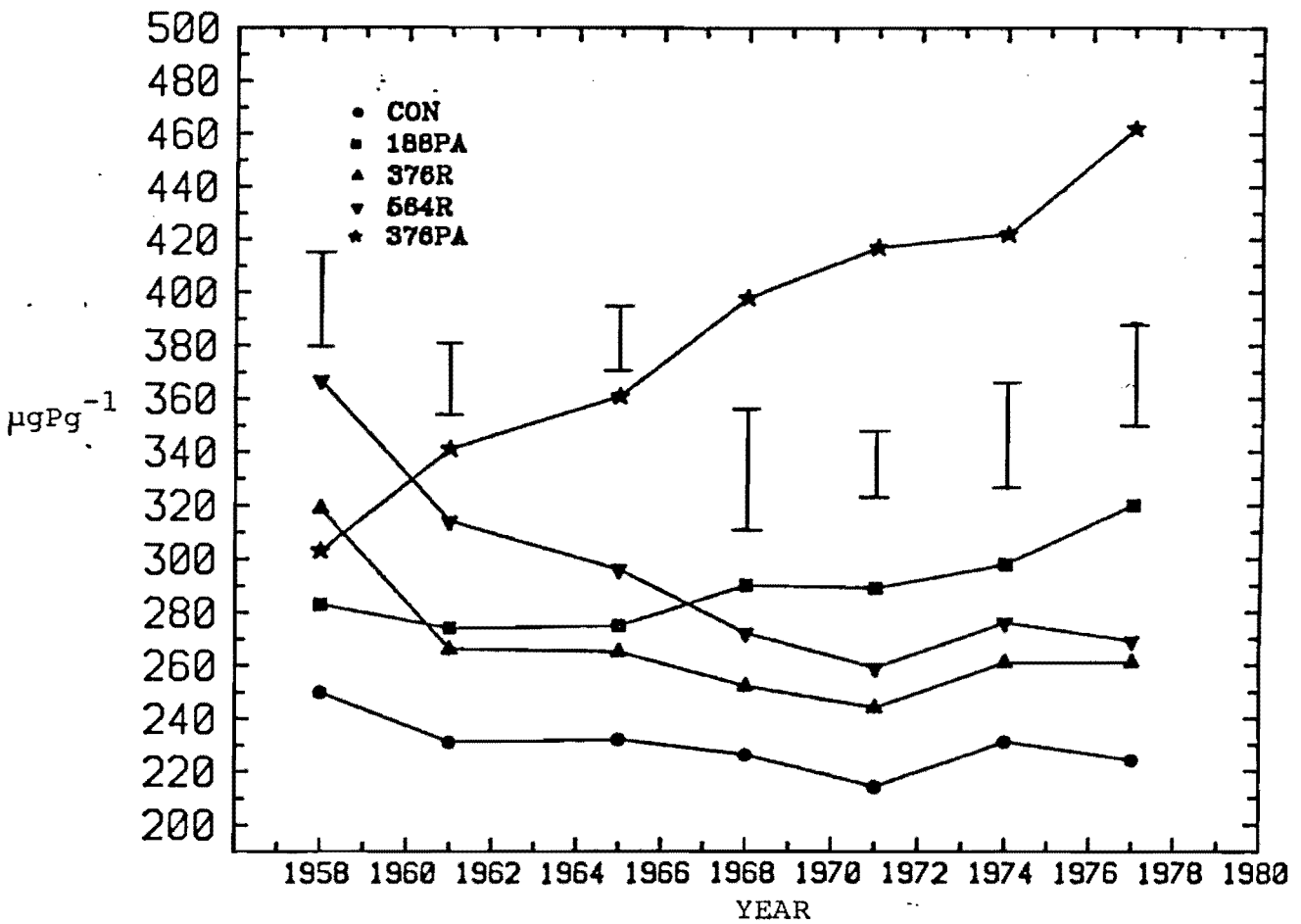
I=Tukey's honestly significant difference(HSD) at 5% level.

Figure 3.3.2.1.3. Amounts (μgPg^{-1}) of HCl inorganic P in soils (0-7.5cm) from the different treatments in the Winchmore long-term trial between 1958 and 1977.



I=Tukey's honestly significant difference(HSD) at 5% level.

Figure 3.3.2.1.4. Amounts (μgPg^{-1}) of NaOH II inorganic P in soils (0-7.5cm) from the different treatments in the Winchmore long-term trial between 1958 and 1977.



I=Tukey's honestly significant difference(HSD) at 5% level.

Figure 3.3.2.1.5. Amounts (μgPg^{-1}) of total extracted inorganic P in soils (0-7.5cm) from the different treatments inthe Winchmore long-term trial between 1958 and 1977.

Table 3.3.2.1.1 Regression analysis of changes in $NaHCO_3$ inorganic P in soils from the long-term Winchmore trial with time (1958-77) and as a result of lime addition in 1972. ($Y_p = a_p + b_{1p}T + b_{2p}T^2 + b_3L$).

Trends with time (1958-77)	Treatment	Regression Coefficients	
		Linear (b_{1p})	Quadratic (b_{2p})
	Control	0.63 NS[a]	-0.02 NS[a]
	188PA	0.80 (NS)[b]	-0.44 (NS)[b]
	376PA	3.21 (***)	-1.20 (***)
	376R	-0.45 (**)	0.35 (NS)
	564R	-2.07 (***)	0.42 (NS)
Effect of lime addition in 1972 (b_3L)[c]		-0.34 NS	

Students t-test significance levels: * = 5%; ** = 1%; *** = 0.1%; NS = not significant.

[a] = significance of the linear and quadratic trend coefficients of the control.

[b] = significance of the linear and quadratic trend coefficients compared with the control.

[c] = step function adjustment (regression coefficient) averaged over all treatments.

Table 3.3.2.1.2 Regression analysis of changes in NaOH I inorganic P in soils from the Winchmore long-term trial with time (1958-77) and as a result of lime addition in 1972. ($Y_p = a_p + b_{1p}T + b_{2p}T^2 + b_3L$).

Trends with time (1958-77)	Treatment	Regression coefficient	
		Linear (b_{1p})	Quadratic (b_{2p})
	Control	-2.13 NS[a]	0.39 NS[a]
	188PA	1.19 (*)[b]	0.33 (NS)[b]
	376PA	8.89 (***)	-1.91 (**)
	376R	-3.77 (NS)	1.83 (NS)
	564R	-7.93 (***)	2.83 (**)
Effect of lime addition in 1972 (b_3)[c]		-10.21 **	

Students t-test significance levels: * = 5%; ** = 1%; *** = 0.1%; NS = not significant.
[a] = significance of the linear and quadratic trend coefficients of the control.
[b] = significance of the linear and quadratic trend coefficients compared with control.
[c] = step function adjustment (regression coefficient) averaged over all treatments.

Table 3.3.2.1.3 Regression analysis of changes in HCl inorganic P in soils from the Winchmore long-term trial with time (1958-77) and as a result of lime addition in 1972. ($Y_p = a_p + b_{1p}T + b_{2p}T^2 + b_3L$).

Trends with time (1958-77)	Treatment	Regression Coefficient	
		Linear (b_{1p})	Quadratic (b_{2p})
	Control	-0.65 NS[a]	0.21 NS[a]
	188PA	2.39 (***)[b]	0.88 (NS)[b]
	376PA	8.50 (***)	1.32 (**)
	376R	-2.12 (*)	0.70 (NS)
	564R	-3.09 (***)	0.72 (NS)
Effect of lime addition in 1972 (b_3)[c]		8.03 ***	

Students t-test significance levels: * = 5%; ** = 1%; *** = 0.1%; NS - not significant.

[a] = significance of the linear and quadratic trends coefficients of the control.

[b] = significance of the linear and quadratic trends coefficients compared with the control.

[c] = step function adjustment (regression coefficient) averaged over all treatments.

Table 3.3.2.1.4 Regression analysis of the changes in NaOH II inorganic P in soils from the Winchmore long-term trial with time (1958-77) and as a result of lime addition in 1972. ($Y_p = a_p + b_{1p}T + b_{2p}T^2 + b_3L$).

Trends with time (1958-77)	Treatment	Regression Coefficient	
		Linear (b_{1p})	Quadratic (b_{2p})
	Control	-1.47 *[a]	0.63 NS[a]
	188PA	1.04 (***)[b]	0.89 (NS)[b]
	376PA	3.53 (***)	0.24 (NS)
	376R	-1.62 (NS)	1.30 (NS)
	564R	-2.16 (NS)	0.70 (NS)
Effect of lime addition in 1972 (b_3)[c]		5.88 **	

students t-test significance levels: * = 5%; ** = 1%; *** = 0.1%; NS = not significant.
[a] = significance of linear and quadratic trend coefficients of the control.
[b] = significance of linear and quadratic trend coefficients compared with the control.
[c] = step function adjustment (regression coefficient) averaged over all treatments.

Table 3.3.2.1.5 Regression analysis of changes in total extracted inorganic P in soils from the Winchmore long-term trial with time (1958-77) and as a result of lime addition in 1972. ($Y_p = a_p + b_{1p}T + b_{2p}T^2 + b_3L$).

Trends with time (1958-77)	Treatment	Regression Coefficients	
		Linear (b_{1p})	Quadratic (b_{2p})
	Control	-3.72 NS[a]	1.16 NS[a]
	188PA	5.27 (**)[b]	1.66 (NS)[b]
	376PA	24.21 (***)	-1.47 (NS)
	376R	-7.95 (NS)	4.15 (NS)
	564R	-15.36 (***)	4.95 (*)
Effect of lime addition in 1972 (b_3)[c]		3.72 NS	

students t-test significance levels: * = 5%; ** = 1%; *** = 0.1%; NS = not significant.

[a] = significance of the linear and quadratic trend coefficients of the control.

[b] = significance of the linear and quadratic trend coefficients compared with the control.

[c] = step function adjustment (regression coefficient) averaged over all treatments.

given in Tables 3.3.2.1.1 to 3.3.2.1.5.

These data showed that changes in inorganic P which occurred in the various treatments between 1958 and 1977 were influenced by P fertiliser inputs. Between 1958 and 1977 the amounts of inorganic P in the various soil P fractions decreased in the residual treatments (376R, 564R) which had not received P fertiliser over this period, while soil inorganic P increased in those treatments (188PA, 376PA) which had received annual applications of P fertiliser throughout. Amounts of soil inorganic P in the various treatments in 1958, which represents the earliest sampling date of soils used for the present fractionation study, and subsequent changes which occurred between 1958 and 1977 are discussed below.

In 1958 amounts of total extracted inorganic P in soils from the different treatments (Figure 3.3.2.1.5) were directly related to the respective quantities of fertiliser P applied between 1952 and 1957 as they followed the order of: ~~546~~564R > 376R = 376PA > 188PA > control. These results indicated that significant accumulations of inorganic P in the soil had occurred as a result of continued annual applications of fertiliser P between 1952 and 1957. Pasture production data from the Winchmore trial (Figure 3.3.2.1.6) showed that between 1952 and 1957 the dry matter yields in the 564R, 376R (PA) and 188PA treatments were in fact similar, which

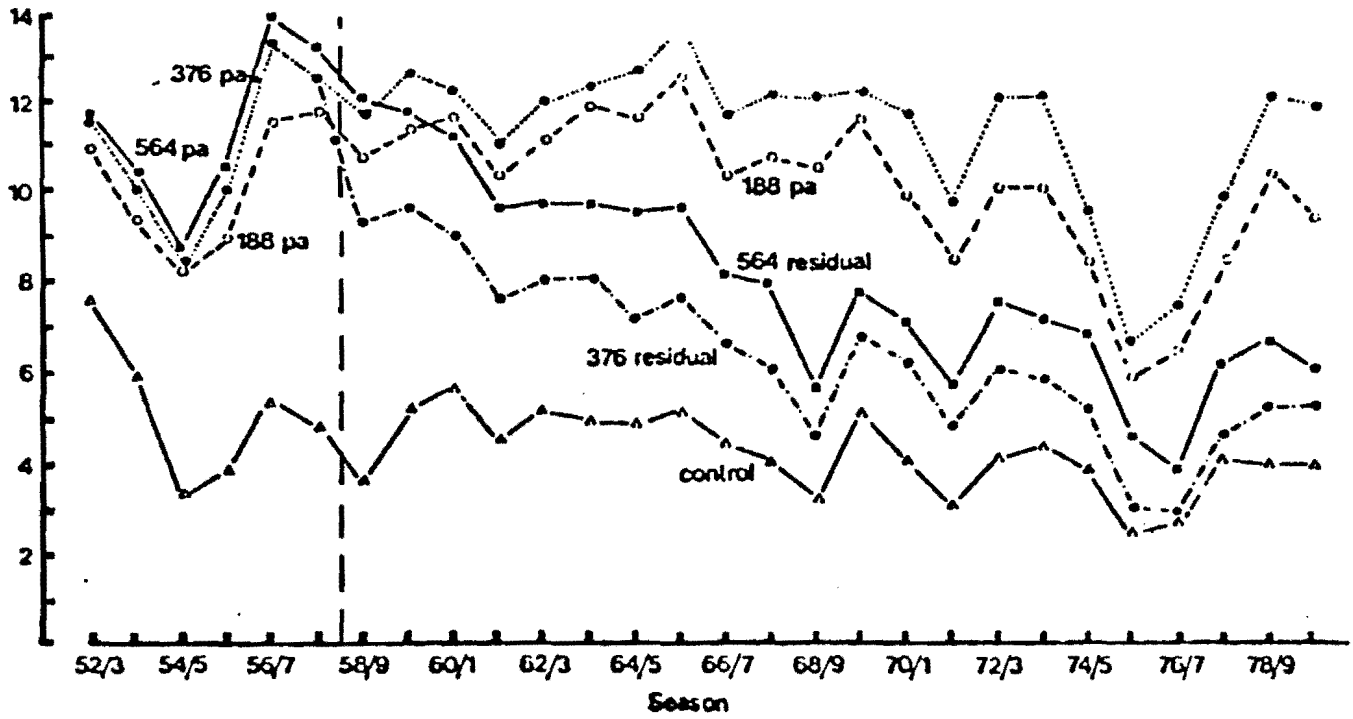


Figure 3.3.2.1.6. Annual pasture dry matter production (t ha^{-1}) on the different treatments in the long-term superphosphate trial at Winchmore between 1952 and 1979 (Quin and Rickard, 1979).

partly explains the greater levels of inorganic P present in the 564R and 376R (PA) soils compared with the 188PA soil.

Amounts of inorganic P in the different soil P fractions in the various treatments in 1958 are shown in Table 3.3.2.1.6. Most increases in soil inorganic P which occurred as a result of P fertiliser additions between 1952 and 1957 were present in the NaOH I fraction. For example, the NaOH I P accounted for 46-56% of the total differences in inorganic P between that of the control and those of the fertilised soils, compared with 9-16%, 21-24% and 6-17% for the $NaHCO_3$, HCl and NaOH II P fractions respectively.

Alkali extractable inorganic P in soil includes adsorbed and mineral forms of P associated with iron and aluminium (see section 2.1.3). The observed increases in $NaHCO_3$, NaOH I and NaOH II P in the fertilised soils (Table 3.3.2.1.6) probably resulted from the conversion of soluble fertiliser P to adsorbed and mineral forms of iron and aluminium bound P. The HCl fraction of soil P is made up mainly of sparingly soluble calcium phosphate minerals such as apatite (see section 2.1.3). The observed increases in HCl P in the fertilised soils (Table 3.3.2.1.6) may have originated from the amorphous calcium phosphates (e.g. $CaHPO_4.2H_2O$) formed within the fertiliser granule following

Table 3.3.2.1.6 Amounts of inorganic P (μg P g^{-1}) in soils from the different treatments in the Winchmore long-term trial sampled in 1958.

Treatment	Soil P Fractions				
	$NaHCO_3$	NaOH I	HCl	NaOH II	Total
Control	16.6	110.8	29.6	93.0	250.0
188PA	19.5 (9)[a]	127.5 (50)	37.8 (24)	98.5 (17)	283.3
376PA	25.2 (16)	140.5 (56)	40.8 (21)	96.2 (6)	302.7
376R	26.0 (14)	142.5 (46)	45.2 (23)	105.0 (17)	318.7
564R	35.5 (16)	171.5 (52)	54.0 (21)	105.8 (11)	366.8

[a] = percentage of differences in total extracted inorganic P compared with control.

initial dissolution of the monocalcium phosphate ($Ca(H_2PO_4)_2.H_2O$) (Sample et al., 1980). In addition, small amounts of unreacted apatite ($Ca_{10}(PO_4)_6(OH,F)_2$) present in the single superphosphate applied between 1952 and 1957 may have contributed to the increases in HCl P which occurred in the fertilised soils compared with the control soil.

3.3.2.1.1 Changes in Soil Inorganic Phosphorus with Time.

3.3.2.1.1.1 Control and Residual Fertiliser Treatments. Between 1958 and 1977 amounts of total extracted soil inorganic P decreased in the control, 376R and 564R treatments (Figure 3.3.2.1.5) although these were greater in the 376R and 564R soils than in the control soil. For example, the overall decreases in total extracted inorganic P between 1958 and 1977 were 98 μg P g^{-1} in the 564R soil compared with 58 and 25 μg P g^{-1} in the 376R and control soils respectively (Table 3.3.2.1.1.1.1).

In general, amounts of soil inorganic P in the control and residual fertiliser (376R,564R) treatments decreased with time over the 1958 to 1977 period, although the greatest decreases occurred in the NaOH I fraction (Table 3.3.2.1.1.1.1). In all soils, the NaOH II P (Figure 3.3.2.1.4) showed large decreases in amounts with time, including the control treatment. This was due to the high values of NaOH II P determined in 1958. The reasons for this

Table 3.3.2.1.1.1.1 Amounts of inorganic P (μg P g^{-1}) in the different soil P fractions in the control and residual fertiliser (376R, 564R) treatments of the Winchmore long-term trial sampled in 1958 and 1977.

Treatment	Year	Soil P Fractions				
		$NaHCO_3$	NaOHI	HCl	NaOHII	Total[a]
Control	1958	16.6	110.8	29.6	93.0	250.0
	1977	20.5	89.0	31.0	84.3	224.8 (-10.1)[b]
376R	1958	26.0	142.5	45.2	105.0	318.8
	1977	22.8	105.3	36.0	97.3	261.3 (-18.0)
564R	1958	35.5	171.5	54.0	105.8	366.8
	1977	21.0	112.8	40.0	95.3	269.0 (-26.7)

[a] = total extracted inorganic P = $NaHCO_3$-P + NaOHI-P + HCl-P + NaOHII-P.

[b] = figures in parentheses are % changes between 1958 and 1977.

are unclear and reanalysis of soil samples in 1958 for NaOH II P gave similar results.

Large decreases in NaOH I P with time in the control and residual fertiliser treatments (Figure 3.3.2.1.2) are consistent with the fact that this was the predominant inorganic P fraction in the soils studied (see section 3.3.1.2). Similarly, NaOH I P was found to represent a large proportion of the increases in inorganic P in the 376R and 564R soils which resulted from applications of P fertiliser between 1952 and 1957 (Table 3.3.2.1.6).

Decreases in inorganic P which occurred in the control and residual fertiliser treatment soils between 1958 and 1977 probably involved the desorption and dissolution of adsorbed and mineral forms of inorganic P which originated from previous P fertiliser applications in response to continued removal of inorganic P from the soil solution by plants (Barrow, 1980; Sample et al., 1980). In the control soil, this residual fertiliser P originated from the 420 kg ha^{-1} of superphosphate which was applied to the whole trial area between 1948 and 1952 (see section 3.2.1). Amounts of inorganic P originating from fertiliser P in the 376R and 564R soils were considerably greater than in the control (see Table 3.3.2.1.6) as a consequence of the additional P fertiliser applied to these soils between 1952 and 1957.

Although decreases in amounts of inorganic P in the control, 376R and 564R soils occurred with time (Figure 3.3.2.1.5), very little of this P was actually lost from the soil. It is most likely that the inorganic P which accumulated in the control, 376R and 564R soils from P fertiliser additions was converted to organic forms in the soil (see later section 3.3.2.2). The depletion of total extracted inorganic P continued to 1977 although after 1968 the relative differences between the residual fertiliser treatments (376R, 564R) and the control remained the same (Figure 3.3.2.1.5). This trend was also shown by the various inorganic P fractions (Figures 3.3.2.1.1 to 3.3.2.1.4). These results suggest that some of the inorganic P which accumulated in the 376R and 564R soils between 1952 and 1958 was present in forms which were not readily utilised by plants. This was probably mainly due to the increased stability of soil-fertiliser P reaction products with time. It is generally accepted that with time the products of the initial reactions of water-soluble fertiliser P (MCP) in the soil, namely adsorbed and precipitated mineral forms of P associated mainly with iron, aluminium and calcium, tend to become more stable and thereby less exchangeable with the soil solution (Devine et al., 1968; Mattingly, 1975; Barrow, 1980; Sample et al., 1980; Barrow, 1983).

The addition of lime ($CaCO_3$) to the trial in 1972 (4t ha^{-1}) appeared to have some effect on the HCl and NaOH II P fractions in the control, 376R and 564R soils. This is shown by small though significant increases in HCl and NaOH II P in these soils between 1971 and 1974 (Figures 3.3.2.1.3 and 3.3.2.1.4; Tables 3.3.2.1.3 and 3.3.2.1.4). These increases may have originated from the mineralisation of organic P which occurred in these soils over this period (see later section 3.3.2.2). This mineralised P was probably converted to HCl and NaOH II P due to presence of large amounts of calcium in the recently limed soils. The mineralised P may have been converted to calcium P forms possibly via adsorption onto lime particles ($CaCO_3$) or adsorption onto clay surfaces as clay-calcium-P complexes (Tisdale and Nelson, 1975; White, 1980).

3.3.2.1.1.2 Annually Fertilised Treatments. In the 188PA and 376PA treatments, amounts of total extracted inorganic P in the soil increased between 1958 and 1977 as a result of continued annual applications of P fertiliser (Figure 3.3.2.1.5; Table 3.3.2.1.5). Increases in total inorganic P between 1958 and 1977 were markedly greater in the 376PA soil (159 μg P g^{-1}) than in the 188PA soil (37 μg P g^{-1}) (Table 3.3.2.1.1.2.1). The enhanced accumulation of inorganic P in the 376PA soil is partly explained by the fact that pasture dry matter

Table 3.3.2.1.1.2.1 Amounts of inorganic P (μg P g^{-1}) in the different soil P fractions in the 188PA and 376PA treatments sampled in 1958, 1971 and 1977.

Soil Inorganic P Fraction	μg P g^{-1} [a]					
	188PA			376PA		
	1958	1971	1977	1958	1971	1977
$NaHCO_3$	19.5	27.2	26.5	25.2	49.0	47.5
NaOH I	127.5	129.0	128.8	140.5	194.8	189.3
HCl	37.8	42.5	58.0	40.8	63.0	103.0
NaOH II	98.5	93.8	107.0	96.2	110.2	122.3
Total[b]	283.3	292.5 (0.7)[c]	320.3 (4.6)[d]	302.7	417.0 (8.8)[c]	462.1 (7.5)[d]

[a] = mean of 4 replicates

[b] = total extracted inorganic P = $NaHCO_3$-P + NaOH I-P + HCl-P + NaOH II-P.

[c] = mean annual increase in total extracted P between 1958 and 1971.

[d] = mean annual increase in total extracted P between 1971 and 1977.

yields were similar in both the annually fertilised treatments between 1958 and 1977 (Figure 3.3.2.1.6).

In the 188PA treatment the increase in total extracted soil inorganic P which occurred between 1958 and 1971 (i.e. 13 years) was only 9 μg P g^{-1} compared with a net increase of 28 μg P g^{-1} between 1971 and 1977 (i.e. 6 years) (Table 3.3.2.1.1.2.1). The enhanced increase in soil inorganic P after 1971 was probably due mainly to the cessation of net organic P accumulation in this soil around this time (see section 3.3.2.2). This, therefore, resulted in greater accumulation of the annually applied fertiliser P in the soil as inorganic P between 1971 and 1977 than between 1958 and 1971.

The distribution of P between the various fractions in the annually fertilised soils between 1958 and 1971 was markedly different from that between 1971 and 1977, particularly in the 376PA treatment (Table 3.3.2.1.1.2.1). Between 1958 and 1971 most of the increases in total inorganic P in the 376PA treatment occurred in the $NaHCO_3$ and NaOH I fractions, while between 1971 and 1977 they were mainly in the HCl and NaOH II fractions. These differences were probably due to (i) the effects of lime additions in 1972 on the reactions of added water-soluble fertiliser P in the soil, and (ii) changes in the forms of P present in the superphosphate.

It has been postulated that the chemical nature of the reaction products of water-soluble fertiliser P (MCP) in soil is influenced by the predominant cations present. In non-calcareous soils added MCP is believed to be converted mainly to adsorbed and mineral forms of P associated with iron and aluminium, whereas in calcareous soils the major products of MCP reaction are calcium bound forms of P (Sample et al., 1980). Increases in $NaHCO_3$ and NaOH I P which occurred between 1958 and 1971 in the annually fertilised treatments (Table 3.3.2.1.1.2.1) indicated that the added fertiliser MCP was mainly converted to iron and aluminium bound forms in these soils. However, the addition of a large quantity of lime in 1972 increased the amount of calcium in the soil which may partly account for the predominance of increases in calcium bound P (HCl-P) in the annually fertilised treatments between 1971 and 1977 (Table 3.3.2.1.1.2.1). Similar trends toward the conversion of water-soluble fertiliser P to calcium-P rather than iron-aluminium-P forms as a result of lime addition were also shown by Pratt and Shoemaker (1955), Laverty and McLean (1961) and Chang and Chu (1961).

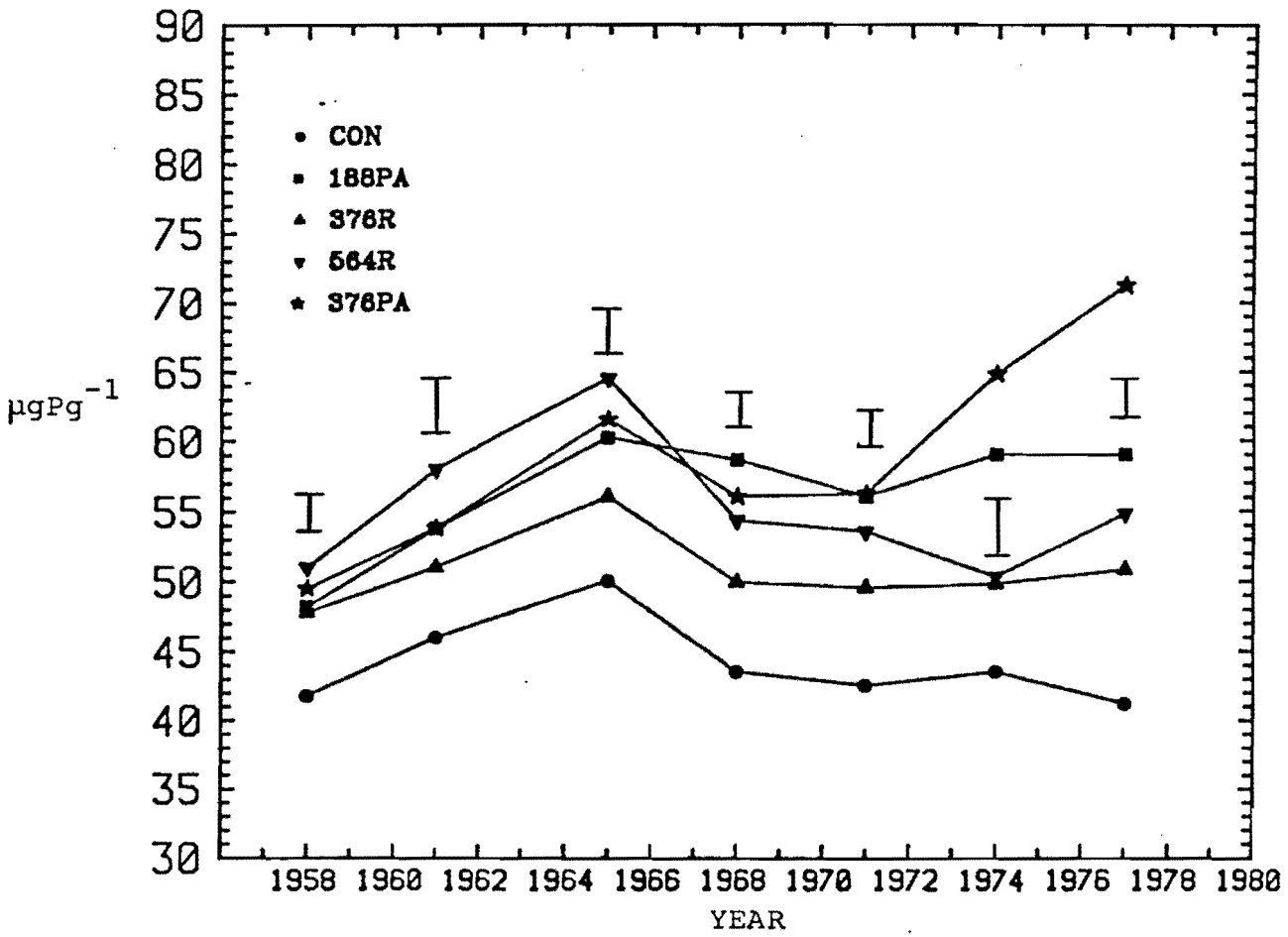
Increases in HCl P which occurred in the 188PA and 376PA soils between 1971 and 1977 (Table 3.3.2.1.1.2.1) may have been partly due to the presence of greater quantities of sparingly soluble unreacted apatite

in the superphosphate applied over this period compared with that applied earlier (i.e. 1958-1971). During the 1950's and 1960's most superphosphate in New Zealand was manufactured from Nauru and Ocean Island phosphate rocks (During, 1972). The superphosphate made from blends of these phosphate rocks generally contained very small quantities of unreacted apatite (White, 1971). During the late 1960's the exhaustion of the Ocean Island deposit and the ongoing depletion of the Nauru reserves resulted in increased quantities of Christmas Island phosphate rock being used for superphosphate manufacture in New Zealand (During, 1984). However, the presence of large amounts of iron and aluminium oxides (c. 5% w/w) in Christmas Island rock inhibits the crystallisation of MCP during the acidulation process and thereby results in a "sticky" product which is unsuitable for bulk storage and does not spread easily (McClellan and Gremillion, 1980; During, 1984). To overcome this problem less sulphuric acid is used in the manufacture of superphosphate from rock blends which contain Christmas Island rock (e.g. 50-50 Nauru-Christmas Island) than when rock blends such as Nauru-Ocean Island are used (White, 1971). As a result superphosphate manufactured in New Zealand in the 1970's contained greater quantities of unreacted apatite than that which was made during the 1950's and 1960's.

Changes in the distribution of soil inorganic P which occurred as a result of lime addition in 1972 (i.e. enhanced increases in HCl and NaOH II P - Table 3.3.2.1.1.2.1) may have been partly due to the formation of artefacts during P fractionation of limed soils. Artefact formation may have been caused by increases in the amounts of calcium in the $NaHCO_3$ and NaOH I extracts in limed soils (i.e. soils taken in 1974 and 1977). This could have resulted in the precipitation of some of the inorganic P in these extracts as calcium phosphates (Williams et al., 1971; Sorn-Srivichai et al., 1984). The precipitated calcium-P would have been extracted from the soil in the subsequent HCl and NaOH II fractions.

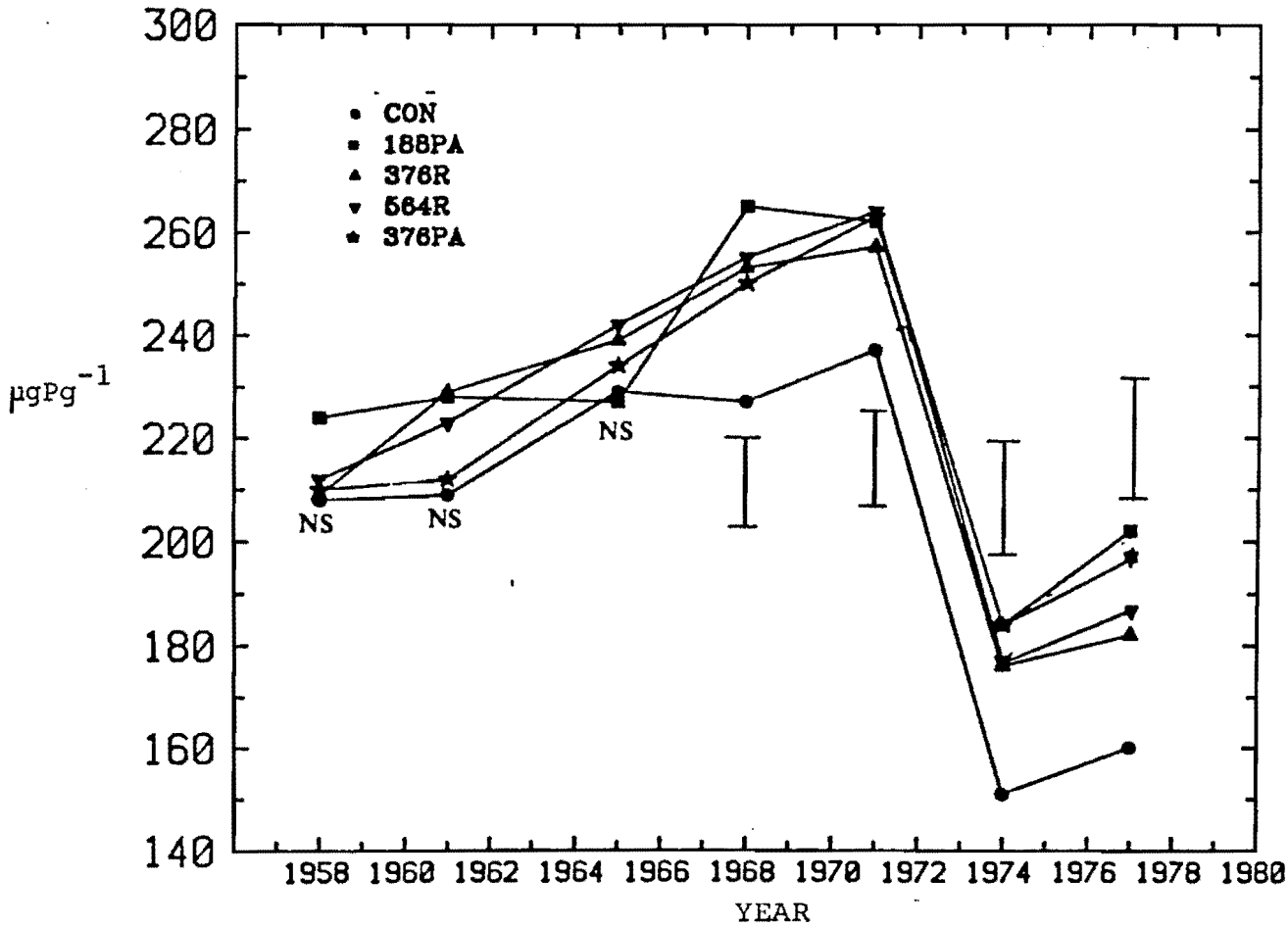
3.3.2.2 Organic Phosphorus. Amounts of organic P in the various soil P fractions ($NaHCO_3$, NaOH I, NaOH II) and the total extracted organic P (i.e. $NaHCO_3$ + NaOH I + NaOH II) in soils from the different treatments in the long-term trial at Winchmore between 1958 and 1977 are shown in Figures 3.3.2.2.1 to 3.3.2.2.4. In addition, results of the regression analysis of changes in the various soil organic P fractions with time (1958-1977) and as a result of the addition of lime in 1972 are given in Tables 3.3.2.2.1 to 3.3.2.2.4.

Between 1958 and 1971, amounts of soil organic P, particularly NaOH I organic P, increased in all treatments. Increases in soil organic P were greater in treatments which had received P fertiliser since



I=Tukey's honestly significant difference(HSD) at 5% level.

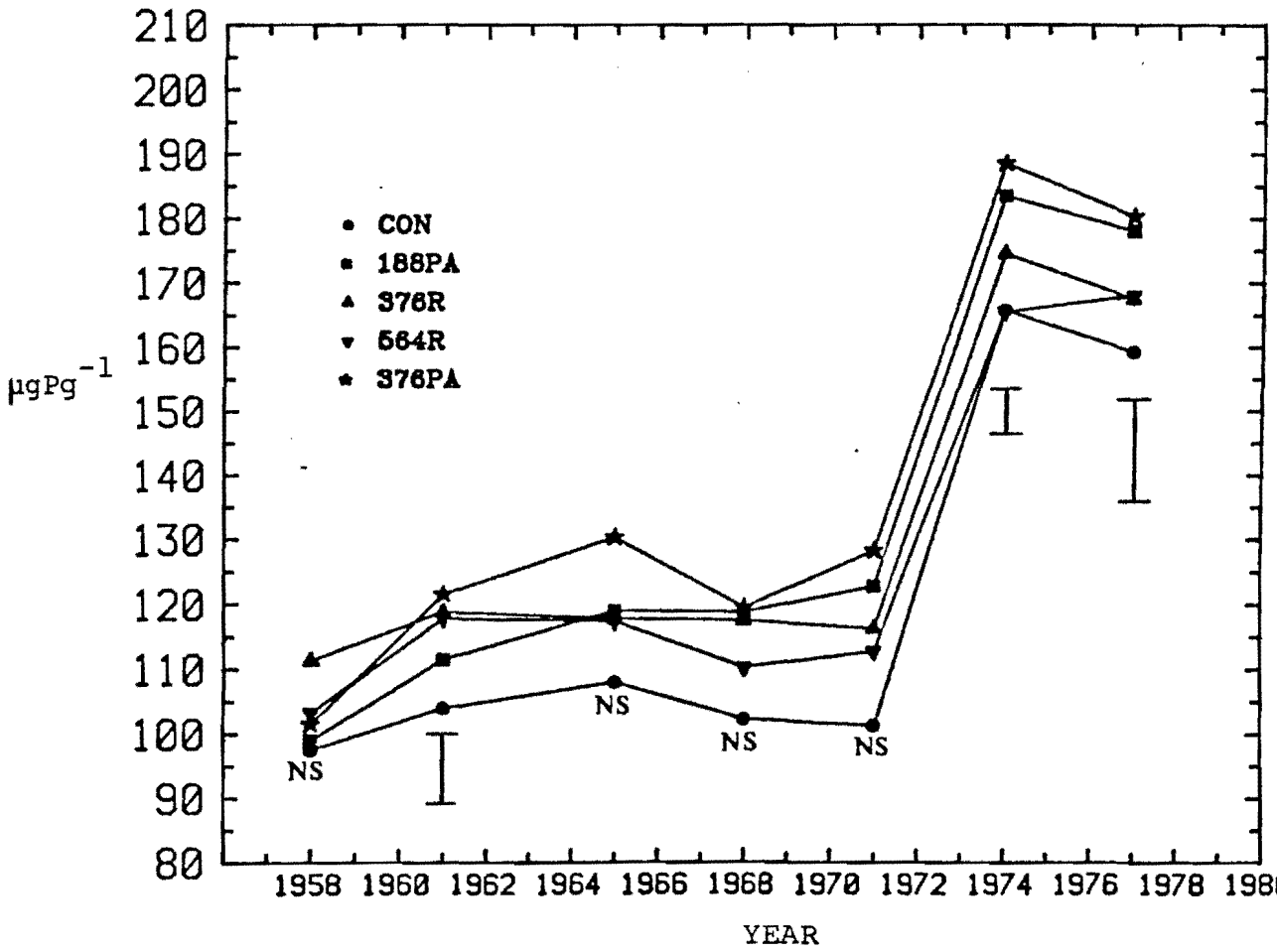
Figure 3.3.2.2.1. Amounts (μgPg^{-1}) of $NaHCO_3$ organic P in soils (0-7.5cm) from the different treatments in the Winchmore long-term trial between 1958 and 1977.



I=Tukey's honestly significant difference(HSD) at 5% level.

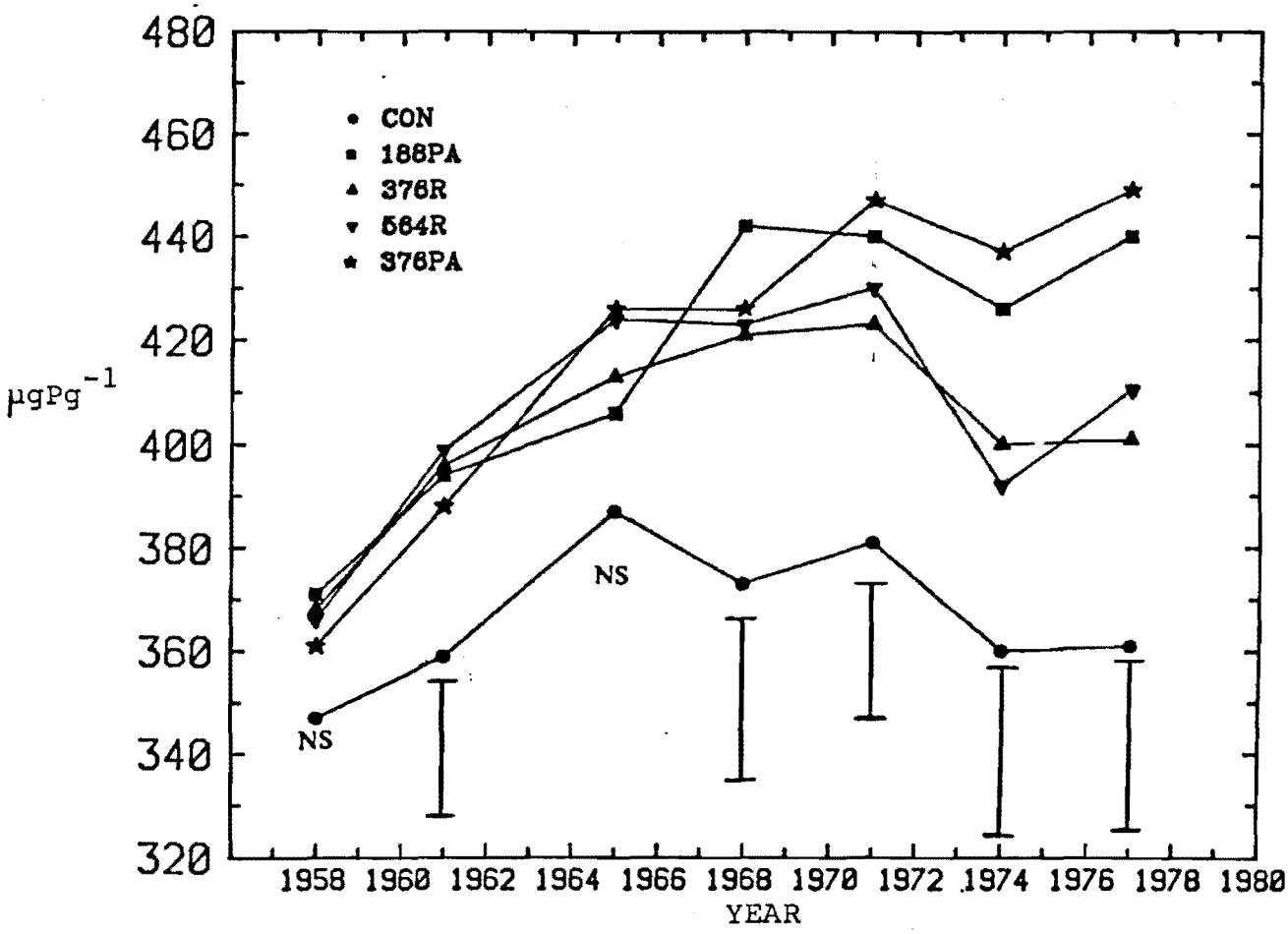
NS=no significant differences between treatment means.

Figure 3.3.2.2.2. Amounts (μgPg^{-1}) of NaOH I organic P in soils (0-7.5cm) from the different treatments in the Winchmore long-term trial between 1958 and 1977.



I=Tukey's honestly significant difference(HSD) at 5% level.
NS=no significant differences between treatment means.

Figure 3.3.2.2.3. Amounts (μgPg^{-1}) of NaOH II organic P in soils (0-7.5cm) from the different treatments in the Winchmore long-term trial between 1958 and 1977.



I=Tukey's honestly significant difference(HSD) at 5% level.

NS=no significant differences between treatment means.

Figure 3.3.2.2.4. Amounts (μgPg^{-1}) of total extracted organic P in soils (0-7.5cm) from the different treatments in the Winchmore long-term trial between 1958 and 1977.

Table 3.3.2.2.1 Regression analysis of changes in $NaHCO_3$ organic P in soils from the Winchmore long-term trial with time (1958-77) and as a result of lime addition in 1972. ($Y_p = a_p + b_{1p}T + b_{2p}T^2 + b_3L$).

Trends with time (1958-77)	Treatment	Regression Coefficients	
		Linear (b_{1p})	Quadratic (b_{2p})
	Control	-1.13 *[a]	-0.74 **[a]
	188PA	0.65 (**)[b]	-0.76 (NS)[b]
	376PA	2.27 (***)	0.11 (*)
	376R	-0.78 (NS)	-0.52 (NS)
	564R	-1.14 (NS)	-0.77 (NS)
Effect of lime addition in 1972 (b_3)[c].		3.86 NS	

Student's t-test significance levels: * = 5%; ** = 1%; *** = 0.1%; NS = not significant.

[a] = significance of the linear and quadratic trend coefficients of the control.

[b] = significance of the linear and quadratic trend coefficients compared with control.

[c] = step function adjustment (regression coefficient) averaged over all treatments.

Table 3.3.2.2.2 Regression analysis of changes in NaOH I organic P in soils from the Winchmore long-term trial with time (1958-77) and as a result of lime addition in 1972. ($Y_p = a_p + b_{1p}T + b_{2p}T^2 + b_3L$)

Trends with time (1958-77)	Treatment	Regression Coefficients	
		Linear (b_{1p})	Quadratic (b_{2p})
	Control	8.55 ***[a]	0.65 NS[a]
	188PA	12.60 (**)[b]	0.95 (NS)[b]
	376PA	14.80 (***)	0.35 (NS)
	376R	11.83 (*)	0.96 (NS)
	564R	13.40 (**)	0.09 (NS)
Effect of lime addition in 1972 (b_3)[c]		-96.35 **	

Student's t-test significance levels: * = 5%; ** = 1%; *** = 0.1%; NS = not significant.
[a] = significance of the linear and quadratic trend coefficients of the control.
[b] = significance of the linear and quadratic trend coefficients compared with control.
[c] = step function adjustment (regression coefficient) averaged over all treatments.

Table 3.3.2.2.3 Regression analysis of changes in NaOH II organic P in soils from the Winchmore long-term trial with time (1958-77) and as a result of lime addition in 1972. ($Y_p = a_p + b_{1p}T + b_{2p}T^2 + b_3L$)

Trends with time (1958-77)	Treatment	Regression Coefficients	
		Linear (b_{1p})	Quadratic (b_{2p})
	Control	-0.69 NS[a]	-0.90 NS[a]
	188PA	2.29 (*)[b]	-1.43 (NS)[b]
	376PA	1.70 (NS)	-2.08 (NS)
	376R	-1.44 (NS)	-1.15 (NS)
	564R	-1.27 (NS)	-1.29 (NS)
Effect of lime addition in 1972 (b_3)[c]		64.33 **	

Student's t-test significance levels: * = 5%; ** = 1%; *** = 0.1%; NS = not significant.

[a] = significance of the linear and quadratic trend coefficients of the control.

[b] = significance of the linear and quadratic trend coefficients compared with control.

[c] = step function adjustment (regression coefficient) averaged over all treatments.

Table 3.3.2.2.4 Regression analysis of changes in total extracted organic P in soils from the Winchmore long-term trial with time (1958-77) and as a result of lime addition in 1972. ($Y_p = a_p + b_{1p}T + b_{2p}T^2 + b_3L$)

Trends with time (1958-77)	Treatment	Regression Coefficients	
		Linear (b_{1p})	Quadratic (b_{2p})
	Control	6.74 **[a]	-1.17 NS[a]
	188PA	16.08 (***)[b]	-0.88 (NS)[b]
	376PA	19.43 (***)	-1.13 (NS)
	376R	11.10 (*)	-1.38 (NS)
	564R	11.73 (*)	-1.67 (NS)
Effect of lime addition in 1972 (b_3)[c]		-30.59 ***	

Student's t-test significance levels: * = 5%; ** = 1%; *** = 0.1%; NS = not significant.

[a] = significance of linear and quadratic trend coefficients of the control.

[b] = significance of linear and quadratic trend coefficients compared with control.

[c] = step function adjustment (regression coefficient) averaged over all treatments.

1952 (i.e. 188PA, 376PA, 376R, 564R) than in the control, although surprisingly there was little difference between the annually fertilised treatments (188PA, 376PA) and the residual fertiliser treatments (376R, 564R).

From 1971 to 1974 marked decreases in organic P occurred in all soils, particularly in the control and residual fertiliser (376R, 564R) treatments. These decreases in soil organic P were undoubtedly related to the application of lime to the long-term trial in 1972, which also caused a dramatic change in the distribution of organic P in all soils. In the annually fertilised soils (188PA, 376PA) between 1974 and 1977, organic P increased to a level approaching that in 1971 before lime application. On the other hand in the control and residual fertiliser (376R, 564R) treatments, overall net decreases in soil organic P occurred between 1971 and 1977.

3.3.2.2.1 Changes in Organic Phosphorus between 1958 and 1971. In 1958, amounts of total extracted organic P were slightly greater in soils under pasture which had received annual applications of fertiliser P between 1952 and 1957 (i.e. 188PA, 376PA, 376R, 564R) than in that of the control soil (Figure 3.3.2.2.4). Most of these increases were ~~large~~ largely due to significant increases in $NaHCO_3$ organic P (c.f. Figure 3.3.2.2.1 with Figures 3.3.2.2.2 and 3.3.2.2.3). This suggests

that the accumulated organic P is recent and of a labile nature (Bowman and Cole, 1978a). Organic P in fertilised soils accumulated largely as a result of increased pasture production compared with the control (Figure 3.3.2.1.6) in the form of plant and animal residues. Levels of pasture production in the various fertilised treatments between 1952 and 1958 were very similar (Figure 3.3.2.1.6) suggesting that similar amounts of soil organic P accumulated in these treatments in 1958 (Figure 3.3.2.2.4).

Between 1958 and 1971 amounts of soil organic P increased in all treatments (Figure 3.3.2.2.4). Increases in total extracted soil organic P over this period were greater in the treatments (188PA, 376PA, 376R, 564R) which had received P fertiliser since 1952 (58-80 $\mu g\ P\ g^{-1}$) than in the control (34 $\mu g\ P\ g^{-1}$) (Table 3.3.2.2.1.1). Although the overall trends with time were similar between fertilised treatments (i.e. 188PA, 376PA, 376R, 564R) (Figure 3.3.2.2.4), increases in total extracted organic P between 1965 and 1971 were slightly greater in the annually fertilised soils (21-34 $\mu g\ P\ g^{-1}$) than in the residual fertiliser soils (6-10 $\mu g\ P\ g^{-1}$) (Table 3.3.2.2.1.1).

In all treatments, increases in total extracted organic P which occurred between 1958 and 1971 were mainly due to increases in the NaOH I organic P fraction (c.f. Figure 3.3.2.2.2 with Figures 3.3.2.2.1 and 3.3.2.2.3). This is consistent with results reported earlier for the distribution of soil P

Table 3.3.2.2.1.1 Amounts (μg P g^{-1}) of total extracted organic P in soils from the different treatments of the Winchmore trial sampled in 1958, 1965, 1971, 1974 and 1977.

Treatment	Year of Sampling				
	1958	1965	1971	1974	1977
Control	347.1	387.0 (+5.7)[a]	380.7 (-1.0)[b]	360.3	361.0 (-3.3)[c]
188PA	370.8	406.0 (+5.0)	440.5 (+5.8)	426.3	439.5 (-0.1)
376PA	361.3	425.8 (+9.2)	447.3 (+3.6)	437.0	449.3 (+0.3)
376R	368.3	412.8 (+6.4)	423.0 (+1.7)	399.8	400.8 (-3.7)
564R	365.8	423.8 (+8.3)	430.0 (+1.0)	391.5	410.3 (-3.3)

[a] = mean annual changes in organic P (μg P g^{-1} $year^{-1}$) between 1958 and 1965.

[b] = mean annual changes in organic P (μg P g^{-1} $year^{-1}$) between 1965 and 1971.

[c] = mean annual changes in organic P (μg P g^{-1} $year^{-1}$) between 1971 and 1977.

fractions (see section 3.3.1.2).

Overall increases in NaOH I organic P between 1958 and 1971 were greater in the fertilised treatments than in the control, although differences observed were only significant over the 1965 to 1971 period (Figure 3.3.2.2.2). Between 1958 and 1965 $NaHCO_3$ organic P increased in all treatments (Figure 3.3.2.2.1), but these increases were counterbalanced by decreases in this fraction which occurred between 1965 and 1971 thus producing little or no overall change in $NaHCO_3$ organic P between 1958 and 1971. Small increases in NaOH II organic P occurred in the fertilised treatments between 1958 and 1971, particularly in the 376PA treatment (Figure 3.3.2.2.3).

Increases in soil organic P which occurred in all treatments between 1958 and 1971 (Figure 3.3.2.2.4) probably resulted from the biological immobilisation of inorganic P via plant, animal and microbial residues. Some of the inorganic P taken from the soil by plants is converted to organic P forms such as inositol phosphates, nucleic acids and phospholipids (Dalal, 1977). In a pasture grazed by sheep, most of the inorganic and organic P in plants is returned to the soil in plant debris and animal faeces (Holford, 1977; Blair et al., 1977; Quin and Rickard, 1979). Similarly, some inorganic P may be taken up by micro-organisms in the soil and converted to organic P in microbial detritus (Alexander, 1977).

The long-term trial at Winchmore was established on an area of browntop pasture which had not received P fertiliser for many years (see section 3.2.1). The fertility status of the soil was very low and as such the pasture production and level of soil organic P were very low. However, the introduction of border-dyke irrigation and the sowing of a new ryegrass-clover pasture together with superphosphate fertiliser additions significantly increased pasture production. An appreciable accumulation of organic matter, including organic P, has been noted to occur when previously P deficient soils are sown with legume based pastures (Barrow, 1980). Thus increases in dry matter yield of pasture have ~~lead~~ led to greater accumulation of soil organic P. This is supported by the finding in the control treatment which showed increases in soil organic P between 1958 and 1971 (Figure 3.3.2.2.4) corresponding to the improved pasture production which resulted from the additions of P fertiliser (420 kg ha^{-1} superphosphate) made to the whole trial area between 1948 and 1952 (see section 3.2.1). Likewise, large increases in soil organic P in the annual and residual fertilised treatments compared with the control between 1958 and 1971 (Figure 3.3.2.2.4) were closely related to the increased pasture production of fertilised treatments after 1952 (Figure 3.3.2.1.6).

Although significant increases in soil organic P occurred in all treatments between 1958 and 1971 the rates of this organic P accumulation decreased with time. For example, the average annual changes in soil organic P in the control, residual fertiliser (376R, 564R) and annually fertilised (188PA, 376PA) treatments between 1958 and 1965 were +5.7, +7.3 and +7.1 μg P g^{-1} $year^{-1}$ respectively, compared with -1.0, +1.4 and +4.7 μg P g^{-1} $year^{-1}$ between 1965 and 1971 (Table 3.3.2.2.1.1). These results suggest that in each of the treatments the respective rates of organic P accumulation (i.e. a balance between organic P addition and mineralisation) in the soil was slowly reaching a steady-state situation. This is consistent with results reported in other studies in New Zealand which showed that the accumulation of organic P in low-fertility pasture soils receiving P fertiliser decreased with time (Jackman, 1964).

3.3.2.2.2 Changes in Organic Phosphorus between 1971 and 1977. Between 1971 and 1974, significant decreases in total extracted soil organic P occurred in all treatments (Figure 3.3.2.2.4; Table ~~3.3.2.2.4~~ 3.3.2.2.1.1). These were greater in the control and residual fertiliser treatments (20-38 μg P g^{-1}) than in the annually fertilised treatments (10-14 μg P g^{-1}) (Table ~~3.3.2.1.1~~ 3.3.2.2.1.1). The results suggest that some net mineralisation of organic P had occurred in all treatments between 1971 and 1974 due to the addition

of lime (4t ha^{-1}) to the trial in 1972.

Lime addition in 1972 did not appear to significantly affect the amounts of $NaHCO_3$ organic P in all soils (Figure 3.3.2.2.1). However, between 1971 and 1974, there were highly significant decreases in NaOH I organic P in all treatments (Figure 3.3.2.2.2, Table 3.3.2.2.2). The NaOH II organic P showed highly significant increases, although the magnitude of change was smaller than that of NaOH I organic P (Figure 3.3.2.2.3, Table 3.3.2.2.3). Changes in these 2 fractions (NaOH I, NaOH II) accounted for the overall decreases observed in total organic P (Figure 3.3.2.2.4; Table 3.3.2.2.4). Since NaOH I organic P changed more than that of NaOH II organic P, it is likely that most of the organic P mineralised as a result of lime addition in 1972 was derived from the NaOH I fraction.

The net mineralisation of soil organic P due to lime addition in 1972 may have been due to a combination of biological and chemical factors. The pH of the soils on the Winchmore trial immediately prior to liming in 1972 was about 5.8 and increased to 6.5 after liming (Quin and Rickard, 1979). It is possible that the addition of lime to these slightly acid soils could have resulted in increases in biological activity which, in turn, could have been partly responsible for the observed organic P mineralisation. General studies have shown that in acid soils (pH <6) the addition of liming materials resulted in increased microbial activity (Halstead

et al., 1963; Sarathchandra and Upsdell, 1981). Any enhancement in soil microbial activity increases microbial demand for P which may be obtained from the mineralisation of organic P. This may partly account for greater decreases in soil organic P between 1971 and 1974 in the control and residual fertiliser treatments (376R, 564R) than in the annually fertilised treatments (188PA, 376PA) (Table 3.3.2.2.1.1). The presence of low levels of readily available inorganic P in the soils of the control and residual fertiliser treatments (376R, 564R) may have resulted in more organic P being mineralised to satisfy microbial demand for P than in the higher P status soils (188PA and 376PA treatments) (McGill and Cole, 1981).

Increases in soil pH and calcium which result from lime addition may have enhanced the solubility of some organic P species in the soil thereby increasing their susceptibility to microbial and enzymic attack and consequent mineralisation. For example, inositol hexaphosphate (IHP), a major constituent of the organic P in most soils (Anderson, 1980), is believed to be present mainly as sparingly soluble iron, aluminium and calcium salts and is also known to be adsorbed into hydrous oxides and aluminosilicate minerals (Greaves and Webley, 1969; Anderson et al., 1974). It has been found that the calcium salt of IHP is more soluble

than the corresponding iron and aluminium salts (Jackman and Black, 1951; Greaves and Webley, 1969). Lime addition to an acid soil may increase the biological availability and mineralisation of IHP due to conversion of some of the iron and aluminium IHP to the more soluble calcium salts. In addition, since IHP is adsorbed onto soil colloid surfaces by similar mechanisms to that for inorganic P (Anderson et al., 1974), it is possible that increases in soil pH by liming may increase the desorption of adsorbed IHP (Haynes, 1984) and thereby improve its biological availability.

The results which showed decreases in NaOH I organic P with corresponding increases in NaOH II organic P in all treatments between 1971 and 1974 due to lime addition in 1972 (Figures 3.3.2.2.2 and 3.3.2.2.3) may have been caused by (i) actual changes in the chemical nature of organic P-mineral colloid interactions and/or (ii) the formation of artefacts during soil P fractionation.

It is possible that the addition of a large amount of calcium by liming results in the conversion of some of the organic P-mineral complexes. The iron and aluminium P forms (i.e. NaOH I organic P - extractable by alkali alone) were converted to calcium or calcium-iron/aluminium P forms (i.e. NaOH II organic P - extractable by alkali following acid treatment to remove calcium).

Liming may also result in formation of artefacts during the fractionation of soil P. Increased amounts of calcium following liming probably increased the levels of calcium in the NaOH I extract. This may have caused some precipitation of the freshly extracted organic P present (Dyer and Wrenshall, 1941). In the fractionation scheme used in the present study (Figure 3.2.3.1) the precipitated organic P from the NaOH I extract would be recovered after acid treatment to remove calcium (NaOH II fraction).

Following decreases in organic P which occurred in all soils between 1971 and 1974, organic P increased in the annually fertilised soils (188PA, 376PA) between 1974 and 1977 (Figure 3.3.2.2.4). The effect of liming the fertilised soils (188PA, 376PA) on total extracted soil organic P appears to be transient as the levels returned to ~~that~~ those of the pre-liming period after 1974 (Figure 3.3.2.2.4). This did not occur in the control and residual fertiliser (376R, 564R) treatments. Reasons for this are not clear. It is possible that microbial ~~availability~~ activity in annually fertilised soils was higher following liming and this enabled increased microbial immobilisation of added soluble fertiliser P. In the soils of the control and residual fertiliser (376R, 564R) treatments, a longer period may be needed before the organic P levels return to the pre-lime levels.

3.4 GENERAL DISCUSSION

Results of the present study showed that a large proportion of the fertiliser P applied to the annually fertilised treatments (188PA, 376PA) between 1958 and 1971 was converted to organic P in the soil. For example, increases in total extracted organic P between 1958 and 1971 accounted for 99% and 48% of the overall increases in total P in the 188PA and 376PA soils respectively (Table 3.3.1.2.1). Although the proportion of total P accumulated as organic P was greater in the 188PA soil than in the 376PA soil, the actual amounts of organic P which accumulated in the 188PA and 376PA soils between 1958 and 1971 were very similar (70-86 $\mu g\ P\ g^{-1}$) (Table 3.3.2.2.1). Increases in soil organic P which occurred between 1958 and 1971 in the residual fertiliser treatments (55-64 $\mu g\ P\ g^{-1}$) and the control (34 $\mu g\ P\ g^{-1}$) were smaller than those in the annually fertilised treatments (Table 3.3.2.2.1). These increases in soil organic P were probably due to the biological conversion of fertiliser and native inorganic P to organic P forms via plant, animal and microbial residues (i.e. P immobilisation).

In the residual fertiliser treatments (376R, 564R), where annual applications of P fertiliser were ceased in 1957, soil organic P continued to increase between 1958 and 1971 while soil inorganic

P decreased (Figure 3.3.2.1.5). Over the same period pasture production in the residual fertiliser treatments declined (Figure 3.3.2.1.6). This suggests that P was limiting plant growth and plants obtained most of their P from inorganic P forms in the soil. Thus, organic P formed as a result of fertiliser P immobilisation appears to be more stable and less available to plants than the inorganic forms of P in the soil.

In all treatments, rates of organic P accumulation decreased with time between 1958 and 1971, while no further accumulation of soil organic P occurred in the control treatment after 1965 (Table 3.3.2.2.1.1). This suggests that a steady-state condition in soil organic P accumulation was being reached in all treatments (Anderson, 1980).

The above results showed that in the annually fertilised pastures, with time the accumulation of inorganic P in the soil becomes more important than that of organic P because the latter tends to reach a steady state. Under these conditions the continued accumulation of inorganic P may have to be considered when determining the ongoing P fertiliser requirements of established pastures. This assumes that the accumulated inorganic P is plant available unlike the accumulated organic P. The extent to which the accumulated inorganic P under steady-state

conditions is available to plants was not measured in the present study but the accumulated inorganic P was present in all extracted P forms determined (Table 3.3.2.1.1.2.1). Further studies are required to determine the relative plant availability of the different forms of accumulated fertiliser P in different soils.

In the annually fertilised 188PA treatment, the decreasing rate of soil organic P accumulation with time corresponded with an accelerated increase in the rate of inorganic P accumulation, particularly after 1971 (Table 3.3.2.1.1.2.1). Before 1971, increases in soil inorganic P in the annually fertilised treatments occurred mainly in the $NaHCO_3$ and NaOH I P fractions (Table 3.3.2.1.1.2.1). After 1971, soil inorganic P increases in the annually fertilised soils were found mainly in the HCl and NaOH II P fractions (Table 3.3.2.1.1.2.1). This was probably due to the combined effects of lime addition in 1972 and the presence of increased quantities of sparingly soluble P species such as apatite and iron-aluminium phosphates in the single superphosphate applied during the 1970's. As the HCl and NaOH II P fractions were found to be less plant available than the $NaHCO_3$ and NaOH I P fractions in the exhaustive pot trial of the present study (see later Chapter 5), liming appears to affect not only the forms of soil inorganic P but also P availability.

Significant decreases in soil organic P occurred after liming in all treatments. This was attributed to the combined biological and chemical effects of liming which affected the balance between P immobilisation and mineralisation and resulted in favour of the latter. Amounts of organic P attributed to mineralisation as a result of liming were greater in the control (20 μg P g^{-1}) and residual fertiliser treatments (23-38 μg P g^{-1}) than in the annually fertilised treatments (10-14 μg P g^{-1}) (Table 3.3.2.2.1.1). This mineralisation probably contributed to the observed cessation of further net organic P accumulation in the different treatments after 1971. However, the liming effect did not last for more than 2 to 3 years since organic P accumulation resumed in the annually fertilised treatments after 1974 (Figure 3.3.2.2.1.4).

Liming also affected the chemical nature of the soil organic P as shown by decreases in the NaOH I organic P fraction and concomitant increases in the NaOH II organic P fraction which occurred in all treatments between 1971 and 1974 (Figures 3.3.2.2.1.2 and 3.3.2.2.1.3). However, this effect may have been an artefact of soil P fractionation caused by the presence of increased levels of calcium in the soil following liming.

As liming was found to decrease organic P levels and increase its mineralisation, additions of liming materials at regular intervals to maintain a high soil pH (pH = 6.5) may have reduced the accumulation of soil organic P which occurred between 1958 and 1971. Oniani et al. (1973) reported decreased soil organic P accumulation from regular liming of a pasture soil which received annual single superphosphate applications for over 100 years.

Significant decreases in soil inorganic P fractions (i.e. $NaHCO_3$, NaOH I, HCl, NaOH II P) occurred in the residual fertiliser treatments between 1958 and 1977 after P fertiliser applications were stopped in 1957 (Table 3.3.2.1.1). Thus, some of the inorganic P which accumulated as a result of P fertiliser additions between 1952 and 1957 was utilised by plants. Despite decreases in soil inorganic P fractions in the residual fertiliser treatments with time, levels of P in the different fractions remained greater than those in the control treatment (Figures 3.3.2.1.1 to 3.3.2.1.4). This suggests that some of the P compounds formed as a result of reactions between fertiliser P and the soil mineral colloids are very stable and as such are only slowly made available to plants (Barrow, 1980).

CHAPTER 4

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CHAPTER 4

CHEMICAL NATURE OF ORGANIC PHOSPHORUS IN SOILS UNDER IRRIGATED PASTURE

4.1 INTRODUCTION

Results from studies of the changes in soil P which occurred in the long-term trial at Winchmore showed that soil organic P increased with time, particularly in the plots which received superphosphate fertiliser (see Chapter 3). The objective of the study reported here was to examine the chemical nature of organic P in the Winchmore soils and the changes which occurred as a result of P fertiliser additions. This involved extracting the organic P from the soil and determining its chemical composition and molecular weight distribution using ^{31}P nuclear magnetic resonance (^{31}P NMR) spectroscopy and gel filtration chromatography respectively.

4.2 MATERIALS AND METHODS

4.2.1 Soils Used

The soils used in this study were obtained from the long-term superphosphate field trial at Winchmore which was described in detail in section 3.2.1. Topsoil samples (0-7.5cm) from the control (i.e. no P fertiliser since 1952) and 376PA (i.e. 376 kg superphosphate ha^{-1} yr^{-1} since 1952) treatments sampled in 1958, 1971 and 1983 were selected. For each treatment, an equal quantity (5g) of soil was

taken from each of the 4 replicates and bulked together to constitute a composite sample per treatment for each year. The soils were air dried and uniformly ground (<150μm) prior to analysis.

4.2.2 Extraction of Organic Phosphorus from the Soil.

In order to examine the chemical nature of soil organic P it is necessary to extract the organic P from the soil. Quantitative recovery of organic P from the soil generally involves sequential extraction with several alkali and acid reagents (Anderson, 1975). The particular extraction method used in the present study was based on the methods described by Anderson (1960) and Halstead et al. (1966) and involved 3 alkali extractions (i.e. 0.1M NaOH, 0.2M aq. acetylacetone (pH 8.3), 0.5M NaOH) in conjunction with pretreatment of the soil with 0.5M HCl (Figure 4.2.2.1). No organic P was found in the HCl extracts and consequently these extracts were discarded.

To examine the chemical nature of the total extracted organic P in the control and 376PA soils sampled in 1958, 1971 and 1983 the component alkali extracts (i.e. 0.1M NaOH, acetylacetone and 0.5M NaOH) from each soil were combined together (5g soil = 300 mls extract). In addition, the chemical nature of the organic P in the component alkali extractants from one soil (376PA - 1983) was examined by extracting 15g soil and keeping the component extracts

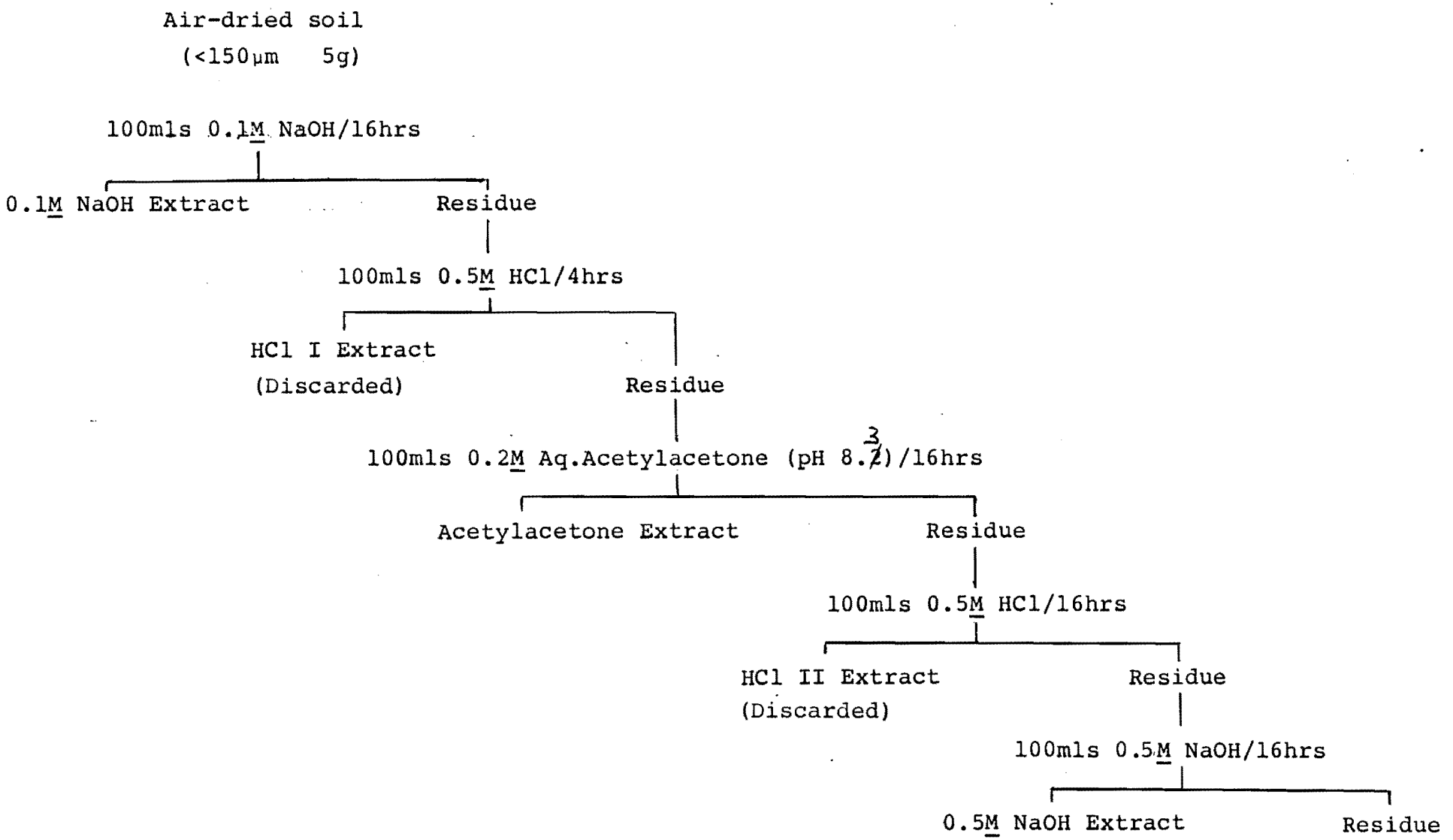

Figure 4.2.2.1 The sequential scheme for the quantitative extraction of soil organic phosphorus.

separate (15 g soil = 300 mls extract^{-1}).

4.2.3 Concentration of Soil Extracts

The soil solution ratio (1:20) used in the sequential extraction scheme (Figure 4.2.2.1) resulted in low P concentrations in the extracts (< 25 µg total P ml^{-1}). Quantitative ^{31}P NMR analysis of soil extracts requires a minimum solution P concentration of 100µg total P ml^{-1} (R.H. Newman, pers. comm.), while considerable dilution of solute occurs during gel filtration (Flodin, 1962). Consequently, it was necessary to concentrate the original soil extracts prior to determining the chemical nature of the extracted soil P by ^{31}P NMR and gel filtration.

Organic matter in the soil (including organic P) is believed to be present mainly in polymeric forms (i.e. macromolecules) (Russell, 1973). Accordingly, it was decided to concentrate the soil extracts using ultrafiltration. Ultrafiltration involves the selective rejection of solute molecules by convective flow through an anisotropic "skinned" membrane. Thus, solute macromolecules which are larger than the uniform minute pores on the membrane are retained in the solution, while molecules which are smaller than the pores pass through the membrane with the solvent (Figure 4.2.3.1). By using a membrane with a low molecular weight cut-off (e.g. MW 500) it is possible to reduce the

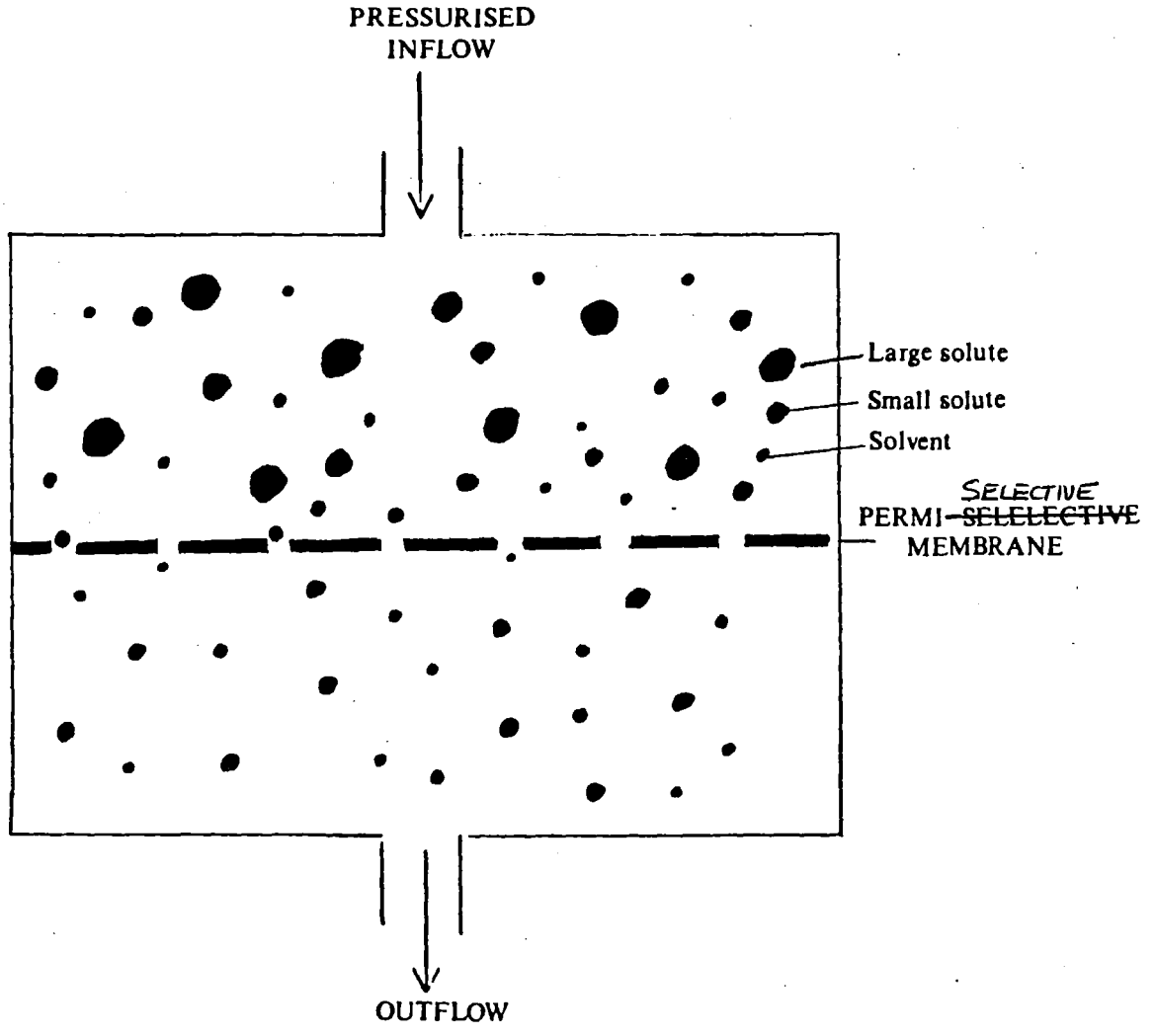

Figure 4.2.3.1. The principle of ultrafiltration through a permiselective membrane.

volume of the soil extracts with minimal passage of P containing organic macromolecules through the membrane.

The membranes used for ultrafiltration are adversely affected by strong alkali conditions (pH >11) and it was therefore necessary to reduce the pH of the alkali soil extracts to 10 prior to ultrafiltration. However, it was found that some colloidal precipitates formed in the extracts when the pH was reduced to 10. Consequently, following pH adjustment with 0.1M HCl the extracts were allowed to stand overnight at room temperature and then centrifuged at 20,000 RPM for 20 minutes to remove the precipitated materials.

Ultrafiltration of the soil extracts was carried out using an Amicon 202 pressurised stirred-cell unit (200ml capacity) fitted with an Amicon Dia-flo UM-05 membrane (nominal molecular weight cut-off at 500) (Amicon Corporation, Lexington, Mass., USA). The ultrafiltration unit was mounted on a magnetic stirrer unit and connected to pressurised source of oxygen-free nitrogen gas (N_2). The complete ultrafiltration system used to concentrate the soil extracts in this study is shown in Figure 4.2.3.2.

Using ultrafiltration the volumes of combined and component soil extracts were reduced from 300mls to 25mls in 2 stages:

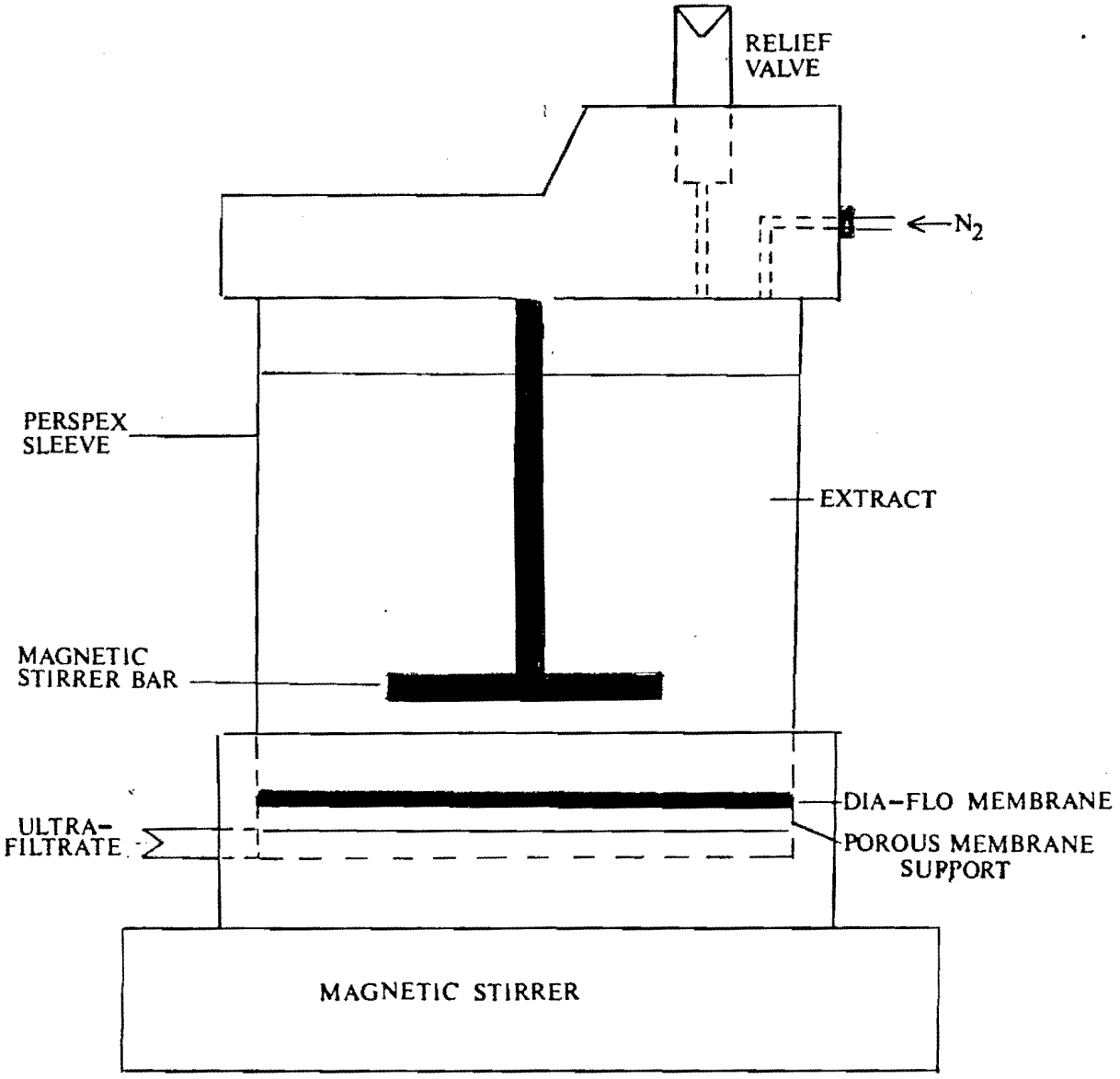

Figure 4.2.3.2. Amicon pressurised stirred-cell system used for ultrafiltration of soil extracts.

(i) Three successive 100ml aliquots of soil extract were placed in the ultrafiltration unit and the volume reduced to 30mls using a gas pressure of 2.9kg cm^{-2} (5-6 hours $100mls^{-1}$). Thus, 300ml extracts were reduced to 90mls by this initial ultrafiltration.

(ii) Final concentration of the soil extracts to 25mls was achieved by further ultrafiltration at a lower gas pressure of 2.2kg cm^{-2} (12-16 hours).

In order to obtain sufficiently high concentrations of P in solution for ^{31}P NMR and gel filtration volumes of the various ultrafiltered extracts were further reduced to 10mls by low temperature (<40°C) rotary evaporation. The concentrated extracts were stored under nitrogen gas at 5°C.

4.2.4 Determination of Phosphorus in Soil Extracts

Aliquots of soil extracts were generally diluted 25-50 fold prior to P analysis. Inorganic P was determined directly using the molybdenum blue method of Dick and Tabatabai (1977), while total P was determined following perchloric-nitric acid digestion using the Murphy-Riley method; organic P was determined by the difference between the total and inorganic P. The various procedures involved in the determination of P in the diluted soil extracts

were described earlier (section 3.2.3).

Results of P analysis of soil extracts were converted to μg P g^{-1} soil according to the quantity of soil and the soil:solution ratio (1:20) used in the extraction scheme (Figure 4.2.2.1).

4.2.5 Determination of Total Soil Organic Phosphorus by Ignition

The amount of organic P extracted from the soil by the sequential extraction scheme (Figure 4.2.2.1) was compared with the total soil organic P determined by ignition. The ignition method used was based on that described by Saunders and Williams (1955). This involved shaking 2g of ignited soil (550°C for 1 hour) with 100mls 1M H_2SO_4 for 16 hours to extract total P. The extraction was repeated on unignited soil for inorganic P. The organic P was determined by the difference between the total and inorganic P.

4.2.6 ^{31}P Nuclear Magnetic Resonance Analysis of Extracted Soil Phosphorus

The ^{31}P NMR Spectra of the concentrated soil extracts were obtained using a Varian FT-80A spectrometer, operating at 32.2 MHz. The samples were run in 10mm diameter glass tubes and consisted of 1.2mls of soil extract and 0.2ml deuterium oxide (D_2O) which provided an internal deutron NMR lock signal.

Each radiofrequency pulse was preceded by a delay of 0.5s, during which the spin decoupler was

switched off to suppress Overhauser enhancements which can distort relative signal areas (Newman and Tate, 1980). Preliminary measurements of the spin-lattice relaxation times (T), for extracts of other pasture soils showed that such a delay would be adequate for signal recovery, being several times as long as the slowest-relaxing signal in each spectrum. Transients from between 14,000 and 34,000 pulses were accumulated, with data acquisition time of 0.5s and a spectral width of 4kHz. Peaks were identified by chemical shifts (δ ppm) which were measured relative to external 85% orthophosphoric acid (H_3PO_4) (Newman and Tate, 1980).

The relative concentrations ($\mu g\ P\ g^{-1}$) of the different P species were estimated by cutting out and weighing the peaks in expanded plots of the ^{31}P NMR spectra. The inorganic orthophosphate and orthophosphate monoester signals were separated by dropping a vertical line to the baseline from the dip between the 2 signals.

4.2.7 Gel Filtration Chromatography of Soil Extracts

The molecular weight distribution of organic P in the concentrated soil extracts ~~were~~ was determined using the Sephadex gel filtration technique. The particular Sephadex gel used was standard grade G-100 (dry bead diameter 40-120 μm) which has a nominal fractionation range of 1000-100,000 (Pharmacia Fine Chemicals, Uppsala, Sweden).

4.2.7.1 Gel Preparation and Column Packing. Sephadex G-100 gel was prepared and packed into a column according to the procedures outlined by Flodin (1962) and described in the manual "Gel Filtration - theory and practice" (Pharmacia Fine Chemicals, Uppsala, Sweden).

Dry Sephadex G-100 (23g) was preswollen in 0.5% w/v NaCl for 24 hours at room temperature. The swollen gel was washed repeatedly with distilled water to remove fine gel particles and immediately prior to column packing the gel slurry was evacuated in a desiccator to remove trapped air bubbles.

The gel was packed into a vertically mounted Glenco glass column (60cm x 2.5cm diameter) fitted with heavy duty plastic adaptors (Glenco Corp., Houston, Texas, USA). Prior to the addition of gel, the column was ¼ filled with distilled water from the bottom to remove air bubbles from the outlet tube and valve. The outlet was then closed and the gel slurry poured into the column in one operation. The gel was allowed to settle with the outlet closed until 2-3cm of consolidated gel had formed. The outlet was opened and the remaining gel in suspension was allowed to settle under gravity feed at a flow rate of approximately 0.5 ml $minute^{-1}$. At this stage, the column was sealed and connected to a solvent reservoir (Tris buffer at pH 9) and an Eyela-MP3 peristaltic pump (Tokyo Rikakai, Tokyo,

Japan) which enabled a constant flow of solvent through the column to be maintained. The flow rate was increased to 1ml $minute^{-1}$ and maintained at this rate for several hours to allow further setting of the gel to occur. The height of the column was then adjusted to 45cm by removing some gel with a pipette. The outlet of the column was connected to a Frac-100 fraction collector (Pharmacia Fine Chemicals, Uppsala, Sweden) which enabled up to 100 consecutive fractions of eluent to be collected. The complete system used to determine the molecular weight distribution of organic P in soil extracts using Sephadex G-100 is shown in Figure 4.2.7.1.1.

The void and total volumes of the column of Sephadex G-100 were determined using coloured high and low molecular weight marker compounds respectively. The void volume (Vo) was determined using "Blue Dextran 2000" (Pharmacia Fine Chemicals, Uppsala, Sweden) which has a molecular weight in excess of 2,000,000 and is therefore totally excluded from the gel particles. The total column volume (Vt) was determined using a yellow coloured solution of 2,4 dinitrophenyl glycine ($(NO_2)_2C_6H_3NH\text{Ø}H_2CO_2H$) which has a very low molecular weight (241) and is therefore able to enter and equilibrate fully with the G-100 matrix.

4.2.7.2 Gel Filtration of Soil Extracts

The solvent used in this study was an alkaline-amino

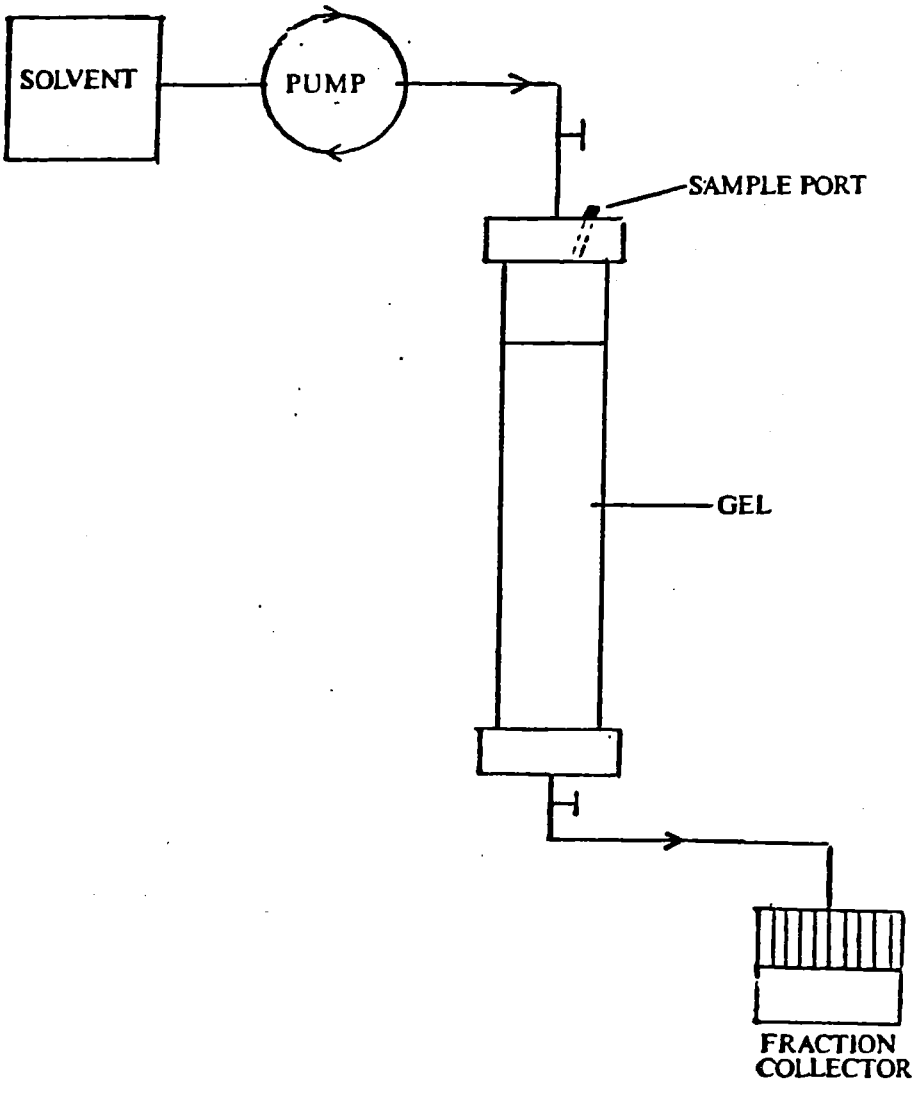

Figure 4.2.7.1.1. Schematic diagram of the system used for Sephadex gel filtration of soil extracts.

buffer (Tris (2 amino-2 (hydroxymethyl)-propane-1.3-diol) buffer at pH9) which was designed to overcome any adsorption of phenolic, heterocyclic and aromatic groupings on the soil organic macromolecules onto the Sephadex gel (Swift and Posner, 1971).

During sample application to the column the outlet valve was closed and solvent flow stopped. A small aliquot (1-5mls) of concentrated soil extract was taken up in a 5ml plastic syringe fitted with 10-15cm of narrow gauge plastic tubing (2mm diameter). The syringe-tubing was inserted through the sample-port at the top of the column (see Figure 4.2.7.1.1) and lowered to within 1 cm of the gel surface. The sample was then gently lowered onto the gel surface and the syringe-tubing withdrawn. The sample-port was then closed, outlet valve opened, and the solvent flow resumed at 1ml minute^{-1}. The automatic fraction collector was calibrated to collect 10ml fractions of eluent from the column.

The colour due to organic matter in each fraction was determined by absorbance at 450nm. Distribution coefficients (Kd) for organic matter were determined according to Flodin (1962), where:

$$Kd = \frac{Ve - Vo}{Vt - Vo}$$

Ve = volume of eluent from the addition of a sample to a concentration maximum of eluted substance (i.e. abs.450nm).

Vo = void volume of column Vt = total volume of column

In view of the small amounts of P present in most of the individual eluent fractions, sets of 5 consecutive fractions were combined for P analysis (i.e. 50ml fractions). The respective amounts of inorganic, total and organic P in aliquots (1-10mls) of the combined column fractions were determined according to the methods outlined in section 3.2.3. The results obtained for P analysis of the column fractions were converted to μg P g^{-1} soil and expressed accordingly.

4.3 RESULTS AND DISCUSSION

4.3.1 Extraction and Concentration of Soil Organic Phosphorus

The sequential extraction scheme used in the present study (Figure 4.2.2.1) extracted 80-87% (mean 84%) of the total soil organic P as determined by the ignition method (Table 4.3.1.1). This proportion could even be greater than 84% due to errors which are known to be associated with the determination of total organic P by the ignition method (see section 2.1.2). Several workers have shown that the ignition value for total organic P in pasture soils may be an overestimate (Oniani *et al.*, 1973; Hawkes *et al.*, 1984). Results for the 376PA - 1983 soil show that the largest proportion (61%) of the organic P was extracted by the 0.1*M* NaOH step (Table 4.3.1.1).

Table 4.3.1.1 Amounts (μg P g^{-1}) of inorganic, organic and total P in original and concentrated extracts from control and annually fertilised (376PA) soils of the Winchmore long-term trial sampled in 1958, 1971 and 1983.

Treatment and Year of Sampling	Extract	Original soil extracts (300mls)					Concentrated soil extracts (10mls)				Total Soil Organic P[a] (μg P g^{-1})
		Inorganic P	Organic P		Total P		Inorganic P	Organic P	Total P		
		(μg P g^{-1})	μg P g^{-1}	% of total organic P[a]	μg P g^{-1}	μg P ml^{-1}	(μg P g^{-1})	(μg P g^{-1})	μg P g^{-1}	μg P ml^{-1}	
Control-1958	combined[b]	266	395	87	661	11	204 (77)[c]	373 (96)[c]	577	288	454
376PA-1958	combined[b]	272	403	88	675	11	220 (81)	384 (94)	604	302	458
Control-1971	combined[b]	204	393	83	597	10	165 (81)	377 (95)	542	271	473
376PA-1971	combined[b]	298	476	83	774	13	243 (82)	457 (96)	700	350	571
Control-1983	combined[b]	178	378	80	556	9	140 (79)	360 (96)	500	250	473
376PA-1983	combined[b]	361	454	86	815	14	270 (75)	440 (95)	710	355	530
376PA-1983	0.1M NaOH	173	285	-	458	23	140 (81)	270 (97)	410	820	-
376PA-1983	Acetylacetone	61	67	-	128	6	50 (82)	65 (95)	115	230	-
376PA-1983	0.5M NaOH	82	113	-	195	10	66 (80)	108 (97)	174	248	-

[a] = determined by ignition method

[b] = 0.1M NaOH + acetylacetone + 0.5M NaOH

[c] = values in parenthesis are amounts of P in concentrated extracts expressed as percentage of those in original extracts.

The ultrafiltration method used in this study provided adequate concentrations of total P (230-830 μg P ml^{-1} - Table 4.3.1.1) in the soil extracts for subsequent quantitative ^{31}P NMR and gel filtration analysis. In addition, the proportion of extracted organic P lost during ultrafiltration was very small (3-6%), although as expected a greater proportion (18-25%) of the extracted inorganic P was lost (Table 4.3.1.1).

The sequential extraction scheme used in this study (Figure 4.2.2.1) removed a greater proportion of the total organic P from the soil for subsequent ^{31}P NMR analysis than in previous other ^{31}P NMR studies in which a single alkali extraction was used (Newman and Tate, 1980; Tate and Newman, 1982; Hawke et al., 1984). Compared to the total amounts determined by ignition, Tate and Newman (1982) recovered an average 58% of the total organic P from 10 New Zealand grassland soils, while Hawkes et al. (1984) found that only 41% was extracted from 4 soils under permanent pasture in England. These were lower than the 84% of total soil organic P extracted in the present study (Table 4.3.1.1).

4.3.2 Nature and Distribution of Soil Phosphorus as Revealed by ^{31}P Nuclear Magnetic Resonance Analysis.

The ^{31}P NMR spectra for the various combined alkali soil extracts (i.e. control - 1958, 1971 and

1983) are shown in Figures 4.3.2.1 to 4.3.2.5, while those for the component alkali extracts (0.1M NaOH, acetylacetone, 0.5M NaOH) from the 376PA - 1983 soil are shown in Figures 4.3.2.6 to 4.3.2.8. In view of the similar amounts of organic P in the control-1958 and 376PA-1958 soil extracts (Table 4.3.1.1) and the limited time available on the NMR spectrometer, only the extract from the control-1958 soil was analysed.

The various soil extracts gave similar ^{31}P NMR spectra and showed 4 major forms of P, namely inorganic orthophosphate, orthophosphate monoesters, orthophosphate diesters and pyrophosphate (Figures 4.3.2.1 to 4.3.2.5). Choline phosphate (an orthophosphate monoester) was resolved in the acetylacetone and 0.5M NaOH extracts from the 376PA-1983 soil (Figures 4.3.2.7 and 4.3.2.8). The ^{31}P NMR signal assignments used were similar to those of Newman and Tate (1980) as summarised in Table 4.3.2.1.

The chemical analysis and ^{31}P NMR analysis both gave similar relative proportions of inorganic and organic P, although values for inorganic P were higher by ^{31}P NMR analysis than by chemical analysis (Table 4.3.2.2). This suggests that most of the P present in the soil extracts was subjected to ^{31}P NMR analysis. A similar finding was reported by Tate and Newman (1982). The difficulty of accurately distinguishing between the adjacent

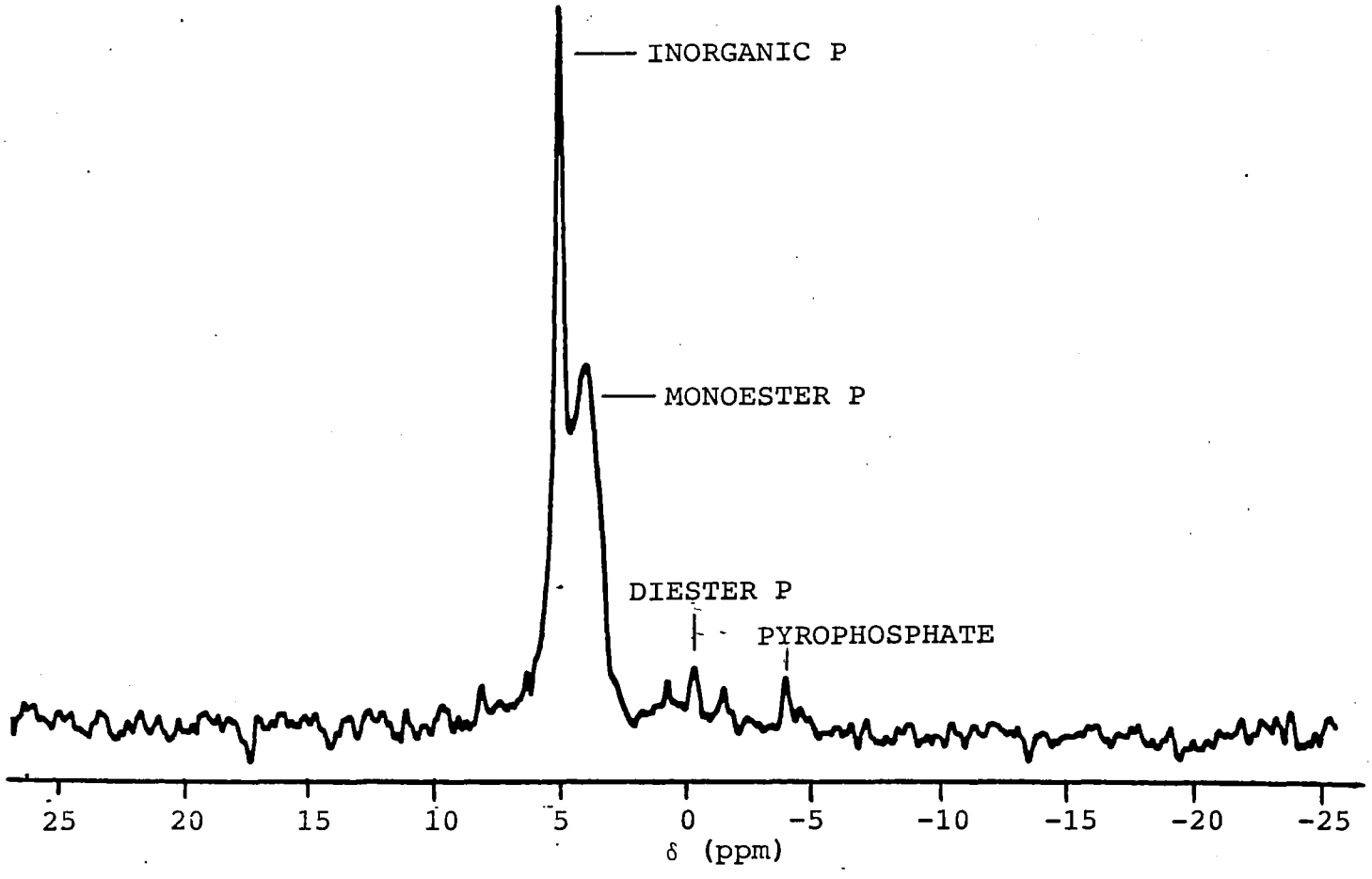

Figure 4.3.2.1. ^{31}P NMR spectrum of the combined extract from the control-1958 soil.

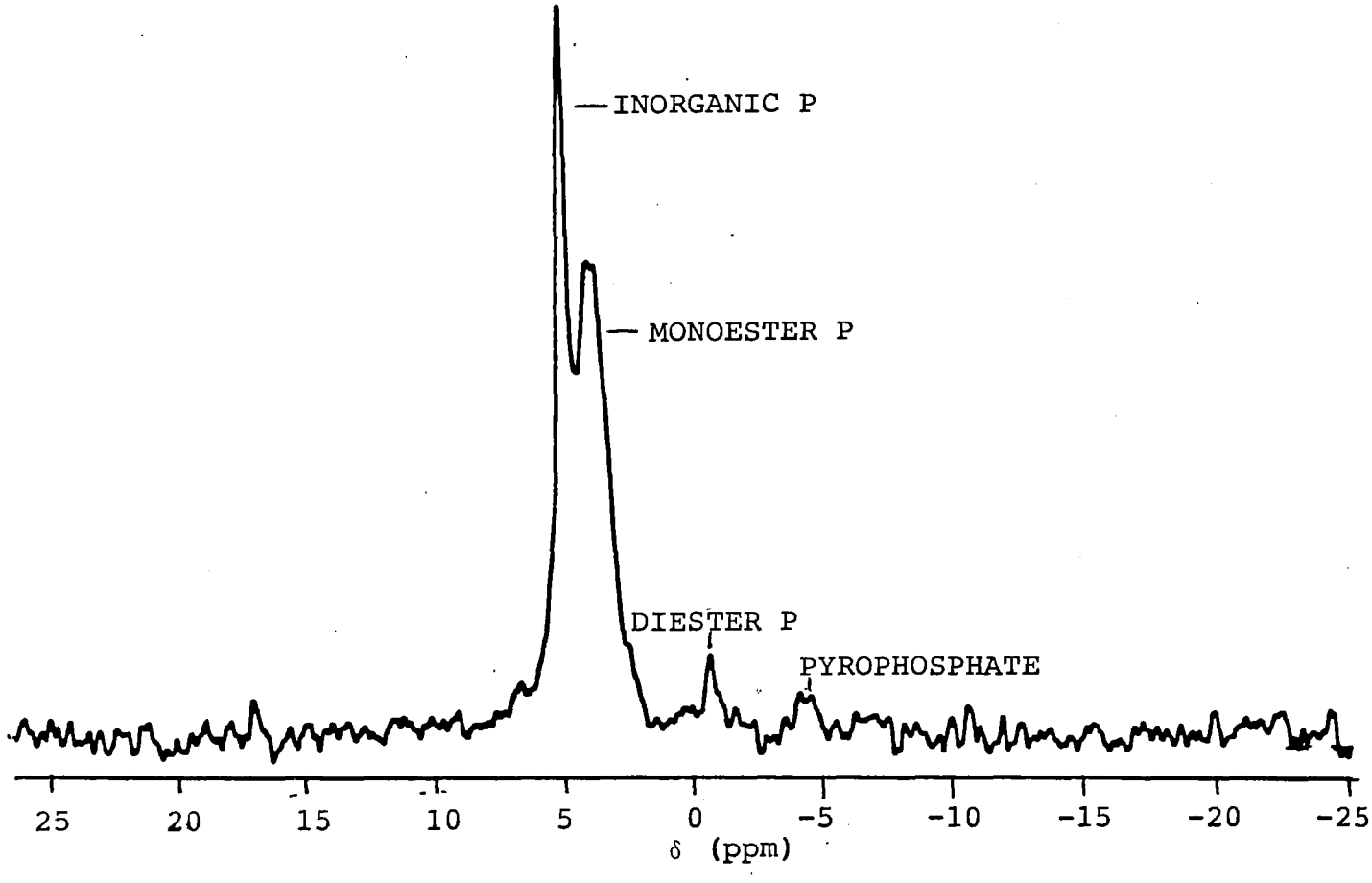

Figure 4.3.2.2. ^{31}P NMR spectrum of the combined extract from the control-1971 soil.

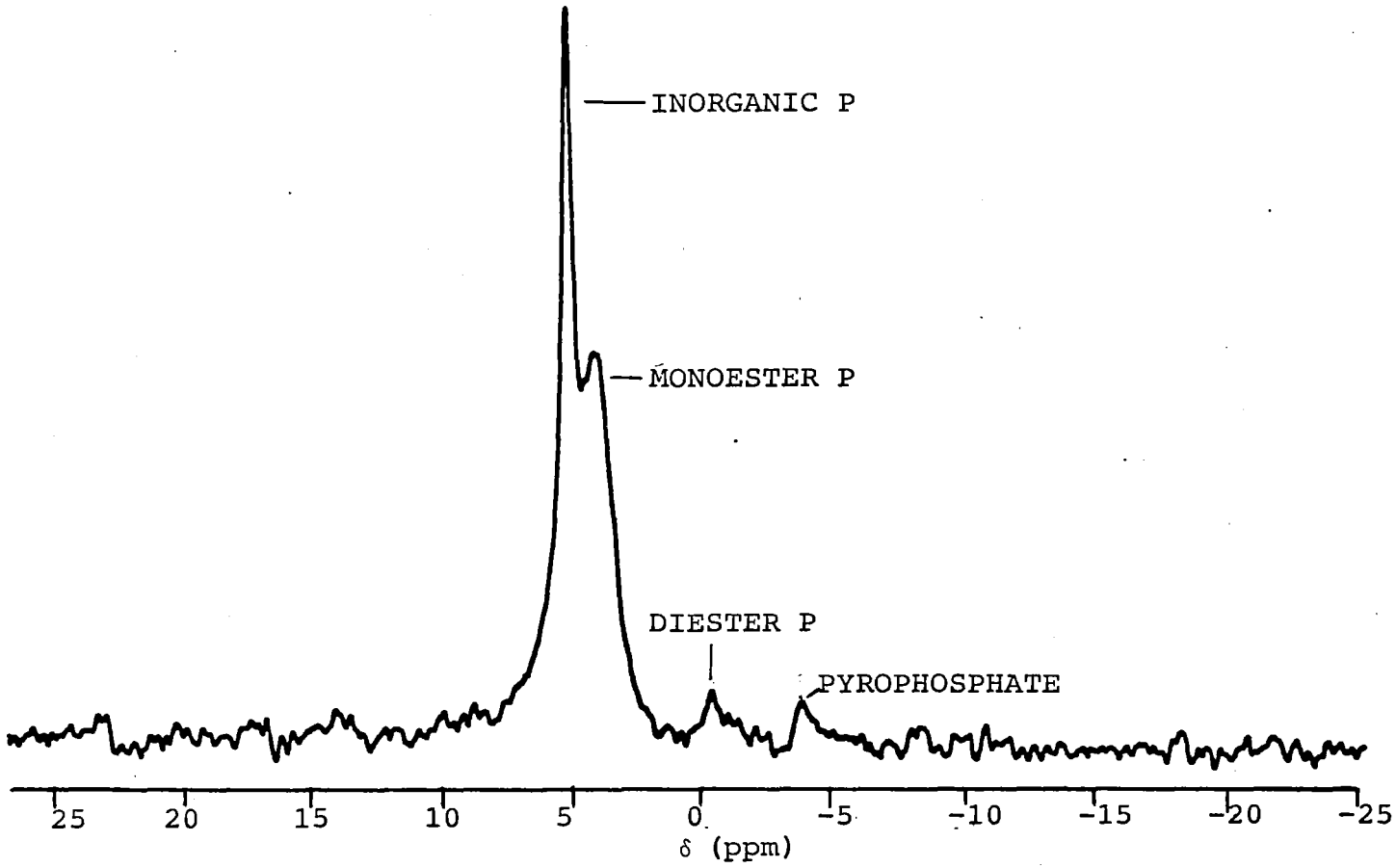

Figure 4.3.2.3. ^{31}P NMR spectrum of the combined extract from the 376PA-1971 soil.

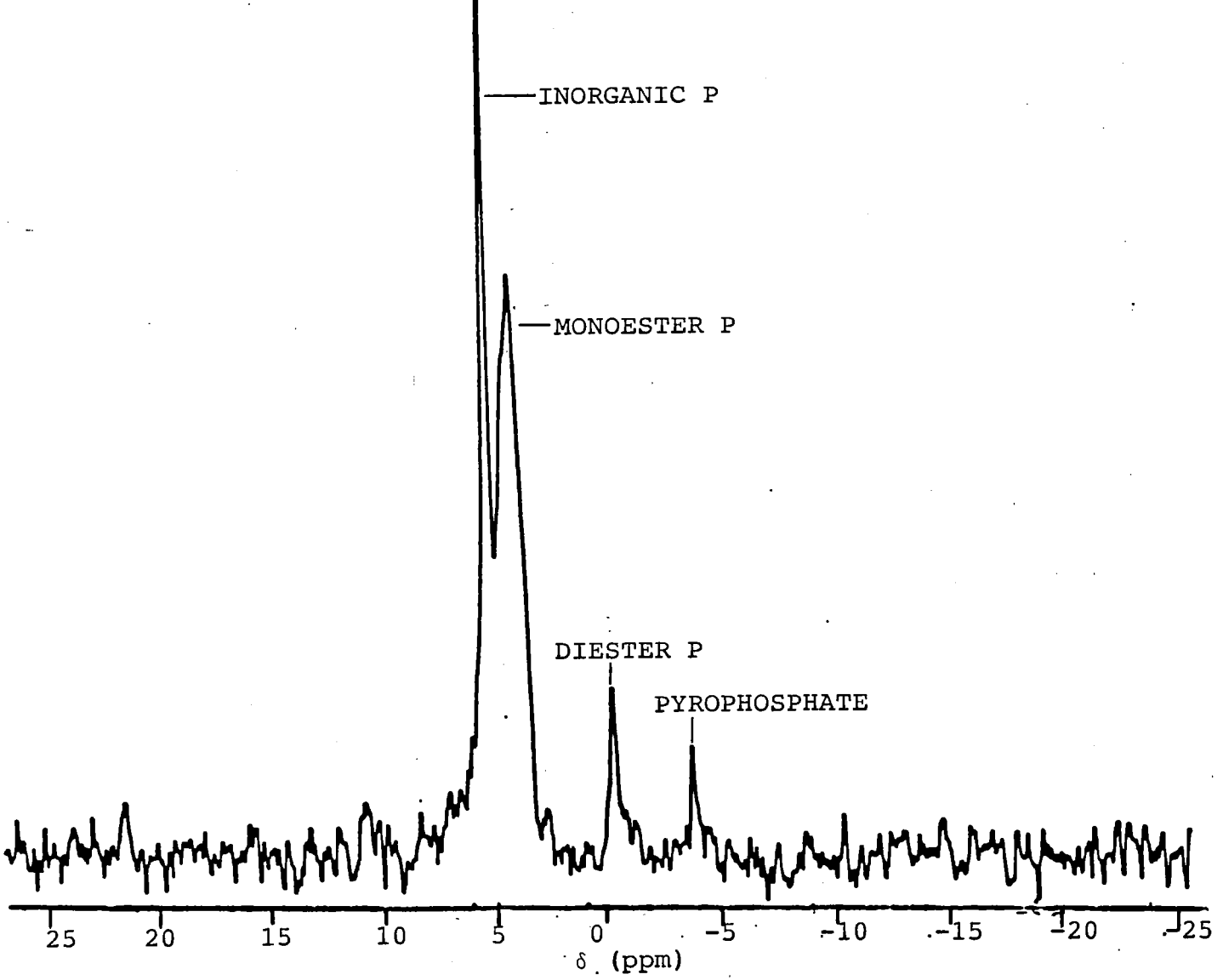

Figure 4.3.2.4. ^{31}P NMR spectrum of the combined extract from the control-1983 soil.

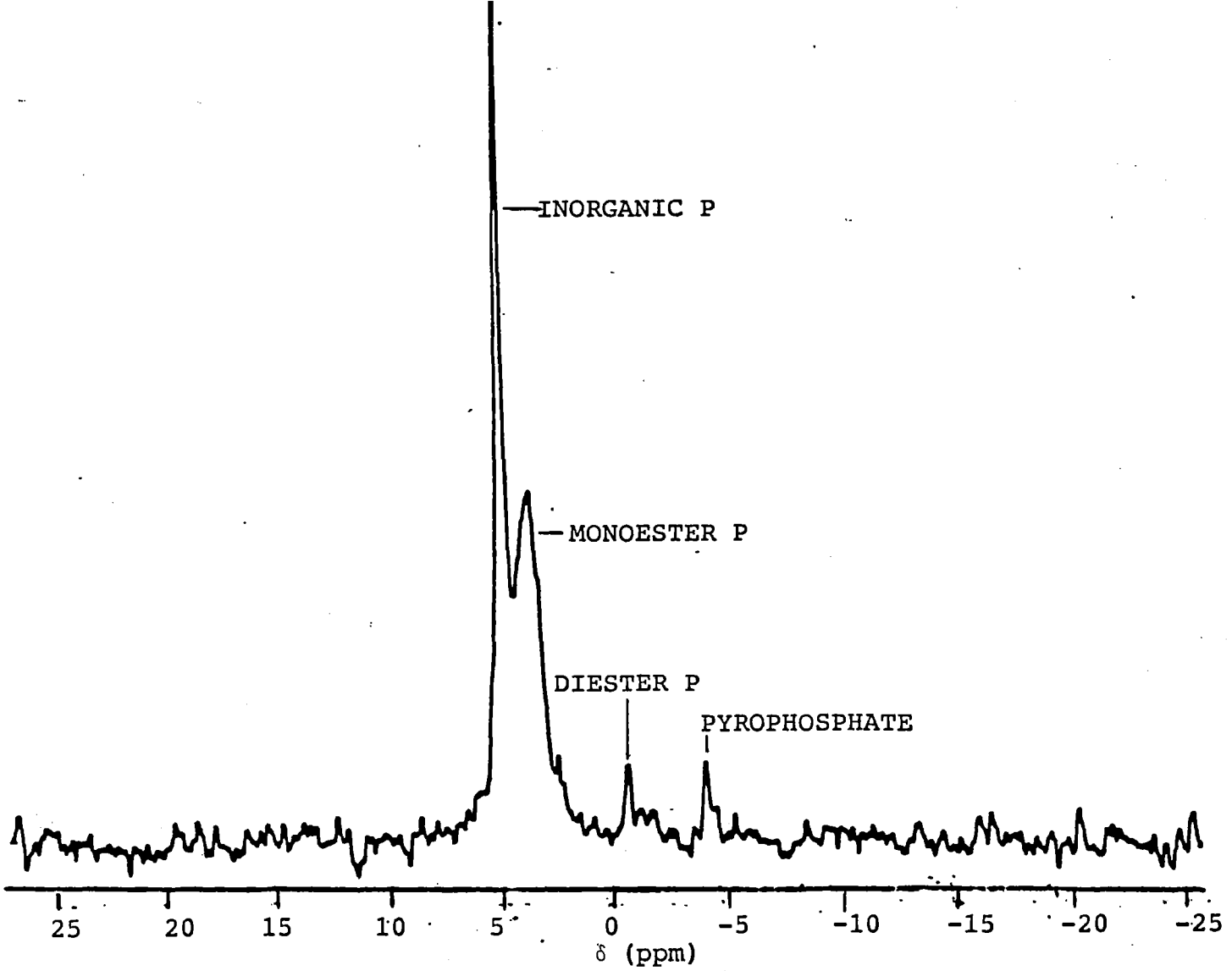

Figure 4.3.2.5. ^{31}P NMR spectrum of the combined extract from the 376PA-1983 soil.

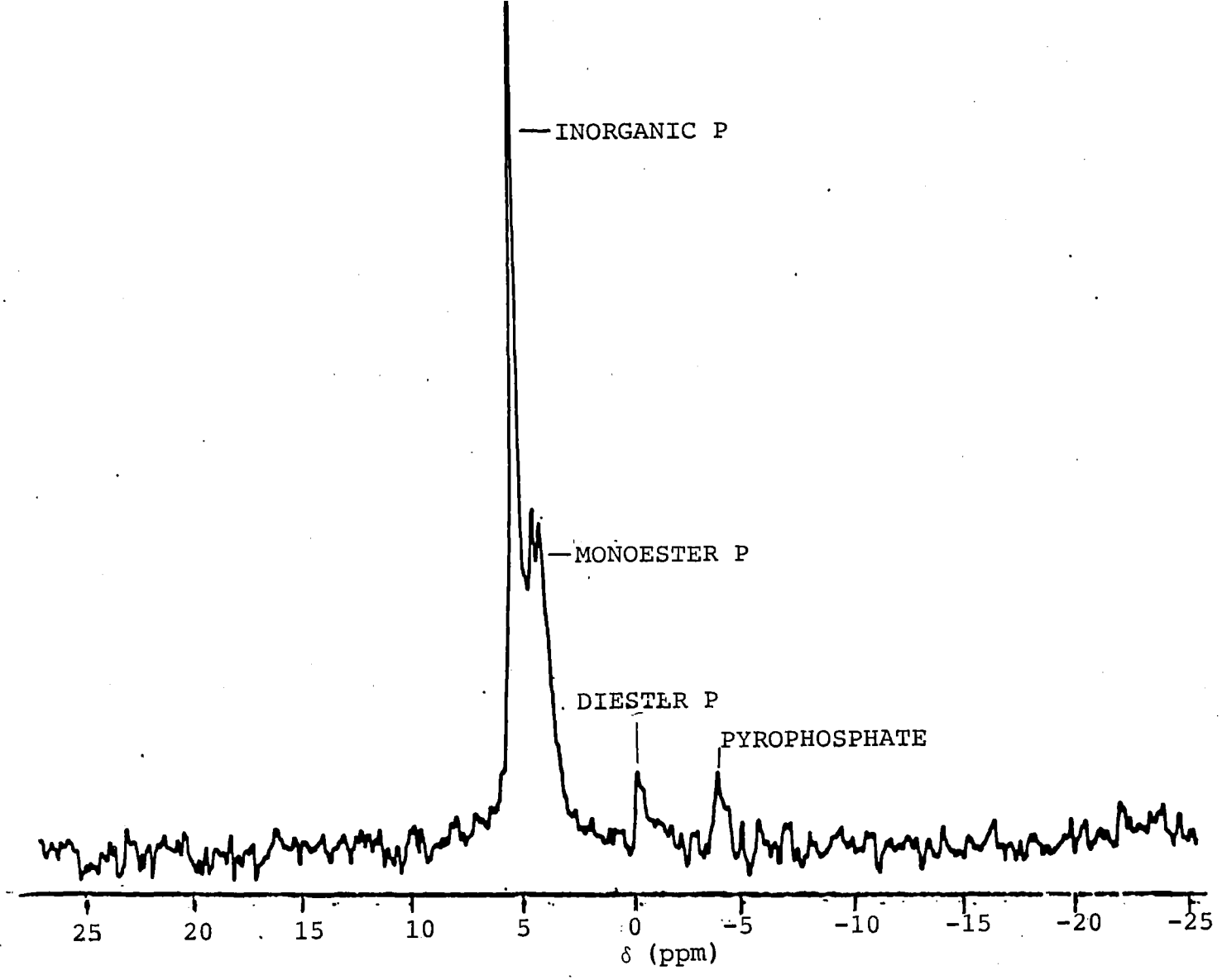

Figure 4.3.2.6. ^{31}P NMR spectrum of the 0.1M NaOH extract from the 376PA-1983 soil.

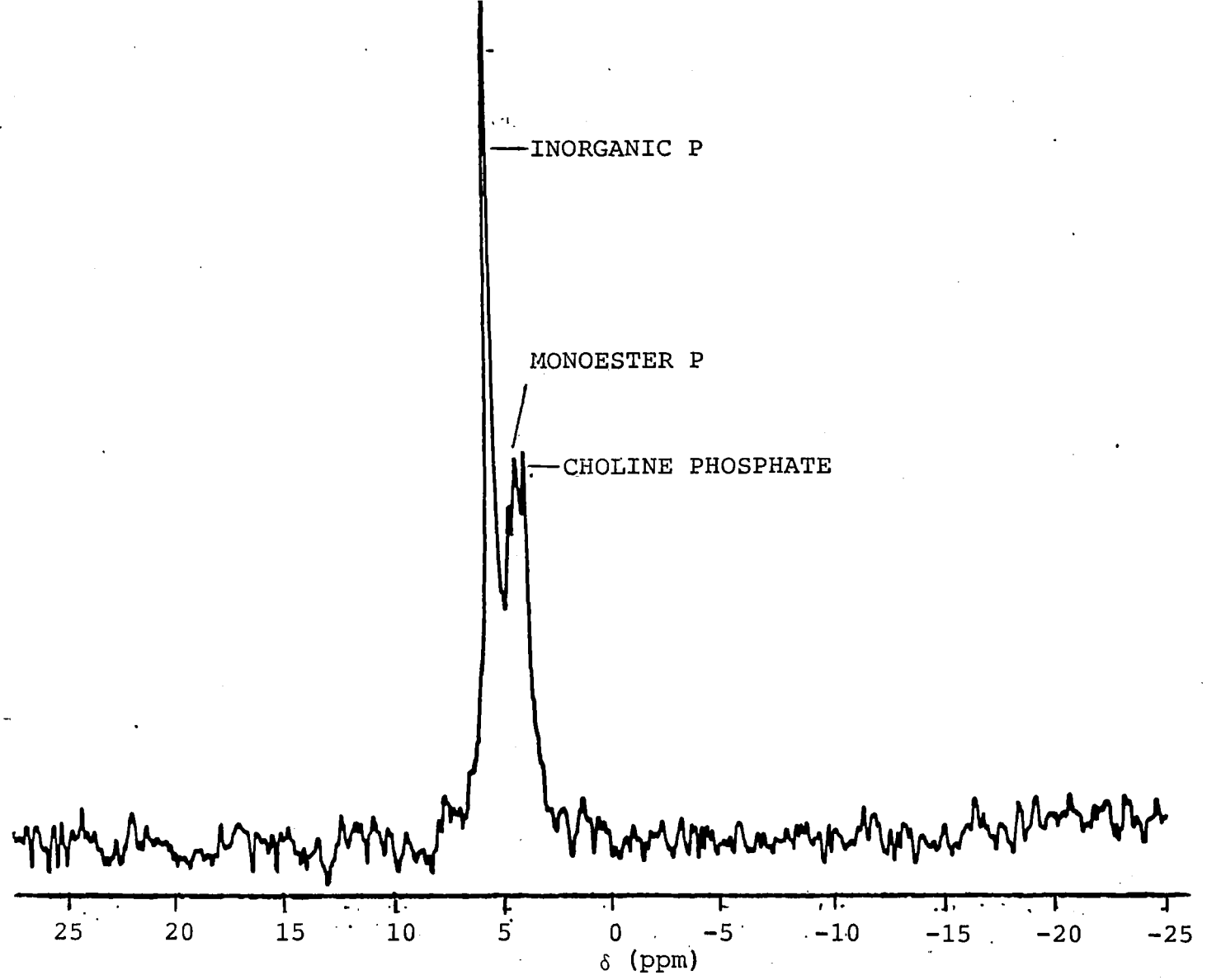

Figure 4.3.2.7. ^{31}P NMR spectrum of the acetylacetone extract from the 376PA-1983 soil.

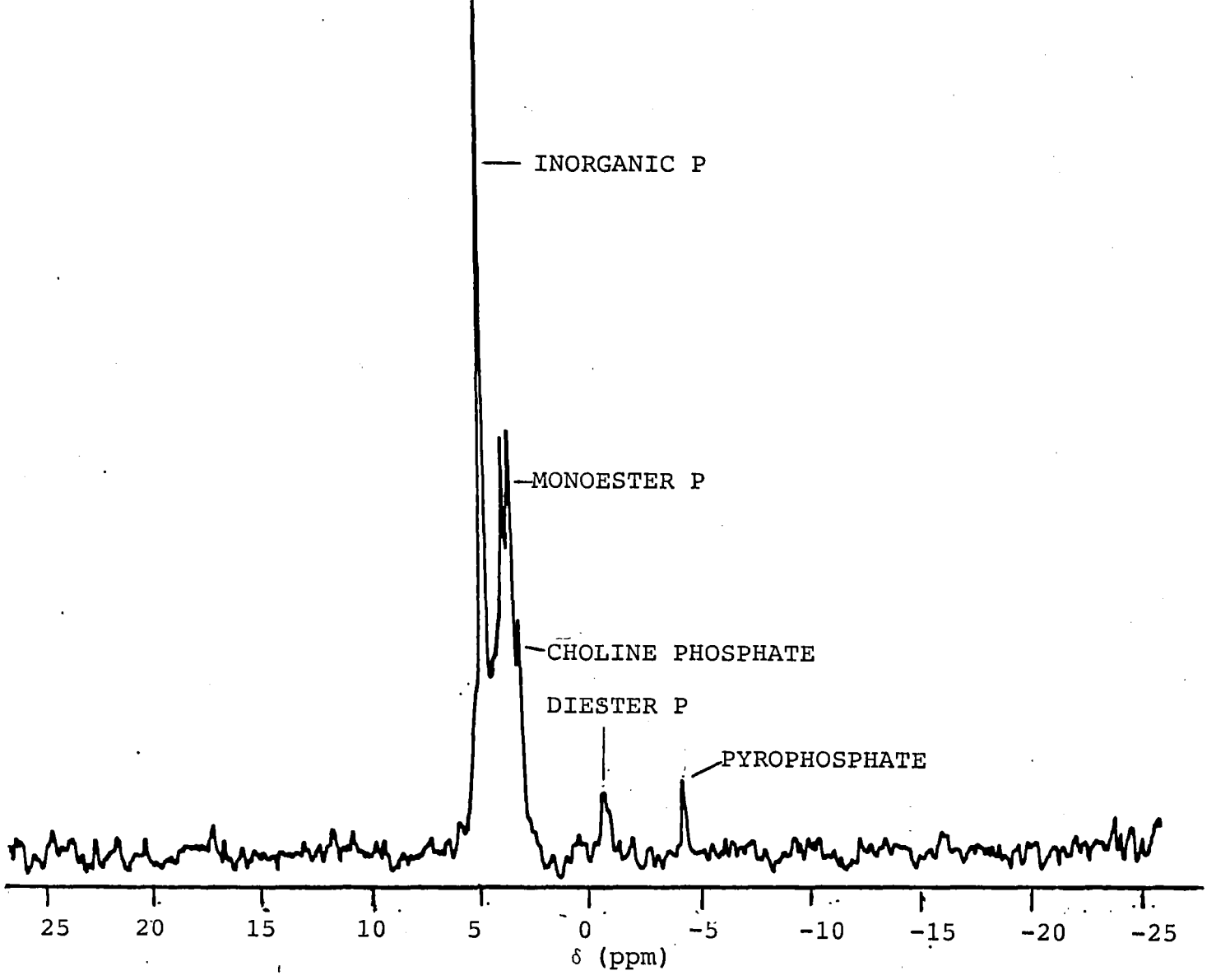

Figure 4.3.2.8. ^{31}P NMR spectrum of the 0.5M NaOH extract from the 376PA-1983 soil.

Table 4.3.2.1 Assignments of ^{31}P NMR chemical shifts for phosphorus compounds in alkaline soil extracts.

Phosphorus Compounds	Structure	Chemical Shift (δ ppm)
Inorganic orthophosphate	PO_4^{3-}	5.3
Orthophosphate monoesters	$ROPO_3^{2-}$	3.5-5.3[a]
Choline phosphate[b]	$(CH_3)_3N^+CH_2CH_2OPO_3^{2-}$	3.6
Orthophosphate diesters	$(RO)(R'O)PO_2^-$	c.-0.8[a]
Pyrophosphate	$P_2O_7^{4-}$	-5.5

[a] = exact values of δ depend on specific structures of the organic substituents R and R'.

[b] = orthophosphate monoester.

Table 4.3.2.2 Relative proportions (%) of inorganic and organic P in combined and component extracts determined by chemical analysis and ^{31}P NMR spectroscopy.

Treatment and Year of Sampling	Extract	Inorganic P		Organic P	
		Chem.	NMR	Chem.	NMR[b]
Control - 1958	Combined[a]	35	40	65	60
Control - 1971	Combined[a]	30	36	70	64
376PA - 1971	Combined[a]	35	30	65	61
Control - 1983	Combined[a]	28	30	72	70
376PA - 1983	Combined[a]	38	41	62	59
376PA - 1983	0.1M NaOH	34	39	66	61
376PA - 1983	Acetylacetone	43	42	57	58
376PA - 1983	0.5M NaOH	38	35	62	65
Mean		35	38	65	62

[a] = 0.1M NaOH, acetylacetone and 0.5M NaOH extracts combined.

[b] = Includes pyrophosphate.

inorganic orthophosphate and orthophosphate monoester P peaks might have accounted for the higher values of inorganic P determined by ^{31}P NMR analysis.

Orthophosphate monoesters constituted the largest proportion of the organic P detected by NMR in each of the soil extracts (Table 4.3.2.3). Orthophosphate diesters and pyrophosphate were present in about the same proportions (Table 4.3.2.3). However, in the various component extracts of the 376PA-1983 soil the forms and relative amounts of P differed. Almost all of the pyrophosphate P was found in the 0.1M NaOH extract, while orthophosphate diester P and pyrophosphate P were not detected in the acetylacetone extract (Table 4.3.2.3).

The predominance of orthophosphate monoesters found in the present study (Table 4.3.2.3) is similar to those reported previously in New Zealand soils (Newman and Tate, 1980; Tate and Newman, 1982). Orthophosphate monoesters are known to include inositol phosphates (Newman and Tate, 1980), and the predominance of orthophosphate monoesters in the Winchmore soils appears to confirm the findings of numerous other studies which have shown that inositol phosphate is the major form of organic P found in most soils (Anderson, 1980).

Orthophosphate diester P, which includes nucleic acid and phospholipid P (Newman and Tate, 1980), are known to be readily mineralised in the soil (Dalal, 1977), which could account for the

Table 4.3.2.3 Amounts (μg g^{-1} soil) of different forms of P determined in the soil extracts by ^{31}P NMR analysis.

Treatment and year of sampling	Extract	IP	MP	CP	DP	PP
Control - 1958	Combined[a]	213	312(90)[c]	ND[b]	17(5)	17(5)
Control - 1971	Combined[a]	195	320(90)	ND	16(5)	16(5)
376PA - 1971	Combined[a]	273	399(94)	ND	14(3)	14(3)
Control - 1983	Combined[a]	150	320(91)	ND	20(6)	10(3)
376PA - 1983	Combined[a]	291	369(88)	ND	28(7)	21(5)
376PA - 1983	0.1M NaOH	160	205(82)	ND	16(6)	29(12)
376PA - 1983	Acetylacetone	48	49(74)	17(26)	ND	ND
376PA - 1983	0.5M NaOH	61	85(75)	19(17)	7(6)	2(2)

IP = inorganic orthophosphate, MP = orthophosphate monoesters, CP = choline phosphate, DP = orthophosphate diesters, PP = pyrophosphate.

[a] = 0.1M NaOH, acetylacetone, 0.5M NaOH extracts combined.

[b] = not detected.

[c] = values in parentheses are amounts expressed as percentage of total organic P (i.e. MP + CP + DP + PP).

small quantities of those P forms found in the soil extracts (Table 4.3.2.3). Greater quantities of pyrophosphate P were detected in the present study (up to 7% total P; Table 4.3.2.3) than that found (<1%) in the soils examined by Tate and Newman (1982). Pyrophosphate is believed to be involved in biological P cycling in the soil, and some may be present as organic esters which are hydrolysed during alkali extraction (Anderson and Russell, 1969). Its presence in the Winchmore soils possibly reflects a higher level of biological activity than that in the acid tussock grassland soils studied by Newman and Tate (1980) and Tate and Newman (1982). Small amounts of pyrophosphate were recently reported in a neutral pasture soil in England by Hawkes et al. (1984).

Newman and Tate (1980) and Tate and Newman (1982) detected choline phosphate in soils under tussock grassland, while none was found in the permanent pasture soils examined by Hawkes et al. (1984). In the present study, choline phosphate constituted a significant proportion (17-26%) of the total organic P in the acetylacetone and 0.5M NaOH extracts of the 376PA-1983 soil (Table 4.3.2.3). This may have been due to (i) the lower amounts of orthophosphate monoester P present in these extracts compared with the 0.1M NaOH and combined extracts (Table 4.3.2.3), and (ii) the effects of acid pretreatment (Figure 4.2.2.1).

The acid pretreatment could have facilitated the desorption of phospholipids held on clay surfaces (Hance and Anderson, 1963), and/or the hydrolysis of phospholipid material (e.g. phosphatidyl choline) producing choline phosphate, which was subsequently extracted by alkali. Apart from choline phosphate, peaks of fine structure in the orthophosphate monoester region were not resolved sufficiently well for identification of individual P species. However, it should be noted that the peak maxima for the 0.1M NaOH (4.08, 4.50 ppm) and 0.5M NaOH (4.00, 4.36 ppm) extracts of the 376PA-1983 soil do not overlap (Figure 4.3.2.9). This suggests that there are differences in the predominant forms of orthophosphate monoesters in these two extracts. The spectra of the combined extracts show a smooth envelope of peaks in the orthophosphate monoester region, resulting from the diversity of monoester P forms present (Figure 4.3.2.9). As discussed earlier (section 3.3.2.2), in the Winchmore long-term trial soil organic P increased as a result of continued annual applications of P fertiliser only in the period before 1971. In 1971 the total increase in soil organic P from P fertiliser application was 80 µg P g^{-1} (Table 4.3.1.1) of which 79 µg P g^{-1} (99%) was detected as orthophosphate monoester P (Table 4.3.2.3). This confirms that orthophosphate monoester P was the

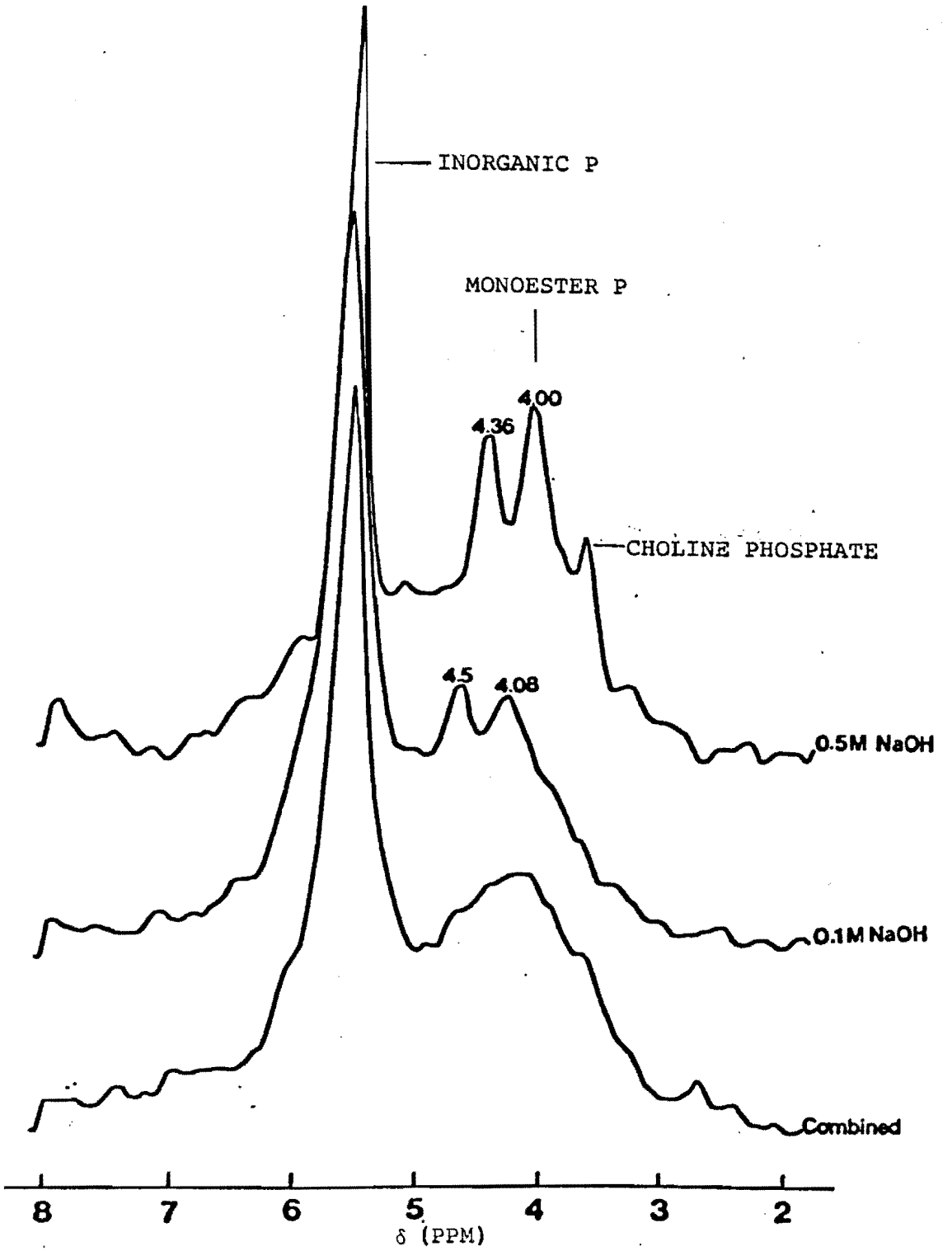

Figure 4.3.2.9. Plot expansions of the inorganic orthophosphate and orthophosphate monoester region from the ^{31}P NMR spectra of combined and component alkali extractions from the 376PA-1983 soil.

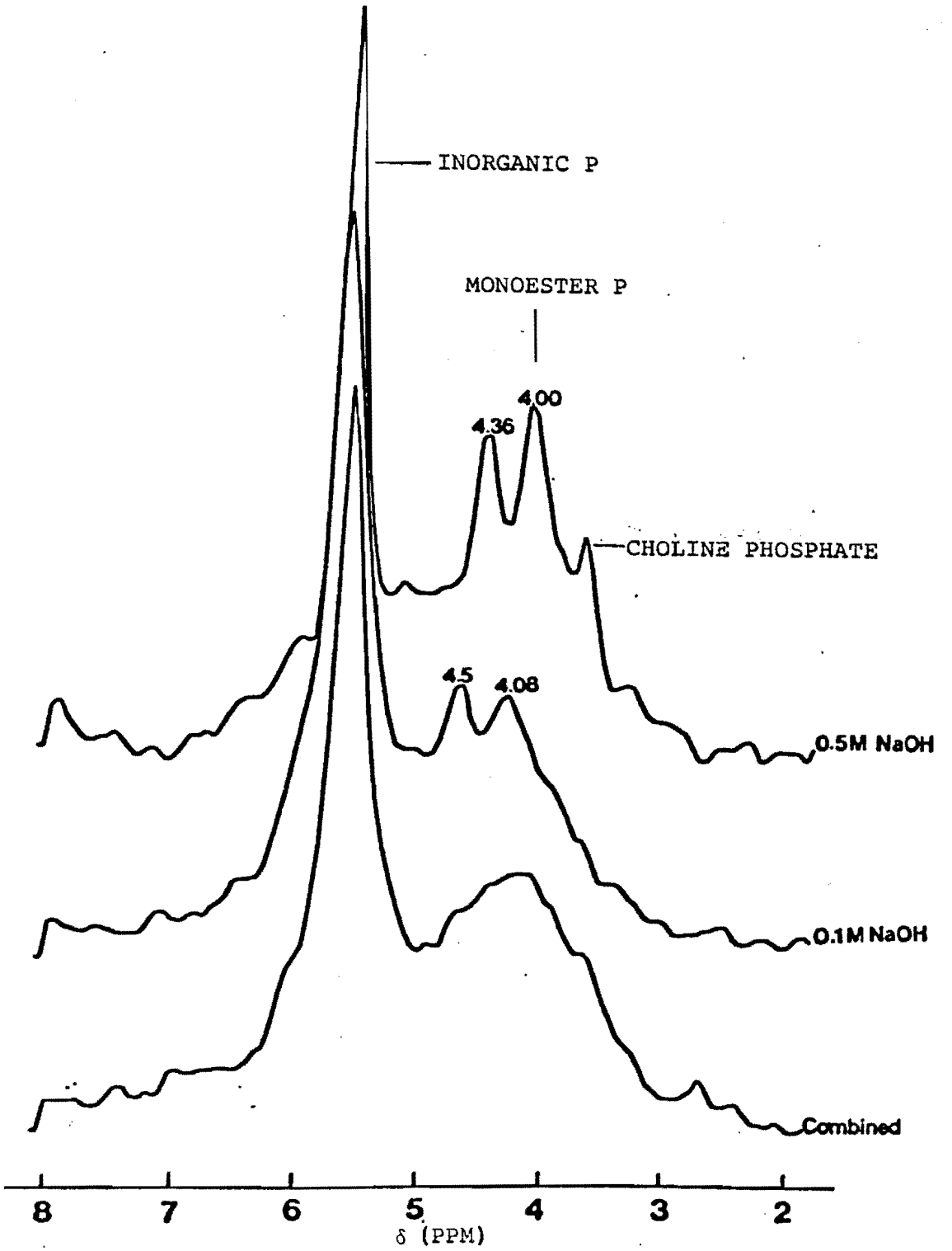

Figure 4.3.2.9. Plot expansions of the inorganic orthophosphate and orthophosphate monoester region from the ^{31}P NMR spectra of combined and component alkali extractions from the 376PA-1983 soil.

predominant form of organic P accumulated in the soils examined in the present study (Table 4.3.2.3). This finding is also consistent with that of Hawkes et al. (1984) who showed that orthophosphate monoester P was the major form of organic P which accumulated in a pasture soil which received P fertiliser annually for 100 years. However, Hawkes et al. (1984) extracted only 55% of the total extractable organic P for ^{31}P NMR analysis, compared to the present study in which almost all (94-97%) of the total extractable organic P was analysed (Table 4.3.1.1).

Results for the 376PA soils indicated that changes in the chemical nature of organic P occurred between 1971 and 1983. This is shown by the decrease in orthophosphate monoester P from 399 μg P g^{-1} in 1971 to 369 μg P g^{-1} in 1983, and the increase in orthophosphate diester P from 14 to 28 μg P g^{-1} over the same period (Table 4.3.2.3). These changes may have been caused by increased microbial activity in the soil as a result of lime addition in 1972 which may have resulted in some net mineralisation of monoester P (e.g. inositol phosphates) and increases in diester P (e.g. nucleic acids, phospholipids). It has been shown that liming increases the mineralisation of inositol phosphate in soil (Pearson et al., 1941), while several studies have indicated that nucleic acid and phospholipid components in soil are mainly of microbial origin (Dalal, 1977; Anderson, 1980).

4.3.3 Molecular Weight Distribution of Soil Organic Phosphorus

4.3.3.1 Distribution of Organic Phosphorus in Combined Extracts from Control and Fertilised (376PA) Soils. Results of the molecular weight fractionation of organic matter and organic P in combined extracts from control and 376PA soils sampled in 1958, 1971 and 1983 using Sephadex G-100 are shown in Figures 4.3.3.1.1 to 4.3.3.1.6.

Most of the added organic matter and organic P was eluted from the gel within the total column volume (Vt) (Figures 4.3.3.1.1 to 4.3.3.1.6). The estimated kd values for the organic matter peaks were 0 and 0.81-0.92 (Figures 4.3.3.1.1 to 4.3.3.1.6) which suggest that minimal chemical interaction occurred between the organic macromolecules and the gel to distort fractionation on a molecular size basis (Flodin, 1962). Furthermore, the recovery of organic P present in various extracts added to the Sephadex G-100 column was between 96% and 104% (Table 4.3.3.1.1), which shows that the irreversible adsorption of soil organic P onto Sephadex gel observed by other workers (Moyer and Thomas, 1970; Steward and Tate, 1972) did not occur in the present study.

In all 6 organic matter extracts 2 adsorption peaks were resolved viz. (i) a peak eluted from the column in the void volume (kd = 0) which

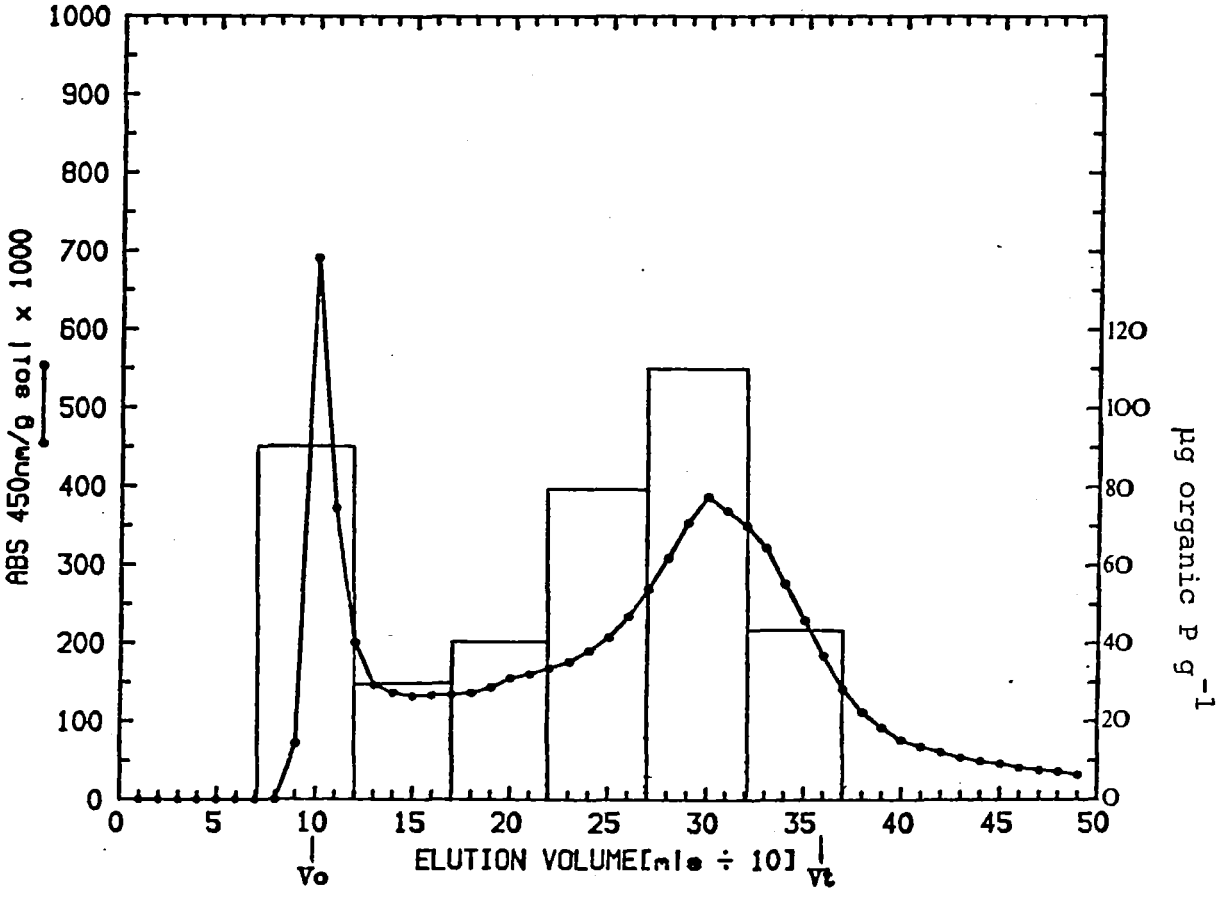

Figure 4.3.3.1.1. Fractionation of organic matter and organic P in the combined extract from the control-1958 soil using Sephadex G-100.

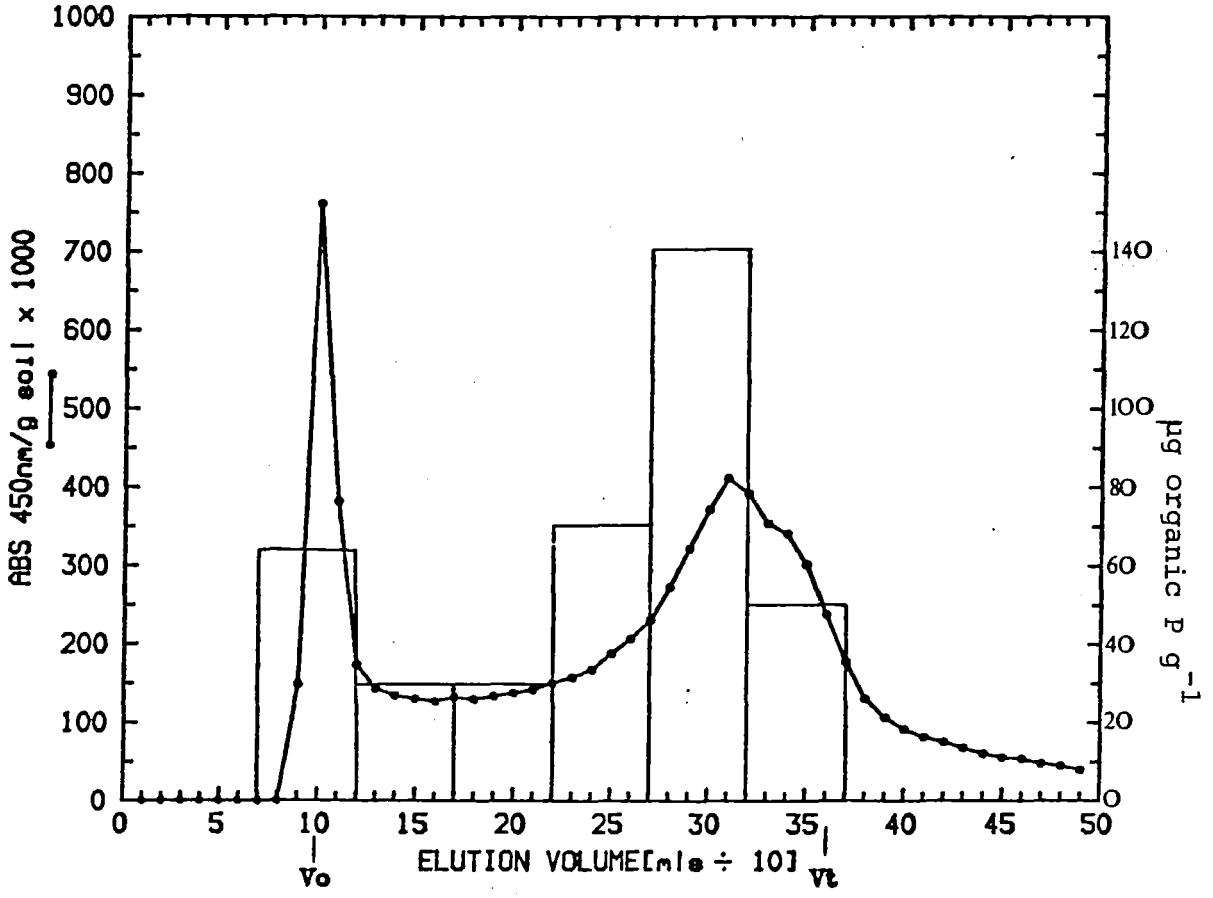

Figure 4.3.3.1.2. Fractionation of organic matter and organic P in the combined extract from the 376PA-1958 soil using Sephadex G-100.

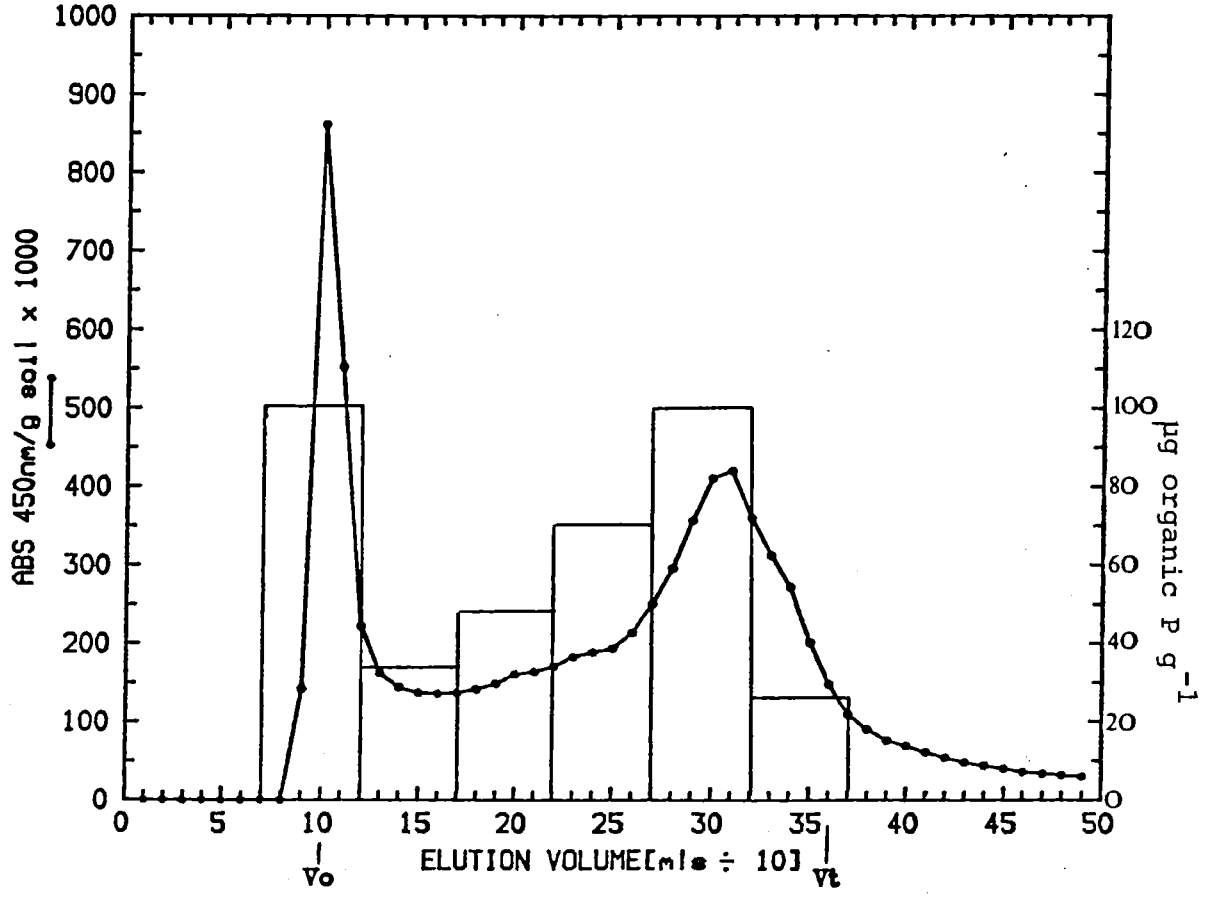

Figure 4.3.3.1.3. Fractionation of organic matter and organic P in the combined extract from the control-1971 soil using Sephadex G-100.

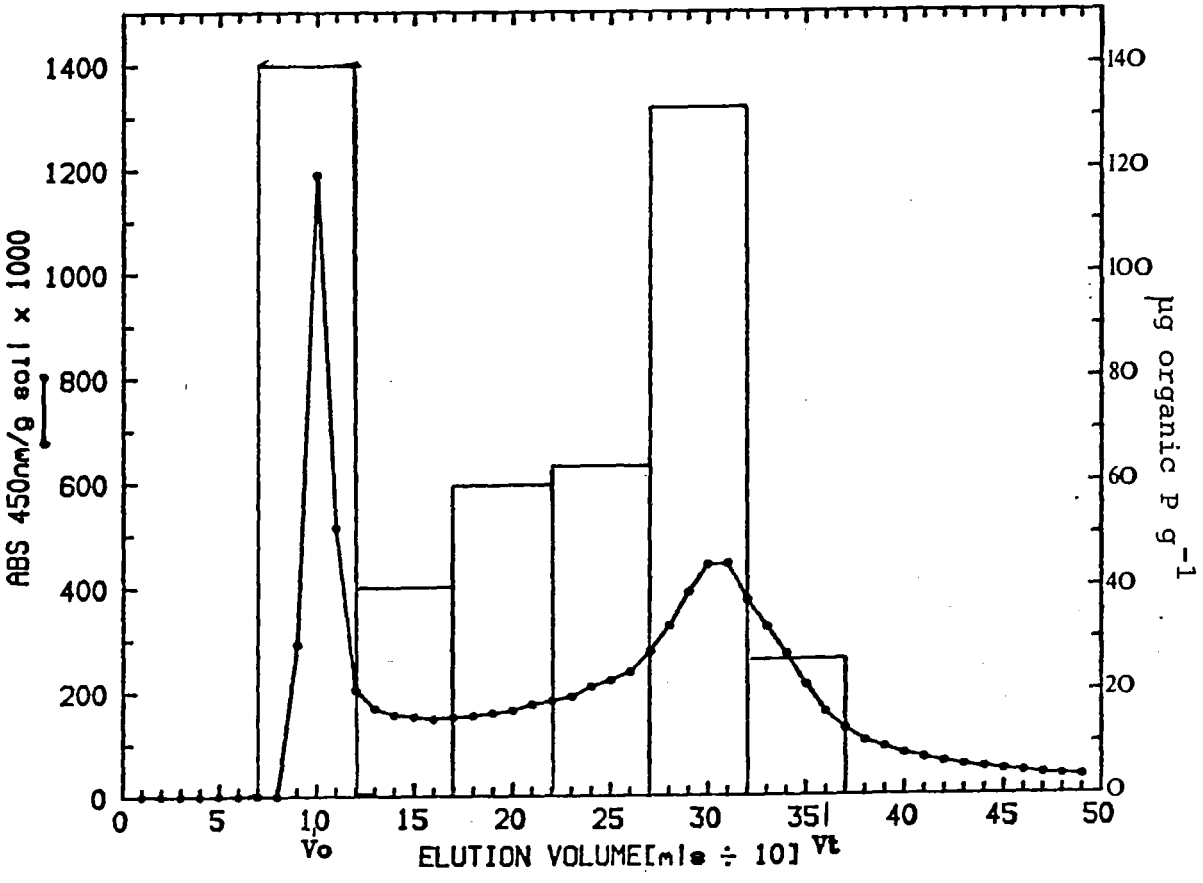

Figure 4.3.3.1.4. Fractionation of organic matter and organic P in the combined extract from the 376PA-1971 soil using Sephadex G-100.

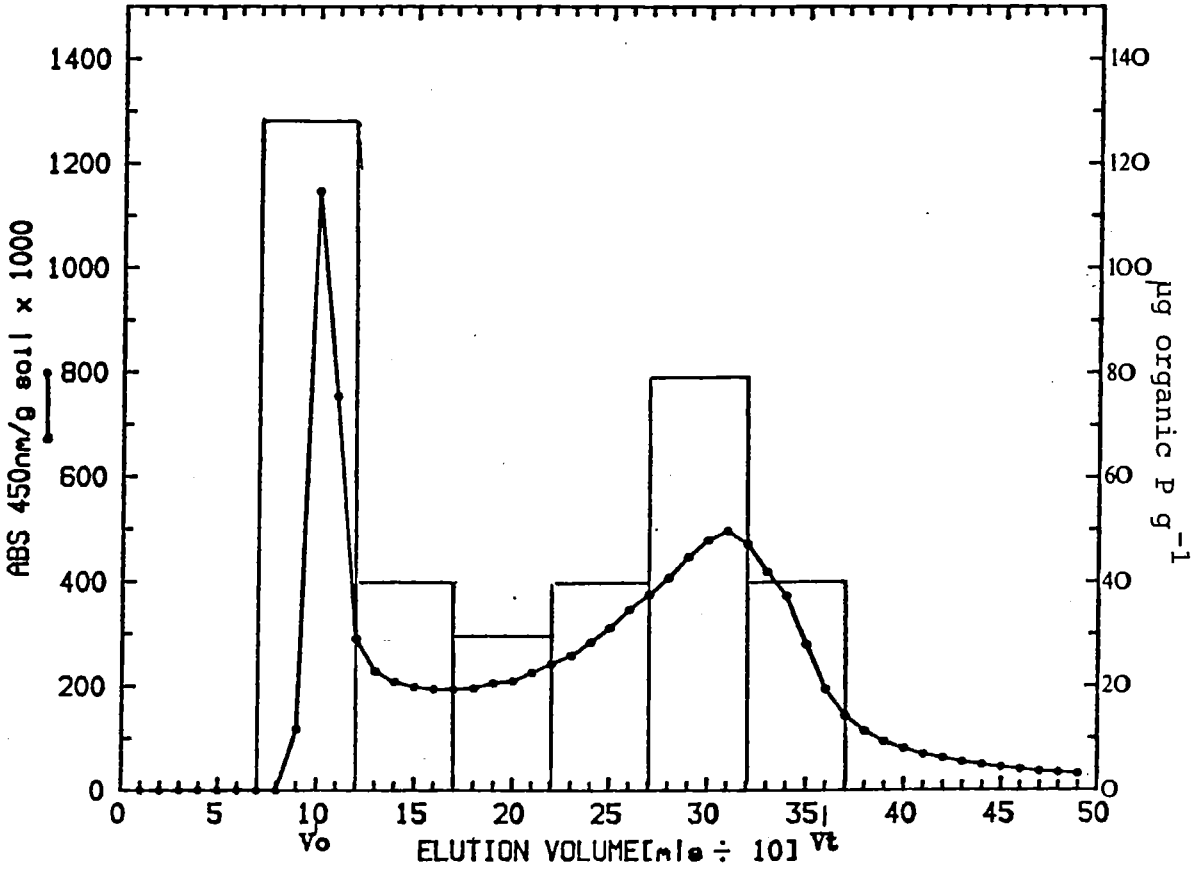

Figure 4.3.3.1.5. Fractionation of organic matter and organic P in the combined extract from the control-1983 soil using Sephadex G-100.

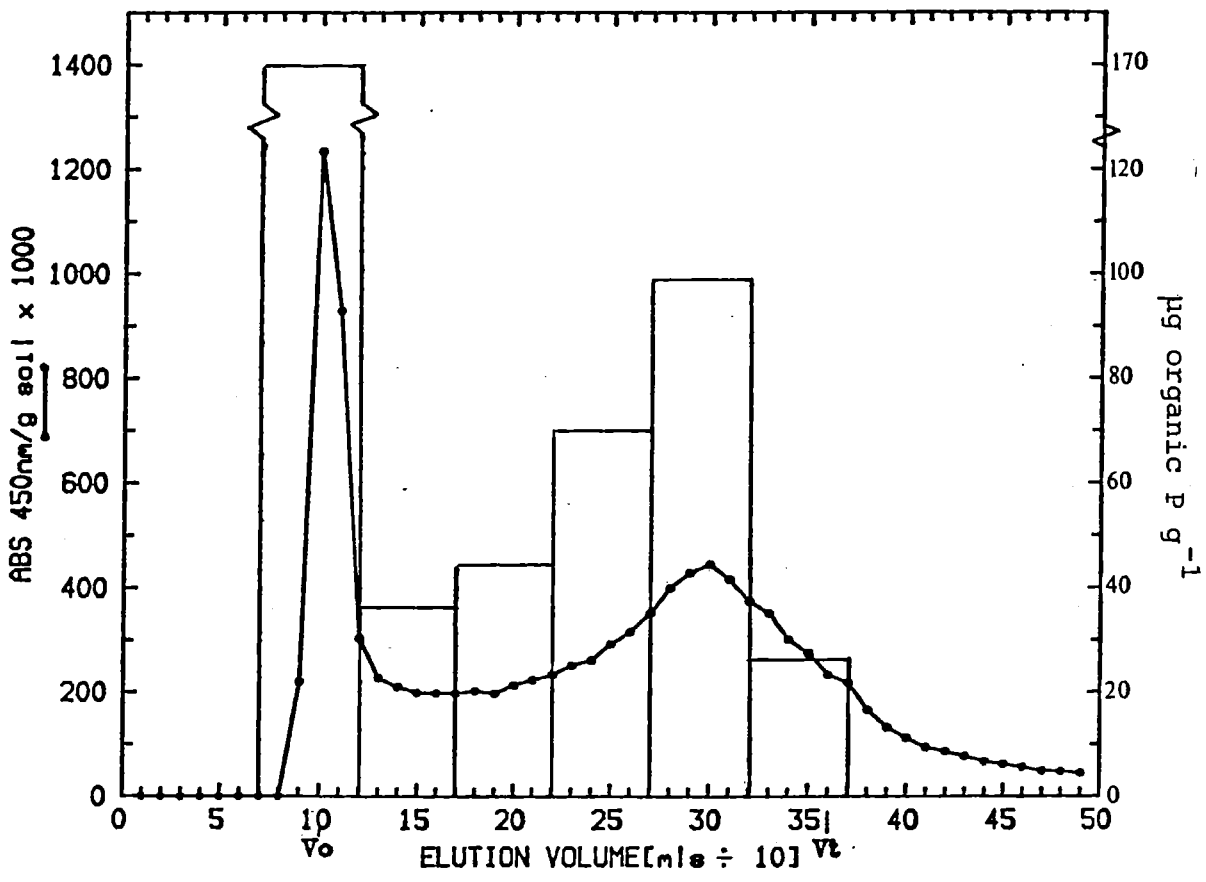

Figure 4.3.3.1.6. Fractionation of organic matter and organic P in the combined extract from the 376PA-1983 soil using Sephadex G-100.

Table 4.3.3.1.1 Recovery of organic P in combined extracts added to the Sephadex G-100 column.

Treatment and year of sampling	µg P		
	Organic P content of added extract (2mls)	Organic P content of eluent (370mls)	Recovery (%)
Control - 1958	369	369	100
376PA - 1958	387	372	96
Control - 1971	379	375	99
376PA - 1971	455	464	102
Control - 1983	360	374	104
376PA - 1983	440	431	98

represented high molecular weight material (>100,000MW) excluded from the gel particles, and (ii) a peak eluted between the void and total column volumes (kd = 0.81-0.92) representing lower molecular weight material (<100,000MW) which entered the gel matrix (Figures 4.3.3.1.1 to 4.3.3.1.6). The organic P elution pattern also showed 2 peaks similar to those of organic matter.

Amounts of organic P in the high and low molecular weight fractions for the various combined extracts are shown in Table 4.3.3.1.2. For the purpose of the present discussion the organic P eluted in the void volume (i.e. <120mls) is designated the high molecular weight fraction (>100,000MW) and that eluted between the void and total column volumes (i.e. 120-370mls) the low molecular weight fraction (<100,000MW). Most (61-83%) of the organic P in the Winchmore soils was present in the low molecular weight fraction while the high molecular weight fraction accounted for only 17-39% of the total organic P (Table 4.3.3.1.2). In both the control and 376PA soils, the proportion of high molecular weight organic P increased with time. For example, between 1958 and 1983 the proportions of organic P in the high molecular weight fraction increased from 17-19% to 38-39% (Table 4.3.3.1.2). These results suggest that organic P was transformed to high molecular weight forms with time. This

Table 4.3.3.1.2 Amounts (μg P g^{-1}) of organic P in the high and low molecular weight fractions of combined extracts from control and 376PA soils sampled in 1958, 1971 and 1983.

Treatment and year of sampling	Molecular Weight Fraction	
	High (>100,000MW)	Low (<100,000MW)
Control - 1958	70(19)[a]	299(81)
376PA - 1958	63(17)	309(83)
Control - 1971	101(27)	274(73)
376PA - 1971	139(30)	325(70)
Control - 1983	142(38)	232(62)
376PA - 1983	168(39)	263(61)

[a] = values in parentheses are amounts expressed as percentage of total organic P (i.e. organic P in >100,000MW + <100,000MW).

transformation may have been due to the actions of soil micro-organisms vis-a-vis the processes of organic matter humification. It is possible that the high molecular weight species represent more stable forms of organic P than low molecular weight organic P (Swift and Posner, 1972). The trend towards the formation of high molecular weight organic P in the soil with time was similar to that observed by Baker (1974) and Goh and Williams (1982), although the time periods involved were much longer (10-20,000 years) than in the present study (<30 years).

Comparisons of the amounts of organic P between the control and those of the 376PA soils in 1971 and 1983 show that the organic P accumulated from annual additions of P fertiliser appeared to be evenly distributed between the high and low molecular weight fractions. For example, in 1971 the difference in total organic P between the control and 376PA soils taken as representing the P_o derived from fertiliser additions was 89 µg P g^{-1}, of which 38 ug P g^{-1} (43%) and 51 ug P g^{-1} (57%) were present in the high and low molecular weight fractions respectively (Table 4.3.3.1.2). The above calculation by difference assumed that fertiliser additions did not cause a priming effect. These results appear to differ from those of Goh and Williams (1982) who found that most of the soil organic P derived from P fertiliser applications accumulated in low molecular weight forms (<50,000MW). However, the results of Goh and Williams (1982) were based on P content relative to

carbon in contrast to the weight of soil basis used in the present study. It is therefore difficult to make a direct comparison between the findings of this study and those of Goh and Williams (1982).

Previous studies on soils from the Winchmore long-term trial showed that organic matter (C,N) increased in the control and annually fertilised treatments up to 1965 (Quin and Rickard, 1981). This is confirmed to some extent by the results of the present study which show that organic matter, as determined by absorbance at 450nm, increased in the high molecular weight fraction (i.e. kd = 0) between 1958 and 1971 in both soils (control, 376PA), while there was little change in the lower molecular weight fraction (kd = 0.81-0.92) over the same period (cf. Figures 4.3.3.1.1 and 4.3.3.1.2 with Figures 4.3.3.1.3 and 4.3.3.1.4). Thus, the distribution of organic P between high and low molecular weight fractions may differ if the results were calculated relative to carbon rather than on a soil weight basis.

The results of this study appeared to indicate that although organic P which accumulated in the 376PA soil was evenly distributed between the high and low molecular weight fractions, the accumulated organic matter was present mainly in the high molecular weight fraction. Mechanisms involved in

the stabilisation (immobilisation) of organic P in the soil have been postulated to be different from those of other organic matter components (C,N) (McGill and Cole, 1981; see section 2.3.4).

4.3.3.2 Distribution of Organic Phosphorus in Component Extracts of Soil (376PA) Sampled in 1983. As with the combined extract from this soil (Figure 4.3.3.1.6) 2 coincident peaks of organic matter and organic P were found in each of the component extracts (0.1M NaOH, acetylacetone and 0.5M NaOH) at the void volume (i.e. >100,000MW) and between the void and total column volumes (i.e. <100,000MW) (Figures 4.3.3.2.1 to 4.3.3.2.3).

Amounts of organic P in the high and low molecular weight fractions for each of the component extracts are shown in Table 4.3.3.2.1. The low molecular weight organic P fraction made up 58% of the total organic P in the 0.1M NaOH extract compared with 79% and 70% in the acetylacetone and 0.5M NaOH extracts respectively (Table 4.3.3.2.1). The greater proportion of low molecular weight organic P in the acetylacetone and 0.5M NaOH extracts may have been due to partial hydrolysis of high molecular weight organic P to lower molecular weight forms during acid pretreatment (Steward and Tate, 1971).

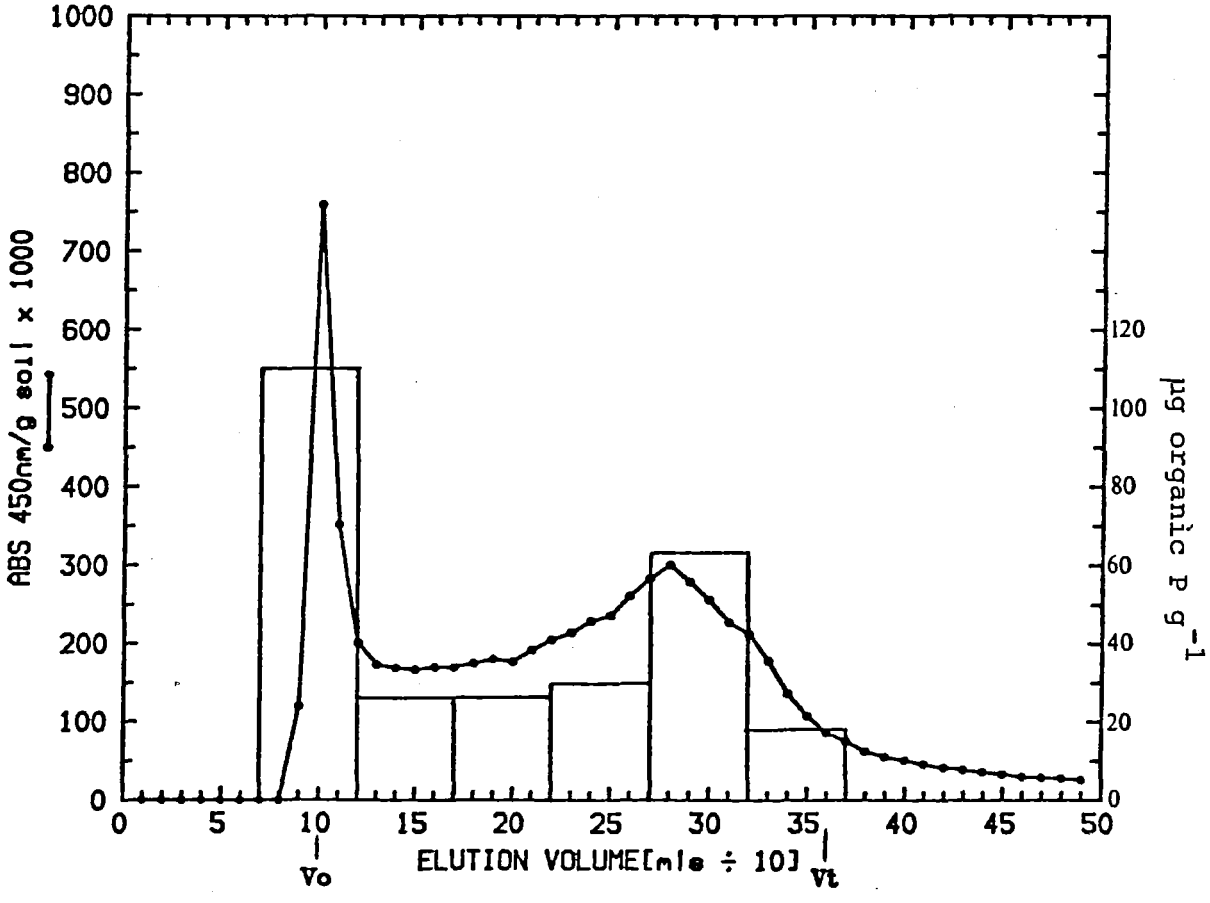

Figure 4.3.3.2.1. Fractionation of organic matter and organic P in the 0.1M NaOH extract from the 376PA-1983 soil using Sephadex G-100.

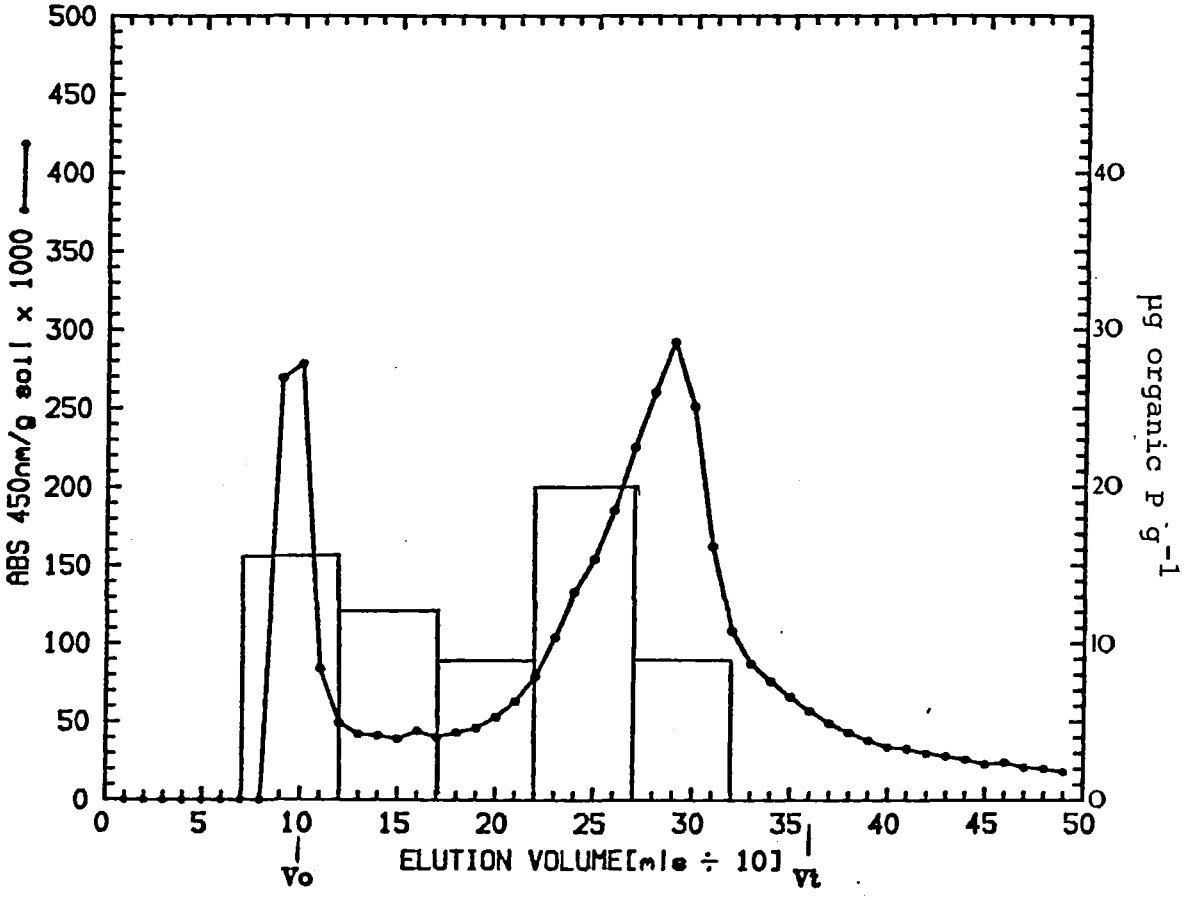

Figure 4.3.3.2.2. Fractionation of organic matter and organic P in the acetylacetone extract from the 376PA-1983 soil using Sephadex G-100.

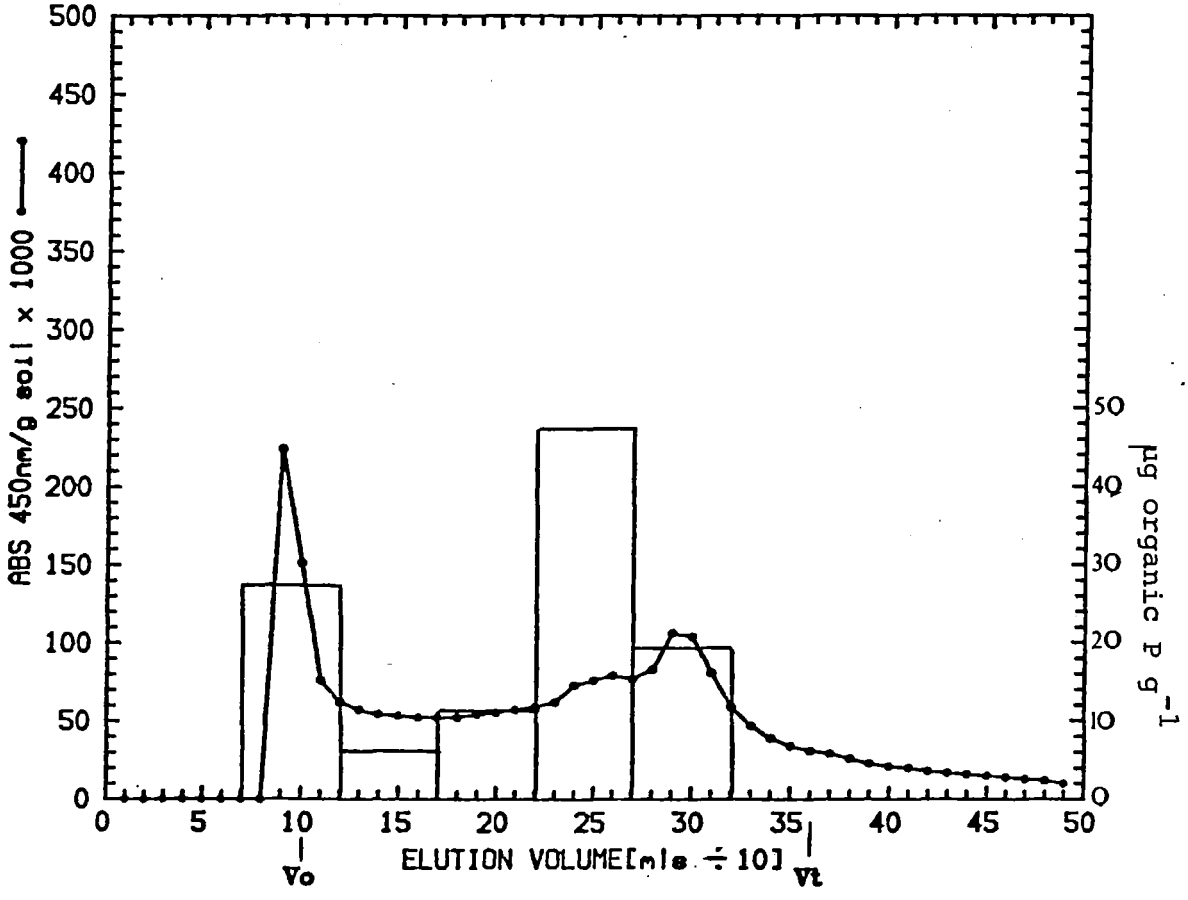

Figure 4.3.3.2.3. Fractionation of organic matter and organic P in the 0.5M NaOH extract from the 376PA-1983 soil using Sephadex G-100.

Table 4.3.3.2.1 Amounts (μg P g^{-1}) of organic P in the high and low molecular weight fractions of 0.1M NaOH, acetylacetone, and 0.5M NaOH extracts from the 376PA soil sampled in 1983.

Molecular weight fraction	Extract		
	0.1M NaOH	Acetylacetone	0.5M NaOH
High (>100,000MW)	112 (42)[a]	13 (21)	27 (25)
Low (<100,000MW)	157 (58)	50 (79)	81 (75)

[a] = values in parentheses are amounts expressed as percentage of total organic P (i.e. organic P in >100,000MW + <100,000MW fractions).

4.4 GENERAL DISCUSSION

Most of the methods which have been developed to determine the chemical composition of soil organic P involve detailed partition chromatography of extracted P species. In most soils which have been investigated using these techniques, less than half of the total organic P has been identified as known P compounds (Dalal, 1977; Anderson, 1980). On the other hand, ^{31}P NMR spectroscopy is a comparatively simple technique which enables the quantitative determination of various types of organic P present in soil extracts. In the present study, sequential alkali extraction and subsequent concentration of the soil extracts using ultrafiltration enabled most (80-87%; mean 84%) of the total soil organic P to be analysed by ^{31}P NMR (Table 4.3.1.1). This represents a considerable improvement compared with previously reported ^{31}P NMR studies of soil P in which a single alkali extraction (0.5M NaOH) was used and on average only 53% (31-90%) of the total soil organic P was extracted and subjected to ^{31}P NMR analysis (Tate and Newman, 1982; Hawkes et al., 1984).

Results of the present study showed that most (88-94%) of the total organic P in the Winchmore soils was present as orthophosphate monoester P (Table 4.3.2.3). This P fraction also constituted almost all of the organic P which accumulated in the soil

as a result of continued annual additions of P fertiliser (Table 4.3.2.3). Orthophosphate monoester P includes organic P species such as inositol polyphosphates (Newman and Tate, 1980) which are known to mineralise very slowly in the soil (Greaves and Webley, 1969; Bowman and Cole, 1978a). The predominance of orthophosphate monoester P, together with results obtained which showed that total soil organic P increased with time in the control and annually fertilised treatments (Table 4.3.1.1), implies that organic P represents a stable form of P in pasture soils.

The ^{31}P NMR analysis of soil extracts is somewhat limited in that it is only possible to resolve broad categories of organic P, namely orthophosphate monoesters, orthophosphate diesters and phosphonates (Newman and Tate, 1980). For example, in the present study most of the organic P was found as orthophosphate monoesters and orthophosphate diesters (Table 4.3.2.3), although some of the pyrophosphate P detected may have been present as organic esters in the soil which were hydrolysed during alkali extraction. Apart from the choline phosphate ($(CH_3)_3N^+ CH_2CH_2 OPO_3^{2-}$) detected in the acetylacetone and 0.5M NaOH extracts of the 376PA soil sampled in 1983 (Table 4.3.2.3), it was not possible to resolve specific organic P species within the orthophosphate monoester and diester P fractions. However,

ongoing developments in the use of solid-state ^{31}P NMR spectroscopy in soil analysis may enable more detailed resolution of the organic P species present in soils than that possible using ^{31}P NMR analysis of soil extracts (R.H. Newman, pers. comm.). In addition, in situ determination of soil P using solid state ^{31}P NMR may overcome organic P hydrolysis and changes in the chemical nature of the organic P which can occur during its extraction from the soil (Thomas and Bowman, 1966; Anderson, 1975; Hong and Yamane, 1980; Williams et al., 1981; Emsley and Niazi, 1983).

Molecular weight fractionations of extracted organic matter showed that most (61-83%) of the organic P in the control and annually fertilised soils was found in the low molecular weight fraction (i.e. <100,000MW) (Table 4.3.3.1.2). However, the proportion of high molecular weight organic P (i.e. >100,000MW) increased with time in both soils from 17-19% in 1958 to 38-39% in 1983 (Table 4.3.3.1.2). The shift towards higher molecular weight forms of organic P with time may have occurred as a result of microbial humification of soil organic matter (Kononova, 1968). This suggests that some of the organic P was incorporated into more complex and possibly more stable organic macro molecules in the soil with time.

Despite changes in the molecular weight distribution of soil organic P with time, within each of the years in which samples were analysed

(i.e. 1958, 1971, 1983), relative proportions of high and low molecular weight organic P in the control and annually fertilised soils were very similar (Table 4.3.3.1.2). This suggests that the organic P which accumulated in the fertilised soils was evenly distributed between the high and low molecular weight organic P fractions. In spite of the similar distribution pattern of organic P between high and low molecular weight fractions, each fraction may have contained different chemical forms of organic P. Further studies are therefore required to determine the relationships between the chemical form and molecular weight of organic P in the soil. This would involve the quantitative determination of organic P species such as inositol phosphates, nucleic acids and phospholipids in different molecular weight fractions of concentrated soil extracts.

CHAPTER 5

AN EXHAUSTIVE POT TRIAL EVALUATING THE PLANT AVAILABILITY OF INORGANIC AND ORGANIC PHOSPHORUS FRACTIONS PRESENT IN LONG-TERM FERTILISED PASTURE SOILS.

CHAPTER 5

AN EXHAUSTIVE POT TRIAL EVALUATING THE PLANT AVAILABILITY OF INORGANIC AND ORGANIC PHOSPHORUS FRACTIONS PRESENT IN LONG-TERM FERTILISED PASTURE SOILS

5.1 INTRODUCTION

Changes in soil P in the long-term field trial at Winchmore reported earlier (see Chapter 3) showed that decreases in soil inorganic P fractions (i.e. $NaHCO_3$, NaOH I, HCl and NaOH II P) occurred in the residual fertiliser treatments (376R, 564R) between 1958 and 1977 following the cessation of P fertiliser inputs in 1957 (Figures 3.3.2.1.1 to 3.3.2.1.4). This showed that in the long-term plants utilised some forms of inorganic P which had accumulated in the soil from annual applications between 1952 and 1957. Furthermore, increases in soil organic P which occurred in the residual fertiliser treatments between 1958 and 1971 (Figure 3.3.2.2.4) indicated that organic forms of P in this soil were very stable.

The objective of the present pot trial experiment was to investigate the short-term plant availability of soil inorganic and organic P fractions. This involved repeated cropping of 3 Lismore silt loam soils taken from under pastures

which had received different amounts of fertiliser P for 30-80 years and thereby contained different levels of accumulated inorganic and organic P.

5.2 MATERIALS AND METHODS

5.2.1 Soils Used

Topsoil (0-7.5cm) was collected in April 1982 from the 2 annually fertilised treatments in the Winchmore long-term trial, namely the 188PA treatment (188 kg superphosphate $ha^{-1}yr^{-1}$ since 1952) and the 376PA treatment (376 kg superphosphate $ha^{-1}yr^{-1}$ since 1952) (see section 3.2.1). Equal amounts (around 3kg) of field moist topsoil were taken on a random basis from each of the 4 replicate borders of the 188PA and 376PA treatments using a 2.5cm diameter steel soil corer. Soils from the replicate borders were bulked together and sieved through <4mm to remove plant debris.

In addition, topsoil from an irrigated pasture adjacent to the Fairton Freezing Works (Canterbury Frozen Meat Ltd) near Ashburton, approximately 7km from the Winchmore trial site was used. Other studies (Ross et al., 1982) showed that the soil at the Fairton site was the same as that at Winchmore, namely a Lismore silt loam (Udic Ustochrept) (see section 3.2.1). This pasture has been regularly irrigated with effluent from the adjacent meat works for over 80 years.

Keeley and Quin (1979) estimated that around 65 kg P ha^{-1} had been applied to this pasture in effluent annually, although up to 40% of the total P in the effluent was present in organic forms. At the Fairton site, about 12kg of field moist topsoil (0-7.5cm) was taken on a random basis from 2 adjacent borders using a 2.5cm diameter steel corer and sieved through <4mm before use.

5.2.2 Exhaustive Pot Trial

Before setting up the exhaustive pot trial, each soil (i.e. 188PA, 376PA, Fairton) was mixed thoroughly. Four subsamples (50g) were taken to determine amounts of P in the different soil P fractions prior to exhaustive cropping (see section 5.2.3).

The exhaustive pot trial used in this study involved growing 3 successive crops of perennial ryegrass (Lolium perenne var. Grasslands Nui) in plastic pots (15cm diameter x 12cm depth) containing 600g field moist soil (= 400g air dry soil). Each crop of ryegrass consisted of sowing 20-30 ryegrass seeds which were subsequently thinned to 8 evenly spaced plants pot^{-1}, and included 3 successive harvests of the above ground herbage (tops), following which the roots were harvested.

The tops were harvested every 4-5 weeks when the plants were 8-10cm high. Harvesting the roots involved carefully separating the root mass from the

soil, taking care to ensure that most of the soil was recovered for subsequent cropping. Following removal of the roots, the soil was remixed thoroughly, repotted and resown with ryegrass as before.

Harvested roots were washed with water to remove adhering soil particles, and the roots and tops were dried at 80°C for 20 hours and weighed. The 3 individual tops harvests of each crop were bulked together and finely ground (<1mm) prior to P determination using the method of Quin and Woods (1976). The dried root material from each crop was also finely ground (<1mm) prior to P analysis.

The three treatments used in the exhaustive pot trial included:

(i) Control: no nutrients added to the pot.

(ii) N100: nutrients added as solution containing N, K, S, Ca, Mg, Cu and Zn.

(iii) N200: same as N100 except twice the amount of N was used.

The 2 different nutrient solution treatments used were specifically designed to examine the effect of different levels of applied N on soil P fraction availability via its expected depressive effect on soil pH. The 3 treatments (control, N100, N200) were replicated 4 times and the pots arranged in a randomised block design.

The composition of the nutrient solutions are shown in Table 5.2.2.1. For each crop of ryegrass, 50mls of the appropriate nutrient solution was applied to each pot immediately following thinning.

The first 2 crops of ryegrass were grown under controlled environmental conditions in a growth cabinet (temperature - 24°C (day), 18°C (night); daylength - 16 hours; light intensity - 16,000 lux; relative humidity - 70%), while the final crop was grown in a glasshouse where the temperature was kept below 30°C. Throughout the trial the soil in the pot was maintained by daily watering at approximately 70% of field capacity by weight.

After the final crop, the soil from each pot was mixed thoroughly and a 50g subsample was taken for P analysis (see section 5.2.3).

5.2.3 Soil Phosphorus Fractionation

Prior to P analysis, soils taken before and after cropping were air dried and finely ground (<150 μm). The soil P fractionation scheme used in this study was that described previously for the long-term Winchmore superphosphate field trial described in section 3.2.3 and shown in Figure 3.2.3.1. Amounts of inorganic, total and organic P in the soil extracts and the total soil P were determined according to the methods detailed earlier in sections 3.2.4 and 3.2.5 respectively.

Table 5.2.2.1 Composition of nutrient solutions used in the N100 and N200 treatments in the exhaustive pot trial.

		N100 Solution		N200 Solution	
Nutrient	Salt	g salt ℓ^{-1}	mg nutrient 50mls^{-1}	g salt ℓ^{-1}	mg nutrient 50mls^{-1}
N	$CO(NH_2)_2$	7.53	177(100)[a]	15.06	354(200)[a]
K	KCl	13.47	354(200)	13.47	354(200)
S	Na_2SO_4	12.26	88(50)	12.26	88(50)
Ca	$CaCl_2$	1.00	18(10)	1.00	18(10)
Mg	$Mg\ Cl_2$	1.41	18(10)	1.41	18(10)
Cu	$CuSO_4$	0.44	9(5)	0.44	9(5)
Zn	$ZnSO_4$	0.45	9(5)	0.45	9(5)

[a] = figures in parentheses are equivalent rates of nutrient addition (kg ha^{-1}) calculated on an area basis (pot area = 1.77×10^{-6}ha.)

5.2.4 Statistical Analysis

Analysis of variance (anova) of the plant (dry matter yield, P uptake) and soil P data was carried out using the Genstat V Statistical Package (Lawes Agricultural Trust, Rothamsted Experimental Station, 1982). The significance of the observed differences between treatment means for (i) dry matter yields and P uptake, and (ii) various P fractions in the original, control, N100 and N200 soils was determined using Tukey's honestly significance difference (HSD) procedure (Steele and Torrie, 1980).

5.3 RESULTS AND DISCUSSION

5.3.1 Dry Matter Yields and Phosphorus Uptake

Dry matter yields and P uptake of tops and roots for each of the 3 successive crops of ryegrass from the control, N100 and N200 treatments in the 188PA, 376PA and Fairton soils are shown in Tables 5.3.1.1 and 5.3.1.2, while the corresponding cumulative tops, roots and total (i.e. tops + roots) dry matter yields and P uptake are shown in Table 5.3.1.3.

Within each treatment (i.e. control, N100, N200), levels of dry matter production and P uptake (Tables 5.3.1.1 and 5.3.1.2) followed the order: Fairton > 376PA > 188PA. This reflects the relative P status of these soils accumulated from the

Table 5.3.1.1 Dry matter yields (g pot^{-1}) of tops, roots and tops + roots for each of the 3 successive crops of ryegrass from the control, N100 and N200 treatments in the 188PA, 376PA and Fairton soils.

Soil	Treatment	Tops			Roots			Total (tops + roots)		
		1	2	3	1	2	3	1	2	3
188PA	Control	2.28	1.05	0.77	1.91	0.80	0.43	4.19	1.85	1.20
	N100	4.98	2.62	0.73	3.39	1.74	0.36	8.37	4.36	1.09
	N200	5.32	2.51	2.93	3.59	1.27	1.26	8.91	3.74	4.19
	HSD	0.99	0.46	0.68	0.84	0.55	0.27	0.77	1.24	1.13
376PA	Control	2.70	1.21	0.87	2.25	0.71	0.52	4.95	1.92	1.39
	N100	6.52	3.36	1.68	4.56	2.41	0.80	11.08	5.77	2.48
	N200	8.32	4.99	2.75	4.97	3.33	1.47	13.29	8.32	4.22
	HSD	0.69	0.49	0.75	0.59	0.71	0.44	0.93	1.71	1.10
Fairton	Control	3.87	1.97	1.46	3.07	1.29	0.73	6.94	3.26	2.16
	N100	7.12	4.04	2.90	4.92	2.22	1.53	12.04	6.26	4.43
	N200	10.39	6.27	3.87	4.39	3.65	2.23	15.78	9.92	6.10
	HSD	2.24	0.61	0.88	1.27	0.57	1.01	1.49	1.37	1.53

HSD = Tukey's honestly significant difference at 5% level.

Table 5.3.1.2. P uptake (P-mg P pot^{-1}) and P concentrations (%P) of tops and roots for each of the 3 successive crops of ryegrass from the control, N100 and N200 treatments in the 188PA, 376PA and Fairton soils.

		Tops						Roots					
Crops		1		2		3		1		2		3	
Soil	Treatment	P	%P	P	%P	P	%P	P	%P	P	%P	P	%P
188PA	Control	4.68	0.20	3.39	0.32	1.70	0.22	2.77	0.14	1.29	0.16	0.61	0.14
	N100	5.98	0.12	4.17	0.16	0.90	0.12	3.65	0.11	1.75	0.10	0.44	0.12
	N200	6.60	0.12	4.07	0.16	3.22	0.11	3.68	0.10	1.44	0.11	1.26	0.10
	HSD	1.50	-	NS	-	0.86	-	NS	-	NS	-	0.34	-
376PA	Control	7.65	0.28	5.66	0.47	2.18	0.25	4.38	0.20	1.48	0.21	0.68	0.12
	N100	10.62	0.16	8.58	0.26	2.72	0.16	6.16	0.14	3.78	0.16	1.00	0.13
	N200	11.24	0.14	9.18	0.18	3.29	0.12	5.99	0.12	4.29	0.13	1.90	0.13
	HSD	2.37	-	2.81	-	NS	-	1.65	-	1.41	-	0.53	-
Fairton	Control	17.3	0.44	13.50	0.68	5.72	0.39	7.55	0.24	3.11	0.24	1.35	0.18
	N100	20.4	0.29	19.02	0.47	8.93	0.31	11.90	0.24	4.66	0.21	2.90	0.19
	N200	32.0	0.31	28.19	0.45	9.67	0.25	12.58	0.23	7.84	0.22	4.11	0.18
	HSD	7.45	-	4.19	-	2.52	-	3.33	-	1.46	-	1.79	-

HSD = Tukey's honestly significant difference at 5% level.
NS = no significant difference between treatment means.

Table 5.3.1.3 Cumulative tops, roots and total dry matter yields (DM - g pot^{-1}) and phosphorus uptake (P - mg P pot^{-1}) after 3 successive crops of ryegrass for the control, N100 and N200 treatments in the 188PA, 376PA and Fairton soils.

		Tops		Roots		Total (tops + roots)			
Soil		DM	P	DM	P	DM	%	P	%
188PA	Control	4.10	9.77	3.14	4.67	7.25	100	14.44	100
	N100	8.33	11.05	5.49	5.84	14.06	194	16.88	117
	N200	10.76	13.89	6.12	6.38	16.87	233	20.27	140
	HSD	1.08	1.94	0.84	0.96	1.74	-	2.43	-
376PA	Control	4.78	15.49	3.49	6.55	8.27	100	22.04	100
	N100	11.56	21.92	7.77	10.94	19.32	234	32.87	149
	N200	16.07	23.71	9.77	12.18	25.84	312	35.89	163
	HSD	0.71	2.65	1.04	2.41	1.07	-	4.85	-
Fairton	Control	7.30	36.5	5.08	13.11	12.38	100	49.6	100
	N100	14.06	48.3	8.66	19.46	22.72	184	67.8	137
	N200	20.53	69.9	11.27	24.66	31.80	257	94.6	191
	HSD	2.43	8.52	1.28	2.45	3.32	-	9.27	-

HSD = Tukey's honestly significant difference at 5% level.

respective amounts of P applied over the previous 30-80 years (see section 5.2.1). Comparing the 3 soils, the level of readily available $NaHCO_3$ inorganic P ~~were~~ was greater in the Fairton soil (244 μg P g^{-1}) than those in the Winchmore soils (30-54 μg P g^{-1}) (Table 5.3.2.1.1).

Within each treatment, tops and roots dry matter yields and P uptake for the 3 successive crops (Tables 5.3.1.1 and 5.3.1.2) followed the order: first crop > second crop > third crop. In all 3 soils, results for the above-ground herbage (tops) showed that the magnitude of decreases in dry matter yield between the first and second crops were greater than the corresponding decreases in P uptake in all treatments (Tables 5.3.1.1 and 5.3.1.2). This was due to the fact that concentrations of P in tops were greater in the second crop than in the first crop (Table 5.3.1.2). In all treatments dry matter yields for the third crop were less than those obtained in the second crop, except for the N200 treatment of the 376PA soil (Table 5.3.1.1).

The continued decreases in dry matter yields with successive cropping are probably due to the continued depletion of available P in the soil. Plants in the first crop are likely to have access to a larger pool of readily available soil P than in subsequent crops which will have to rely increasingly on less readily available forms of P.

Decreases in dry matter yield which occurred between the second and third crops may have been partly due to less favourable growing conditions experienced in the glasshouse compared with those in the growth cabinet in which the first and second crops were grown. Thus, while the temperature, humidity, daylength and light intensity were maintained at optimum levels in the growth cabinet (see section 5.2.2), the environmental conditions probably varied widely in the glasshouse which may, in turn, have restricted plant growth.

Within each soil, the addition of nutrients other than P (i.e. N, K, S, Ca, Mg, Cu, Zn) in the N100 and N200 treatments increased dry matter production and P uptake compared with those in the control treatment (Table 5.3.1.3). Furthermore, the additional quantity of N provided by the N200 treatment compared with the N100 treatment significantly increased dry matter yields and P uptake, except in the P uptake of the 376PA soil (Table 5.3.1.3). For example, the relative increases in P uptake compared with the controls in the N100 treatments were 17, 49 and 37 49% in the 188PA, 376PA and Fairton soils respectively, while the corresponding relative increases in the N200 treatments were 40, 63 and 91% (Table 5.3.1.3). This suggests that with the higher total P level in the Fairton soil than in the Winchmore soils

(Table 5.3.2.1) the additional N becomes more beneficial. In the N100 and N200 treatments in the Fairton soil, P was not limiting as the P concentration in tops (0.25-0.47%) were higher than the level (0.20%) generally assumed to represent P deficiency in temperate grasses such as perennial ryegrass (Mays et al., 1980). On the other hand, concentrations of P in tops from the N100 and N200 treatments in the Winchmore soils were generally lower than 0.20%, except in the 2nd crop of the N200 treatment in the 376PA soil (0.26%) (Table 5.3.1.2). This suggests that in these treatments P was a major limiting factor in plant growth.

5.3.2 Changes in Soil Phosphorus Fractions Due to Plant Uptake

Amounts of total extracted inorganic P, total extracted organic P and residual (non-extracted) P in the original (uncropped) 188PA, 376PA and Fairton soils and those after the 3rd cropping in the control, N100 and N200 treatments are shown in Table 5.3.2.1. As expected, amounts of total P in the different soils followed the order: Fairton > 376PA > 188PA (Table 5.3.2.1). This reflects the relative quantities of P applied to the soils over the previous 30-80 years (see section 5.2.1).

Table 5.3.2.1 Amounts (μg P g^{-1}) of total extracted inorganic P, total extracted organic P and residual (non-extracted) P in the original (uncropped) 188PA, 376PA and Fairton soils and those after the 3rd cropping in the control, N100 and N200 treatments.

		Total Extracted P							
		Inorganic P[a]		Organic P[b]		Residual P[c]		Total P	
Soil	Treatment	μg P g^{-1}	%	μg P g^{-1}	%	μg P g^{-1}	%	μg P g^{-1}	%
188PA	original	346.1	100	445.6	100	82.3	100	847.0	100
	control	325.3	94	446.7	100	86.3	105	858.3	98
	N100	303.6	88	453.4	102	83.5	101	840.5	96
	N200	299.9	87	447.8	98	84.5	103	832.2	95
	HSD	15.0	-	NS	-	NS	-	22.5	-
376PA	original	521.3	100	461.1	100	96.5	100	1078.9	100
	control	476.6	91	463.2	100	92.8	96	1032.6	96
	N100	455.6	87	459.9	100	95.5	99	1011.0	94
	N200	449.6	86	464.5	101	96.0	99	1010.1	94
	HSD	48.2	-	NS	-	NS	-	56.3	-
Fairton	original	1243.9	100	483.4	100	174.4	100	1901.7	100
	control	1103.4	89	520.5	108	168.3	96	1792.2	94
	N100	1021.3	82	515.8	107	176.0	101	1713.1	90
	N200	975.3	78	519.6	107	172.8	99	1667.7	87
	HSD	33.9	-	NS	-	NS	-	131.3	-

[a] = $NaHCO_3$ (inorganic P) + NaOH I (inorganic P) + HCl P + NaOH II (inorganic P).
[b] = $NaHCO_3$ (organic P) + NaOH I (organic P) + NaOH II (organic P).
[c] = Total P - total extracted P (inorganic + organic P).
HSD = Tukey's honestly significant difference at 5% level.

The greatest differences between the various soils occurred in the total extracted inorganic P fraction. For example, amounts of total extracted inorganic P in the original (uncropped) 188PA, 376PA and Fairton soils were 346, 521 and 1243 $\mu g\ P\ g^{-1}$ respectively (Table 5.3.2.1). The differences between soils for total extracted organic P and residual P were smaller and insignificant.

Almost all of the decreases in soil P which resulted from growing 3 successive crops of ryegrass occurred in the extracted inorganic P fractions. Levels of extractable organic P and residual P appeared not to change much after successive cropping (Table 5.3.2.1).

Decreases in total extracted inorganic P due to cropping were greater in the Fairton soil (140-260 $\mu g\ P\ g^{-1}$) than those in the 376PA (45-72 $\mu g\ P\ g^{-1}$) and 188PA (21-46 $\mu g\ P\ g^{-1}$) soils (Table 5.3.2.1). Within each soil, significant decreases in total extracted inorganic P occurred in the N100 and N200 treatments compared with that of the control treatment, particularly in the Fairton soil (Table 5.3.2.1). Furthermore, a significant difference occurred in total extracted inorganic P between the N100 (1021 $\mu g\ P\ g^{-1}$) and N200 (975 $\mu g\ P\ g^{-1}$) treatments in the Fairton soil, but not in the Winchmore soils (188PA, 376PA) (Table 5.3.2.1). These findings are consistent with the corresponding trends in total plant P uptake between the different

soils reported earlier (see section 5.3.1).

5.3.2.1 Soil Inorganic Phosphorus Fractions. Amounts of inorganic P in the different extracted P fractions ($NaHCO_3$, NaOH I, HCl, NaOH II) in the original (uncropped) 188PA, 376PA and Fairton soils and those after the 3rd cropping in the control, N100 and N200 treatments are shown in Table 5.3.2.1.1.

The $NaHCO_3$ and NaOH I fractions were the major forms of extracted soil inorganic P which were depleted significantly after the 3rd successive crop of ryegrass. Relative decreases in inorganic P were generally greater in the $NaHCO_3$ than in the NaOH I fraction. For example, the amounts of $NaHCO_3$ and NaOH I inorganic P in the various cropped soils represented 53-82% and 79-90% of those present in the corresponding uncropped soils respectively (Table 5.3.2.1.1).

Amounts of $NaHCO_3$ and NaOH I inorganic P (Table 5.3.2.1.1) removed by plant uptake followed the order: Fairton > 376 PA > 188PA.

Within each soil, decreases in $NaHCO_3$ and NaOH I inorganic P were greater in the N100 and N200 treatments than those in the corresponding control treatment. These differences were significant for both fractions in the Fairton soil, but only for the $NaHCO_3$ fraction in the 376PA soil (Table 5.3.2.1.1). These findings are consistent with the respective amounts of P removed by plant uptake from the different soils (i.e. Fairton >

Table 5.3.2.1.1 Amounts (μg P g^{-1}) of inorganic P in the different extracted P fractions in the original (uncropped) 188PA, 376PA and Fairton soils and those after the 3rd cropping in the control, N100 and N200 treatments.

		Soil Inorganic P Fractions							
		$NaHCO_3$		NaOH I		HCl		NaOH II	
Soil	Treatment	μg P g^{-1}	%	μg P g^{-1}	%	μg P g^{-1}	%	μg P g^{-1}	%
188PA	original	29.5	100	122.8	100	83.3	100	110.5	100
	control	23.5	82	108.6	88	85.2	102	108.0	98
	N100	21.2	72	103.0	84	74.8	90	104.6	95
	N200	21.5	73	108.8	89	66.8	80	102.8	93
	HSD	2.6	-	13.9	-	NS	-	NS	-
376PA	original	54.0	100	188.3	100	151.5	100	127.5	100
	control	39.2	73	158.8	84	148.3	98	130.3	102
	N100	31.6	58	154.1	82	144.5	95	125.6	98
	N200	30.8	57	144.5	77	149.3	98	125.0	98
	HSD	2.4	-	8.7	-	NS	-	NS	-
Fairton	original	243.5	100	573.9	100	254.5	100	172.0	100
	control	170.0	70	517.8	90	238.8	94	176.8	103
	N100	137.6	56	475.4	83	239.0	94	169.3	98
	N200	129.3	53	464.8	81	214.5	84	166.7	97
	HSD	10.0	-	16.8	-	21.8	-	6.7	-

HSD = Tukey's honestly significant difference at 5% level.

NS = no significant differences between treatments means.

376PA > 188PA) and between the different treatments within each soil (i.e. N200 ≥ N100 > control) (see section 5.3.1).

In the Fairton soil, the amount of HCl P in the N200 treatment (214 μg P g^{-1}) was significantly lower than that in the uncropped soil (254 μg P g^{-1}), control (239 μg P g^{-1}) and N100 (239 μg P g^{-1}) treatments (Table 5.3.2.1.1). This was not apparent in the Winchmore soils or in the NaOH II P fraction of the 3 soils studied.

The significant decrease in HCl P in the N200 treatment of the Fairton soil may have been due to the significantly lower pH of this soil after successive cropping (Table 5.3.2.1.2). Within each treatment, decreases in soil pH which occurred as a result of successive cropping were greater in the Fairton soil than in the Winchmore soils (Table 5.3.2.1.2). This may have been due to the combined effects of (i) greater plant growth and root production in the Fairton soil than in the Winchmore soils (Table 5.3.1.3) which probably increased the release of hydrogen ions (H^+) from plant roots as a result of the uptake of basic cations (e.g. Na^+, K^+, Ca^{2+}, Mg^{2+}) (Tisdale et al., 1985) and increased release of organic acids by rhizosphere micro-organisms (Tinker, 1980a), and (ii) greater quantities of NH_4^+-N released from organic N mineralisation in the Fairton soil. The latter is supported by the high organic N in the Fairton soil

Table 5.3.2.1.2 Soil pH values for original (uncropped) 188PA, 376PA and Fairton soils and those after the 3rd cropping in the control, N100 and N200 treatments.

Treatment	Soil 188PA	376PA	Fairton
original	6.1	6.1	6.2
control	5.9	5.8	5.7
N100	5.8	5.8	5.5
N200	5.5	5.6	5.1
HSD	0.5	NS	0.3

HSD = Tukey's honestly significant difference at 5% level; NS = no significant differences between treatment means.

and by consideration of the C:N ratio of the Fairton and Winchmore soils. Ross et al. (1982) reported C and N contents of the effluent treated Fairton soil as 6.8% organic C and 0.63% total N, while Quin and Rickard (1981) found that the annually fertilised (188PA, 376PA) Winchmore soils contained 3% organic C and 0.3% organic N. Thus, the C:N ratios of the Fairton and Winchmore soils (10-11) are less than 20 (Tisdale et al., 1985) suggesting that organic N mineralisation would have been favoured over N immobilisation in the present study. Furthermore, the methods employed in the present study, which involved thorough mixing of the soil between successive crops (section 5.2.2), probably encouraged soil organic N mineralisation.

Within each soil, decreases in soil pH were greater in the N100 and N200 treatments than in the control, although this effect was only significant in the Fairton soil. This was probably mainly due to the acidifying effect of the urea-N in the nutrient solutions applied to these treatments (Tomlinson, 1970).

5.3.2.2 Soil Organic Phosphorus Fractions. Amounts of organic P in the different extracted P fractions ($NaHCO_3$, NaOH I, NaOH II) in the original (uncropped) 188PA, 376PA and Fairton soils and those after the 3rd cropping in the control, N100 and N200 treatments are shown in Table 5.3.2.2.1.

Table 5.3.2.2.1 Amounts (μg P g^{-1}) of organic P in the different extracted P fractions in the original (uncropped) 188PA, 376PA and Fairton soils and those after the 3rd cropping in the control, N100 and N200 treatments.

		Soil Organic P Fractions					
		$NaHCO_3$		NaOH I		NaOH II	
Soil	Treatment	μg P g^{-1}	%	μg P g^{-1}	%	μg P g^{-1}	%
188PA	original	43.8	100	226.0	100	175.8	100
	control	43.8	100	214.4	95	188.5	107
	N100	46.3	106	225.8	100	181.3	103
	N200	50.5	115	223.5	99	173.8	93
	HSD	NS	-	8.8	-	NS	-
376PA	original	44.3	100	236.0	100	180.8	100
	control	43.8	99	223.5	95	195.9	109
	N100	44.7	101	224.4	95	190.8	106
	N200	47.0	106	237.0	100	180.5	100
	HSD	NS	-	NS	-	11.4	-
Fairton	original	49.3	100	241.8	100	192.2	100
	control	57.5	117	254.0	105	208.8	109
	N100	53.4	108	252.5	104	209.8	109
	N200	51.5	104	262.3	109	205.8	107
	HSD	NS	-	NS	-	NS	-

HSD = Tukey's honestly significant differences at 5% level;
NS = no significant differences between treatment means.

In general, the removal of 3 successive crops of perennial ryegrass had little effect on the amounts of organic P in the different soil P fractions (Table 5.3.2.2.1). Nonetheless, some small significant changes in soil organic P were observed in particular treatments viz (i) a small decrease in NaOH I organic P in the control treatment of the 188PA soil compared with that in the original soil and the N100 and N200 treatments, and (ii) a small increase in NaOH II organic P in the control treatment of the 376PA soil compared with that in original soil and N200 treatment (Table 5.3.2.2.1). Reasons for these small changes in soil organic P are unclear, especially since they occurred in different soils and were not consistent with changes in organic P observed in other treatments.

In the Fairton soil, small, though not significant, increases in NaOH I and NaOH II organic P occurred in the cropped soils (control, N100, N200) compared with that in the original soil (Table 5.3.2.2.1). This may have been due to a combination of (i) microbial immobilisation of soil inorganic P to organic P in the rhizosphere, and (ii) the accumulation of organic P in root debris (Hedley et al., 1982b). Furthermore, the fact that the pH of the Fairton soil decreased significantly as a result of cropping (Table 5.3.2.1.2) may have reduced microbial activity which, in turn, may have reduced soil organic P mineralisation (see section

2.3.3.1.5). Results of this study appeared to indicate that soil organic P mineralisation did not contribute significantly to plant P uptake over the short term, which confirms the findings of Hedley et al. (1983).

5.4 GENERAL DISCUSSION

Results of the present study showed that the plant availability of the different extracted soil inorganic P fractions, as determined by the relative decreases which occurred after 3 successive crops of perennial ryegrass followed the order: $NaHCO_3$ P > NaOH I P > HCl P = NaOH II P. For example, in the control, N100 and N200 treatments in the 3 soils studied the average relative decreases in $NaHCO_3$ and NaOH I inorganic P were 34% (18-47%) and 16% (10-23%) respectively (Table 5.3.2.1.1). The corresponding decreases in HCl P and NaOH II inorganic P were generally small (<10%) and not significant, except in the N200 treatment in the Fairton soil where a significant decrease in HCl P (16%) occurred (Table 5.3.2.1.1).

These findings may be partly explained by relating the P fractions determined in the present study to those described in the schematic diagram of soil P forms as proposed by Stewart and McKercher (1980) shown in Figure 5.4.1. As described previously (section 3.3.1.2), the $NaHCO_3$ and NaOH I P fractions were suggested to represent

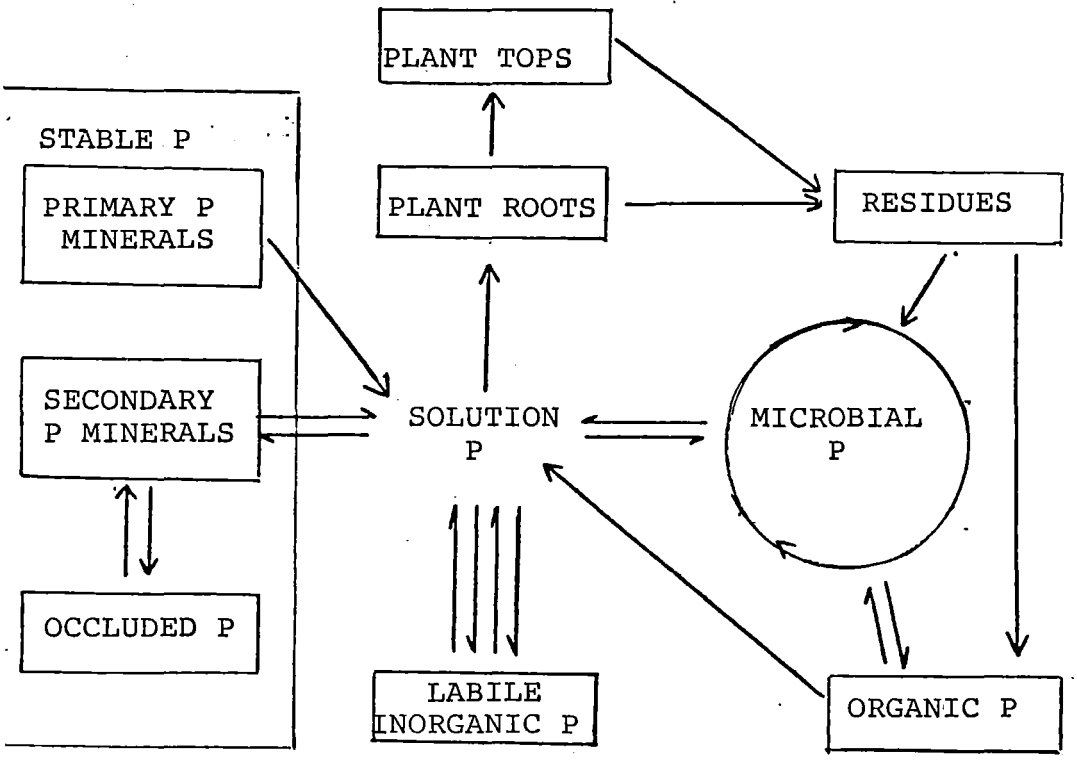

Figure 5.4.1. Schematic diagram of the different forms of P in the soil and their inter-relationships (adapted from Stewart and McKercher, 1980)

similar forms of inorganic P in soil, namely adsorbed P and sparingly soluble minerals associated mainly with iron and aluminium. Thus, the $NaHCO_3$ and NaOH I inorganic P fractions determined in the present study may represent labile and secondary mineral forms of P respectively (Figure 5.4.1). These forms of P are considered to be in direct equilibrium with the soil solution and are therefore most likely to be depleted in response to the continued removal of P from the soil solution by plant uptake. The present results showed that the $NaHCO_3$ P fraction was depleted more than the NaOH I inorganic P (Table 5.3.2.1.1). This suggests that the $NaHCO_3$ and NaOH I inorganic P fractions can be designated as the labile and secondary mineral P forms respectively.

The NaOH II inorganic P fraction is assumed to include occluded forms of P in the soil (section 3.3.1.2). The relatively low plant availability of this P fraction as obtained in the present study is consistent with the existence of a slow equilibrium between the non-labile P and the more labile secondary mineral (NaOH I) P fraction (Figure 5.4.1). Thus, non-labile P is expected to respond less readily to the continued removal of P from the soil solution by plant uptake than the more labile P fractions (Larsen, 1977; Barrow, 1983).

The significant decrease in HCl P which occurred in the N200 treatment in the Fairton soil (Table 5.3.2.1.1) was attributed to the greater decrease in soil pH which occurred in this treatment compared with the other treatments (Table 5.3.2.1.2). This acid soluble P fraction in soils is believed to consist mainly of basic calcium phosphate minerals such as apatites (section 3.3.1.2) which are known to dissolve more readily under low soil pH conditions (Khasawneh and Doll, 1978; Hedley et al., 1982b). Results of the present study suggest that in the short-term the HCl P in soils may contribute significantly to plant P requirements only under low pH conditions.

Present results showed that amounts of soil organic P were largely unaffected by successive cropping (Table 5.3.2.2.1), which suggests that organic P in these soils was very stable. This is consistent with results reported earlier (Chapter 3) for the changes in soil P which occurred in the residual fertiliser treatments (376R, 564R) in the Winchmore long-term field trials. These field results showed that soil organic P continued to accumulate in the residual fertiliser treatments after the cessation of P fertiliser inputs in 1957 while soil inorganic P and pasture production declined.

The high stability of organic P as found in the present study suggests that organic P either contributes very little to plant P uptake or the release of P from the organic forms was not enough to maintain plant growth. Plant P deficiency was apparent in the N100 and N200 treatments of the Winchmore soils (Table 5.3.1.2). According to the conceptual model of organic P turnover in the soil (i.e. immobilisation-mineralisation) as proposed by McGill and Cole (1981) under P deficient conditions plants can utilise organic forms of P in the soil by increased production of exocellular phosphatase enzymes which, in turn, increases organic P mineralisation (see section 2.3.4). It is not known whether such increases in phosphatase activity would be able to supply sufficient P to plants to overcome the observed P deficiency in the Winchmore soils. Although the present results do not demonstrate the existence of the increased phosphatase activity in the P deficient Winchmore soils they show that the increased activity, if it existed, was unable to cause significant organic P mineralisation.

CHAPTER 6

A FIELD TRIAL EVALUATING THE EFFECTS OF LIME, NITROGEN FERTILISER AND CULTIVATION ON THE FORM AND AVAILABILITY OF PHOSPHORUS PRESENT IN THE WINCHMORE ANNUALLY FERTILISED SOILS.

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CHAPTER 6

A FIELD TRIAL EVALUATING THE EFFECTS OF LIME, NITROGEN FERTILISER AND CULTIVATION ON THE FORM AND AVAILABILITY OF PHOSPHORUS PRESENT IN THE WINCHMORE ANNUALLY FERTILISED SOILS.

6.1 INTRODUCTION

The effect of P fertiliser additions on the amounts and forms of soil inorganic and organic P in the long-term trial at Winchmore were described in Chapter 3. The objective of the present study was to examine the effect of some pasture management practices (i.e. liming, nitrogen fertiliser application and cultivation) on the forms and availability of P to pasture plants in selected fertilised plots in the Winchmore trial. This field study involved monitoring the effect of the different treatments on soil P fractions and plant P uptake by regular sampling over a 2 year period.

6.2 MATERIALS AND METHODS

6.2.1 Trial Site and Treatments

Field trials in the present study were sited on the annually fertilised treatments of the existing long-term trial at Winchmore, namely the 188PA treatment (188 kg superphosphate $ha^{-1}yr^{-1}$ since 1952) and the 376PA treatment (376 kg superphosphate $ha^{-1}yr^{-1}$ since 1952) (see section 3.2.1). In December 1981,

a large area (22 x 8m) was fenced off within a selected replicate border of each long-term treatment (i.e. 188PA, 376PA). The enclosed areas were mown and 20 small plots (1 x 5m) were marked out. The trials consisted of 5 treatments with 4 replicates arranged in a randomised complete block design. Treatments included a control (CON), 2 rates of lime (L1 = 2 t ha^{-1}; L2 = 4 t ha^{-1}), urea fertiliser (N) and mechanical cultivation of the topsoil (CR). Ground limestone ($CaCO_3$) was applied in December 1981, while urea ($CO(NH_2)_2$) applications of 200 kg N ha^{-1}, 100 kg N ha^{-1} and 100 kg N ha^{-1} were made in December 1981, September 1982 and March 1983 respectively (total 400 kg N ha^{-1}). In the CR treatment, the topsoil (0-7.5cm) was cultivated in December 1981 using a garden rotary hoe and the plots were kept fallow for 18 months thereafter by regular hoeing. In May 1983, perennial ryegrass (Lolium perenne var. "Grasslands Nui") was sown in the cultivated plots to re-establish pasture prior to the completion of the field trials in December 1983. Throughout the 2 year duration of the field trials (i.e. December 1981 to December 1983) the enclosed areas were not grazed by sheep and did not receive any P fertiliser.

6.2.2 Determination of Pasture Dry Matter Yield and Phosphorus Uptake

The individual plots were harvested as required

(every 6-12 weeks) using a 0.75m wide reel-mower. The fresh herbage from each plot was weighed and converted to dry weight (kg DM ha^{-1}) by drying a subsample (100-200g) at 80° for 20 hours. The clippings from each harvest were not returned to the plots and were discarded away from the trial areas.

A fraction (10g) of the dried herbage was finely ground (<1mm) and duplicate subsamples (0.1g) taken for P determination using the method outlined by Quin and Woods (1976). The herbage P content (% P) thus obtained and the dry matter yield (kg DM ha^{-1}) were used to determine the P uptake (kg P ha^{-1}) at each harvest.

6.2.3 Soil Sampling

Topsoil (0-7.5cm) samples were taken from each plot following treatment application (and cultivation) and immediately prior to each harvest (i.e. every 6-12 weeks). This involved taking 8 small cores (18mm diameter) randomly from within the plot area. The soil cores were bulked together, sieved through 4mm to remove stones and plant debris and sealed to prevent desiccation.

6.2.4 Soil Analyses

All soil analyses were performed on fresh (i.e. field moist) soil (<4mm) and the results of P analyses were expressed on an oven dry (105°C) basis (i.e. µg P g^{-1} soil).

6.2.4.1 Soil pH. Duplicate 2g samples of soil were placed in 10ml graduated plastic tubes and shaken with 5mls of distilled water for 1 hour. The soil suspension was allowed to settle overnight and the pH of the supernatant was determined using a glass electrode (Blakemore et al., 1977).

6.2.4.2 Soil Phosphorus Fractionation. The soil P fractionation scheme used in this particular study was based on that of Stewart et al. (1980) (see section 2.1.3). Some modifications were made to the original fractionation scheme before it was adopted for use in the present study:

(i) The anion exchange resin extraction procedure was eliminated because it was found that it was difficult to quantitatively recover the soil following extraction.

(ii) Residual (non-extracted) P was determined by the difference between the total soil P and the sum of the total P in the various fractions extracted from the soil.

The soil P fractionation scheme developed for use in the present study is shown in Figure 6.2.4.2.1. Inorganic, total and organic P in the various soil extracts were determined according to the procedures outlined in section 3.2.4, and the total soil P was determined by the ignition method described in section 3.2.5.

duplicate samples of sieved (<4mm) field moist soil (=1g oven dry soil) in 50ml centrifuge tubes

	Soil P Fraction
20mls 0.5M $NaHCO_3$ (pH 8.5)/16hrs	$NaHCO_3$ P (IP/OP)
centrifuge (10,000 RPM)	
30mls 0.1M NaOH/16hrs	NaOH P (IP/OP)
centrifuge (10,000 RPM)	
30mls 0.1M NaOH sonified (20kHz sec^{-1}) for 3mins at 5°C (Kerrys Ultrasonics G100) then shaken for 16 hrs	Sonicate-NaOH P (IP/OP)
centrifuge (10,000 RPM)	
30mls 1M HCl/4hrs	HCl P (IP)
centrifuge (10,000 RPM)	
Residue	Residual P

IP = inorganic P; OP = organic P

Residual P = Total soil P - (~~$NaOH_3$~~ $NaHCO_3$ P + NaOH P + sonicate-NaOH P + HCl P).

Figure 6.2.4.2.1 Fractionation scheme for soil inorganic and organic P used in short-term field experiments.

6.2.4.3. Soil Microbial Phosphorus. The microbial P content of the soil was determined using the chloroform fumigation-bicarbonate extraction technique of Brookes et al. (1982) using a recovery correction factor of 0.40.

6.2.5 Statistical Analysis

Analysis of variance (anova) of the dry matter yield, P uptake and soil P data was performed using the Genstat V statistical package (Lawes Agricultural Trust, Rothamsted Experimental Station, 1982) in accordance with the experimental layout of the trial (i.e. randomised block design). The significance of the observed differences between the means of the various treatments at each sampling date and the cumulative dry matter yields and P uptake were determined using Tukey's honestly significant difference (HSD) procedure (Steele and Torrie, 1980).

6.3 RESULTS AND DISCUSSION

6.3.1 Pasture Yield and Phosphorus Uptake

The dry matter yield and P uptake results for the various treatments in the 188PA and 376PA soils at each harvest are shown in Tables 6.3.1.1 and 6.3.1.2 respectively, while the cumulative yield and cumulative P uptake results are shown in Table 6.3.1.3. Dry matter yields and P uptake were greater in the 376PA soil than those in the 188PA

Table 6.3.1.1 Pasture dry matter yield (kg ha^{-1}) and P uptake (kg P ha^{-1}) from different treatments in the 188PA plot at Winchmore between December 1981 and December 1983.

	1/82		3/82		5/82		10/82		12/82		2/83		4/83		9/83		12/83	
Treatment	DM	P	DM	P	DM	P	DM	P	DM	P	DM	P	DM	P	DM	P	DM	P
CON	3650	7.6 (.25)[a]	2570	6.7 (.24)	420	1.0 (.24)	2970	7.4 (.25)	3490	8.7 (.25)	2390	6.4 (.27)	1470	4.0 (.27)	320	0.8 (.25)	3630	9.8 (.27)
L1	3710	9.6 (.26)	2990	7.7 (.26)	550	1.4 (.25)	3110	8.1 (.26)	3620	9.4 (.26)	2710	7.3 (.27)	1310	3.5 (.27)	210	0.6 (.28)	3630	9.8 (.27)
L2	3650	9.5 (.26)	2930	7.6 (.26)	570	1.4 (.24)	3450	9.0 (.26)	3480	9.0 (.26)	2560	7.2 (.28)	1680	4.7 (.28)	270	0.7 (.26)	3710	10.3 (.28)
N	6430	14.0 (.22)	3080	6.8 (.22)	190	0.4 (.21)	4800	10.6 (.22)	4090	11.0 (.22)	3990	10.0 (.25)	2430	6.1 (.25)	490	1.1 (.22)	5140	11.3 (.22)
HSD	1030	4.1	NS	NS	240	0.7	440	1.0	370	0.9	610	1.7	580	1.4	120	0.3	280	0.7
CV%	14.0	23.2	13.5	14.2	32.4	33.6	7.2	6.9	5.6	5.7	12.3	12.2	19.7	19.0	22.0	23.8	4.1	4.0

HSD = Tukey's honestly significant difference at 5% level.

CV% = coefficient of variation.

NS = no significant difference between treatment menas.

[a] = % P in herbage.

Table 6.3.1.2 Pasture dry matter yield (kg ha^{-1}) and P uptake (kg P ha^{-1}) from different treatments in the 376PA plot at Winchmore between December 1981 and December 1983.

	1/82		3/82		5/82		10/82		12/82		2/83		4/83		9/83		12/83	
Treatment	DM	P	DM	P	DM	P	DM	P	DM	P	DM	P	DM	P	DM	P	DM	P
CON	4140	14.0 (.34)[a]	2710	9.2 (.34)	420	1.4 (.33)	3600	12.1 (.34)	3560	12.1 (.34)	4430	15.5 (.35)	2000	7.0 (.35)	340	1.2 (.35)	4720	16.5 (.35)
L1	4340	15.2 (.35)	3050	10.7 (.35)	790	2.8 (.36)	4040	14.1 (.35)	3300	11.6 (.35)	4010	15.2 (.38)	1800	6.8 (.37)	620	2.3 (.37)	4560	17.4 (.38)
L2	4770	17.2 (.36)	3440	12.4 (.36)	660	2.4 (.36)	3840	13.8 (.36)	3580	12.9 (.36)	4260	16.2 (.38)	1820	6.9 (.38)	290	1.1 (.38)	4530	17.2 (.38)
N	7120	21.4 (.30)	3200	9.6 (.30)	480	1.4 (.29)	6140	18.4 (.30)	5650	17.9 (.31)	5520	16.6 (.30)	3150	9.4 (.30)	580	1.8 (.31)	5840	17.6 (.30)
HSD	1050	3.4	NS	NS	NS	NS	500	1.7	760	NS	960	NS	540	2.0	NS	NS	680	NS
CV%	12.1	12.3	15.2	16.5	39.7	40.7	6.7	7.1	12.8	11.6	12.4	12.7	14.5	15.1	45.4	46.2	8.0	8.5

HSD = Tukey's honestly significant difference at 5% level.

CV% = coefficient of variation.

NS = no significant difference between treatment means.

[a] = % P in herbage.

Table 6.3.1.3 Cumulative dry matter yields (DM - kg ha^{-1}) and P uptake (P kg P ha^{-1}) for the different treatments in the 188PA and 376PA plots at Winchmore between December 1981 and December 1983.

Treatment	DM (t ha^{-1})		P (kg ha^{-1})	
	188PA	376PA	188PA	376PA
CON	20.9(100)	25.2(100)	58.8(100)	89.0(100)
L1	21.8(104)	26.5(105)	57.6(107)	96.1(108)
L2	22.3(107)	27.2(108)	59.5(111)	100.0(112)
N	31.5(151)	35.4(140)	71.2(132)	103.6(116)
HSD	2.5	2.4	6.1	11.2
CV%	6.0	4.9	6.4	6.7

HSD = Tukey's honestly significant difference at 5% level.
CV% = coefficient of variation.
Figures in parentheses are yields and P uptake relative to control (=100%).

soil (Table 6.3.1.3). This reflects the higher P status of the 376PA soil as a result of greater P fertiliser inputs over the previous 30 years.

Applications of nitrogen fertiliser (urea) significantly increased dry matter production and P uptake in both soils, although the relative increases were slightly greater in the 188PA soil than those in the 376PA soil (Table 6.3.1.3). In both soils, the concentration of P in the herbage was generally greater in the control treatments (0.24-0.27% P in the 188PA soil; 0.33-0.35% P in the 376PA soil) than those in the nitrogen fertiliser treatments (0.21-0.25% P in the 188PA soil; 0.29-0.31% P in the 376PA soil) (Tables 6.3.1.1 and 6.3.1.2). This is due to dilution effect as the relative improvements in dry matter yield which occurred as a result of N fertiliser addition (40-51%) were greater than the corresponding increases in P uptake (16-32%) (Table 6.3.1.3).

In both soils, liming resulted in small increases in pasture production and P uptake compared with those in the control, although these differences were not significant (Table 6.3.1.3). The relative improvements in DM yield and P uptake were slightly greater in soils which received 4 t lime ha^{-1} (7-12%) than in those which received 2 t lime ha^{-1} (4-8%) (Table 6.3.1.3). These increases may have been due to increases in the availability of soil P, and the enhanced mineralisation of organic P in particular (see later section 6.3.3.2).

6.3.2 Soil pH

Changes in soil pH which occurred in the various treatments in the 188PA and 376PA soils between December 1981 and December 1983 are shown in Figure 6.3.2.1. As expected, liming significantly increased soil pH from 6.1 to about 6.5 and 6.8 in the L1 and L2 treatments respectively (Figure 6.3.2.1).

The initial application of urea fertiliser (i.e. 200kg N ha^{-1} in December 1981) produced little overall effect on soil pH, although subsequent urea applications (i.e. 100kg N ha^{-1} in September 1982 and 100kg N ha^{-1} in March 1983) significantly decreased soil pH from 6.1 to about 5.7 in both soils during the second year of the trials (Figure 6.3.2.1). The latter effect was probably due to hydrogen ions (H^+) released as a result of microbial nitrification of ammonia (NH_3) formed from the hydrolysis of urea in the soil (Tomlinson, 1970). The fact that the initial application of urea had little effect on soil pH suggests that the buffering capacity of the soil was sufficient to counterbalance the small increase in hydrogen ions.

Some variability was observed in the soil pH data in the cultivation treatments in both the 188PA and 376PA soils. Small though significant decreases in soil pH (from 6.0-6.1 to 5.7-5.8) occurred immediately following cultivation (December 1981 to March 1982) and again during the following spring

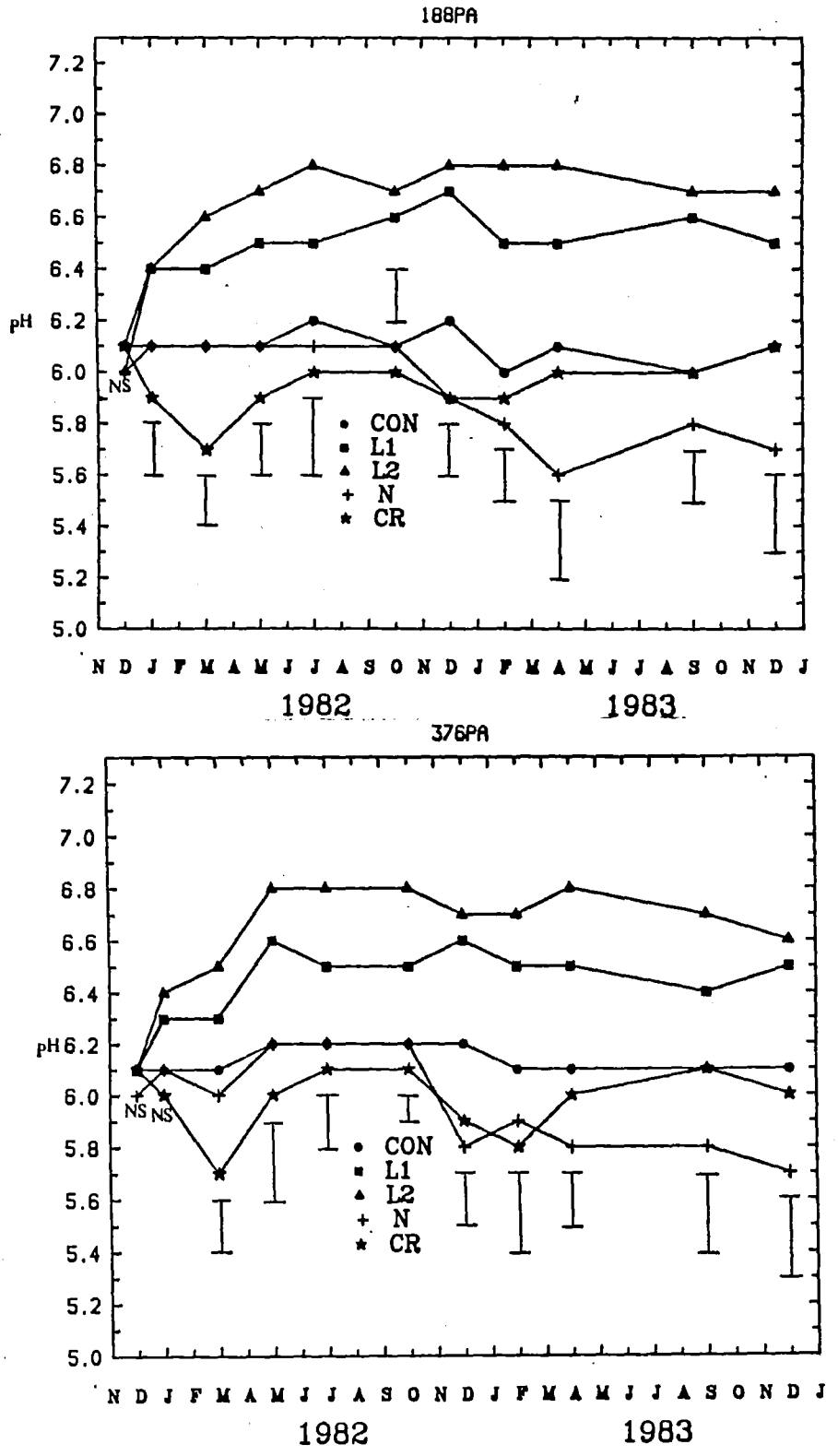


Figure 6.3.2.1. pH of soil (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.

and summer (October 1982-April 1983) (Figure 6.3.2.1). These transitory decreases in pH may have been partly due to increased levels of carbon dioxide (CO_2) in the soil as a result of increased microbial decomposition of plant residues during these periods (i.e. $CO_2 + H_2O \rightleftharpoons HCO_3 + H^+$) (Russell, 1973).

6.3.3 Soil Phosphorus Fractions

Amounts of P in the different inorganic, organic, residual and microbial P fractions in the 188PA and 376PA soils sampled at the beginning of the field trials in December 1981 are shown in Table 6.3.3.1. The largest proportion of the total P in the Winchmore soils was infact found in the residual (non-extracted) P fraction (39-42%), while the NaOH organic P (21-25%), NaOH inorganic P (11-14%) and HCl P (8-12%) fractions made up significant, though smaller, proportions of the total soil P. The remaining inorganic and organic P fractions (i.e. $NaHCO_3$ and sonicate-NaOH P) individually accounted for less than 5% of the total soil P. The high proportion of total P found in the residual P fraction in this particular study is similar to that observed by several other workers using similar soil P fractionation schemes. For example, in calcareous soils, Hedley et al. (1982a) and Tiessen et al. (1983) found that the residual P fraction made up 38-54% of the total P.

Amounts of P in the inorganic and residual P fractions were greater in the 376PA soil than those

Table 6.3.3.1 Amounts (μg P g^{-1}) of P in the inorganic, organic, residual and microbial P fractions in the 188PA and 376PA soils (0-7.5cm) sampled at the beginning of the field trials in December 1981.

Soil P Fraction	188PA Soil[a]	376PA Soil[a]
$NaHCO_3$ inorganic P	24.2(3)	42.9(4)
NaOH inorganic P	99.0(11)	149.3(14)
Sonicate NaOH inorganic P	24.0(3)	32.0(3)
HCl P I	67.2(8)	128.5(12)
$NaHCO_3$ organic P	41.3(5)	45.7(4)
NaOH organic P	222.3(25)	223.0(21)
Sonicate NaOH organic P	36.5(4)	32.4(3)
Residual P	369.3(42)	422.4(39)
Microbial P	60.2(7)	65.9(6)
Total Soil P	883.7	1076.2

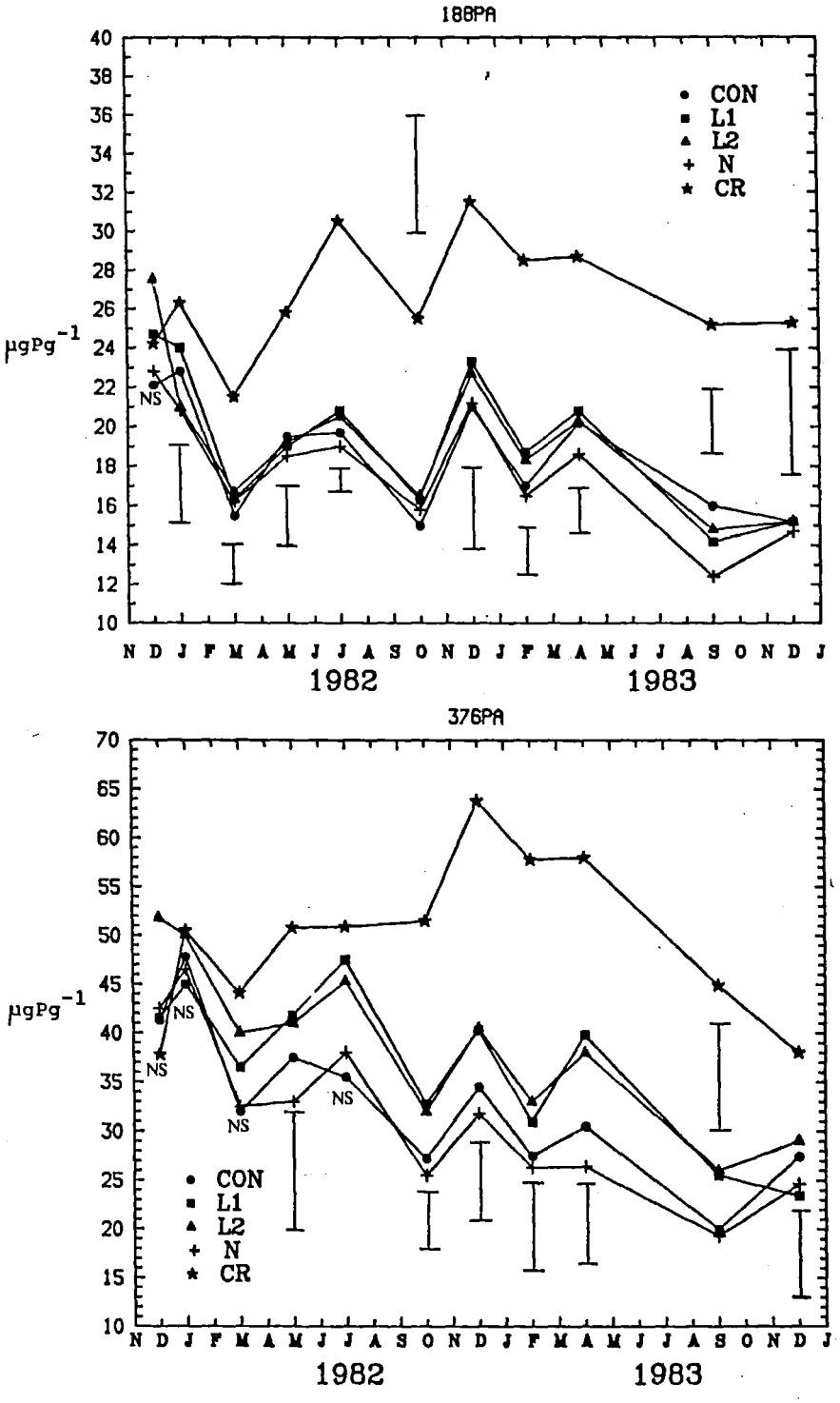
[a] = mean of 20 replicate plots.

Figures in partheses are percentages of total P.

in the 188PA soil, while amounts of P in the organic P fractions were similar in both soils (Table 6.3.3.1). These are consistent with earlier results obtained from the annually fertilised soils in the Winchmore long-term trial (see section 3.2.2), and reflect the greater amounts of fertiliser P applied to the 376PA soil during the previous 30 years. The particular forms of P which make up the various soil P fractions have been described earlier in sections 2.1.3 and 3.3.1.2.

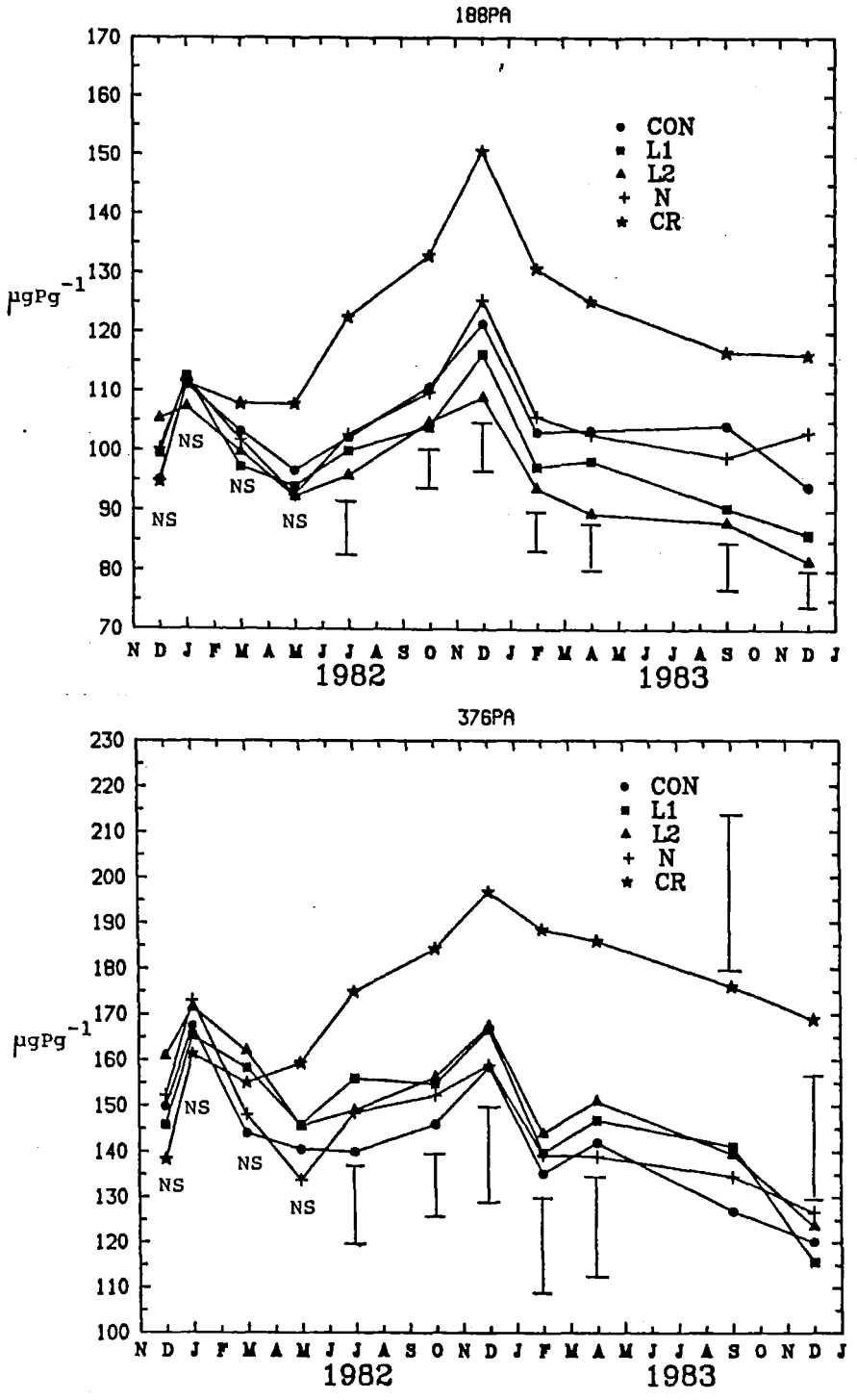
Microbial P made up 6-7% of the total P in the 188PA and 376PA soils (Table 6.3.3.1), which is similar to the proportion of microbial P (about 5% total P) found in a wide range of improved pasture soils by Brookes et al. (1984) and Sarathchandra et al. (1984).

6.3.3.1 Changes in Soil Phosphorus Fractions with Time. Changes in the various soil P fractions with time are shown in Figures 6.3.3.1 to 6.3.3.9. Amounts of P in the various fractions varied with time throughout the 2 year duration of the trials, particularly in the $NaHCO_3$ inorganic P (Figure 6.3.3.1), NaOH inorganic P (Figure 6.3.3.2), NaOH organic P (Figure 6.3.3.6) and microbial P (Figure 6.3.3.9) fractions. There was no evidence of any distinct seasonal pattern in the changes in soil P fractions with time. Reasons for the observed variations in soil P with time are unclear,



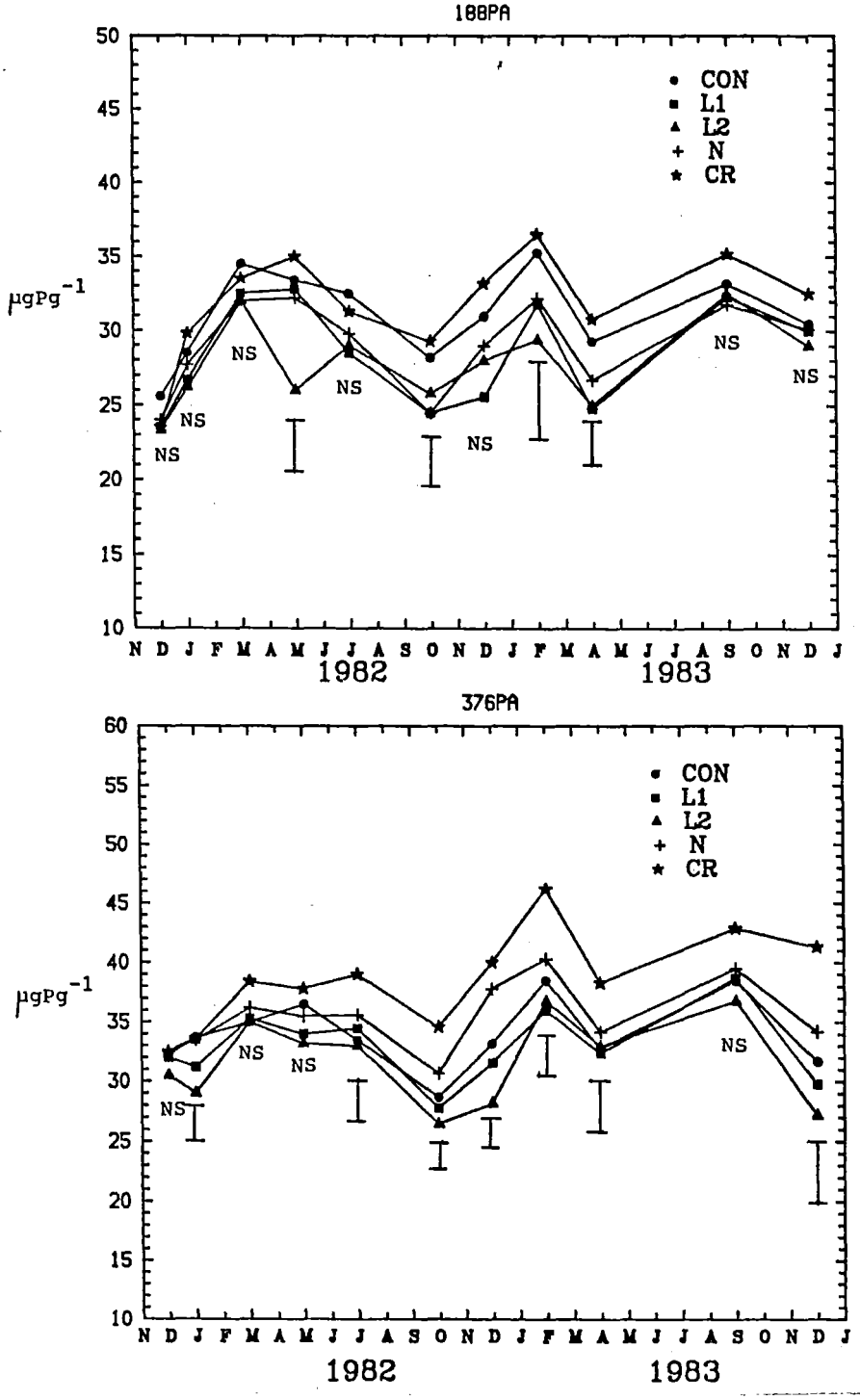
I =Tukey's honestly significant difference(HSD) at 5% level.
NS=no significant differences between treatment means.

Figure 6.3.3.1. Amounts (µg P g^{-1}) of $NaHCO_3$ inorganic P in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.



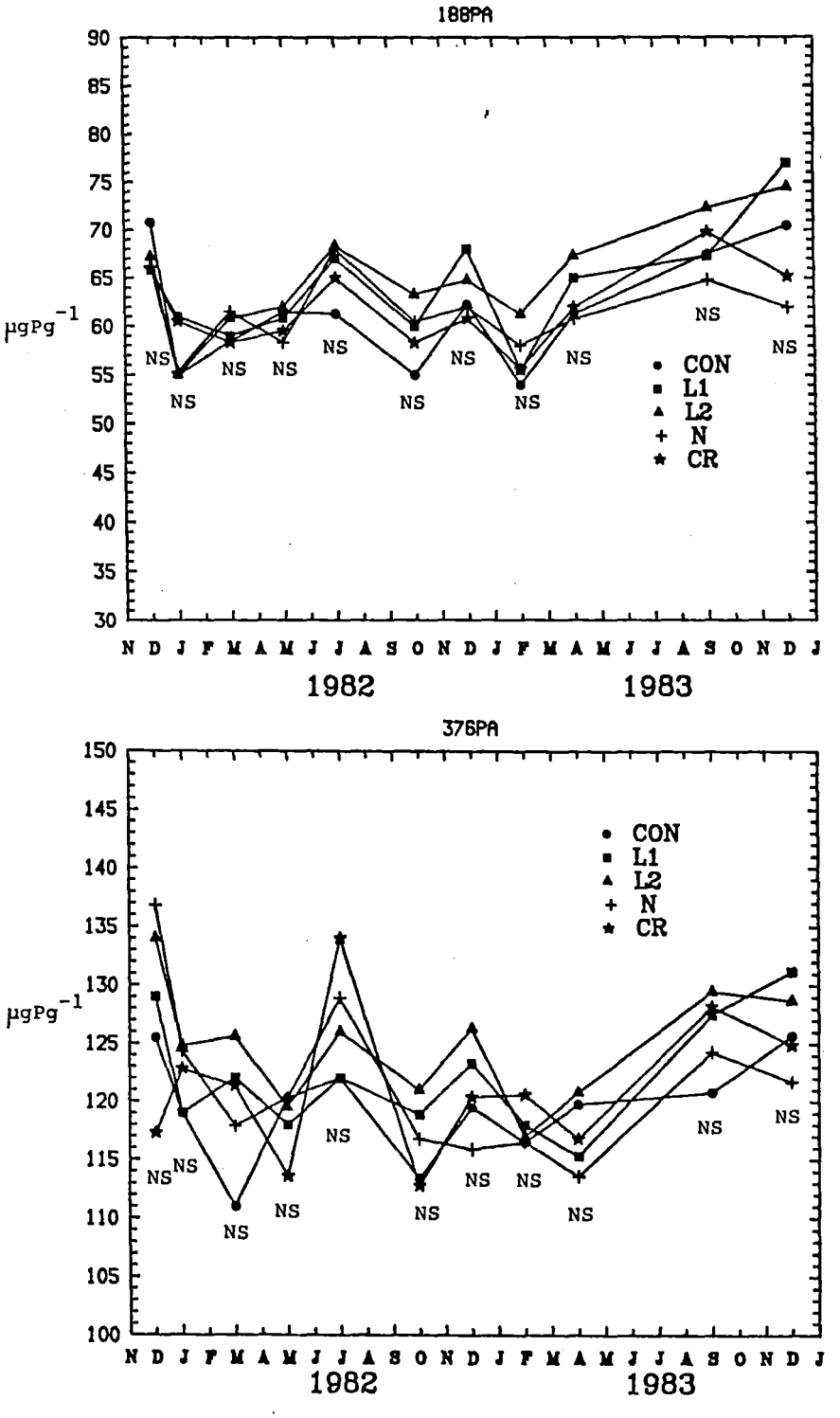
I =Tukey's honestly significant difference(HSD) at 5% level.
NS=no significant differences between treatment means.

Figure 6.3.3.2. Amounts (µg P g^{-1}) of NaOH inorganic P in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.



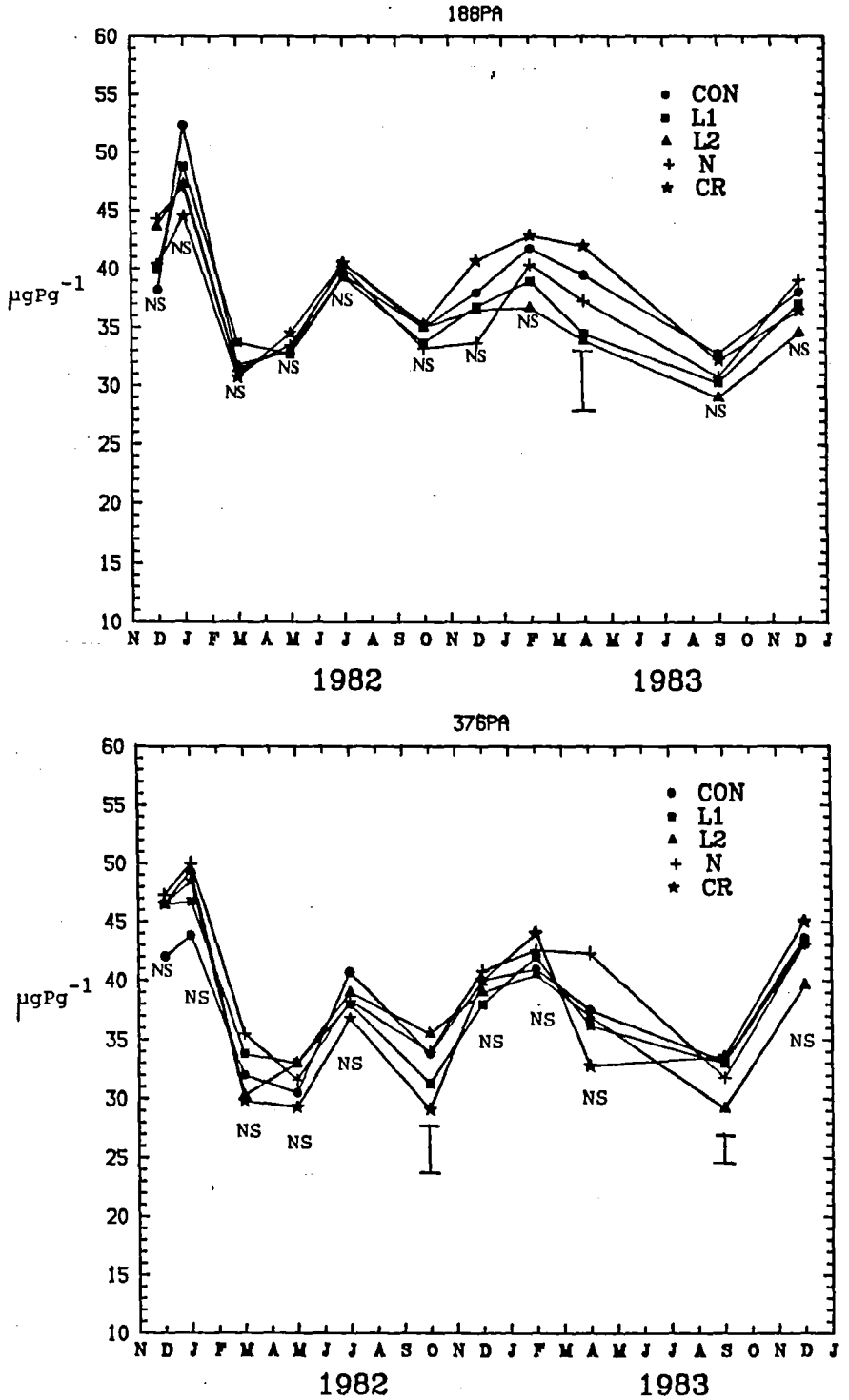
I =Tukey's honestly significant difference(HSD) at 5% level.
NS=no significant differences between treatment means.

Figure 6.3.3.3. Amounts (µg P g^{-1}) of sonicate-NaOH inorganic P in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.



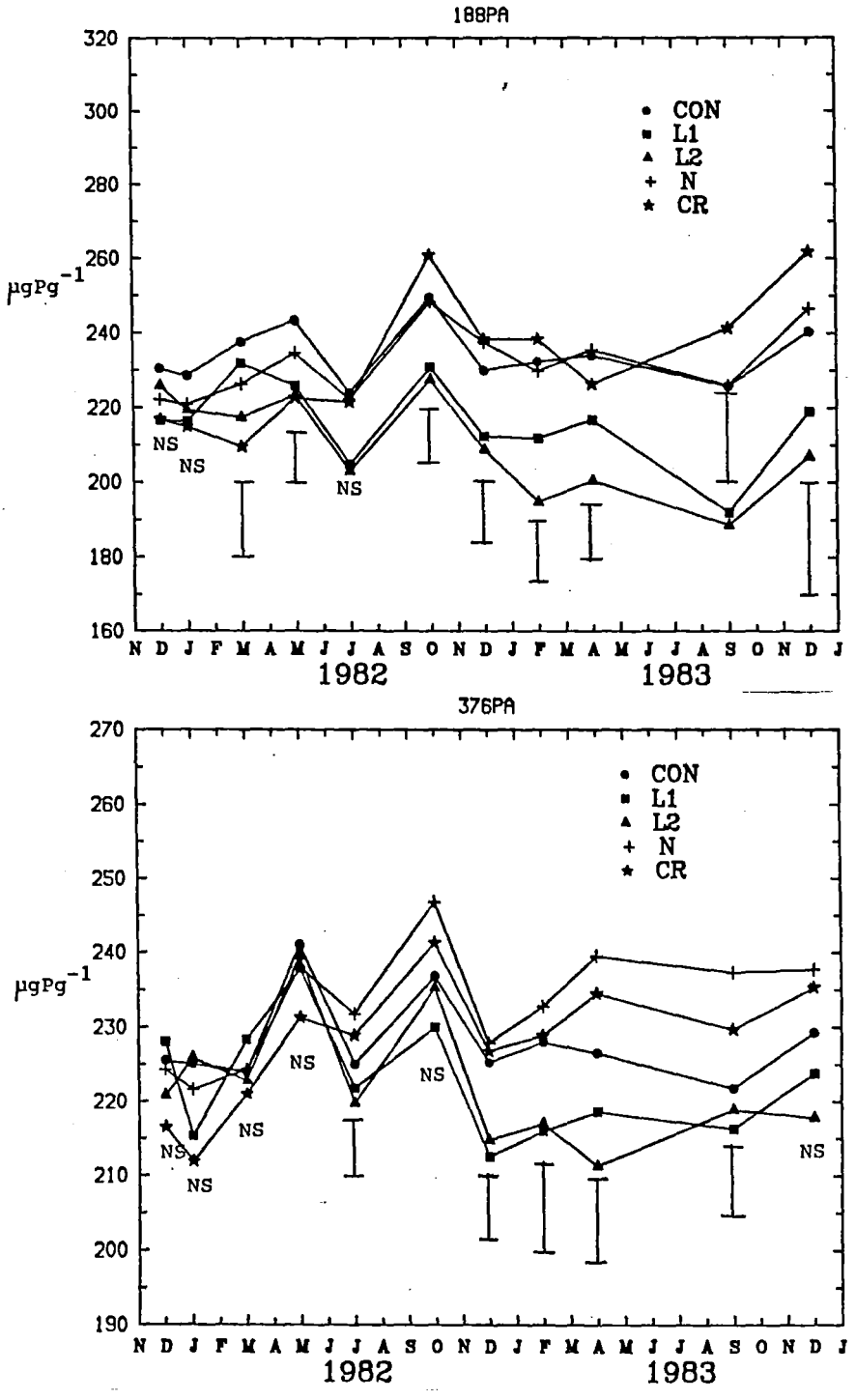
NS=no significant differences between treatment means.

Figure 6.3.3.4. Amounts (µg P g^{-1}) of HCl inorganic P in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.



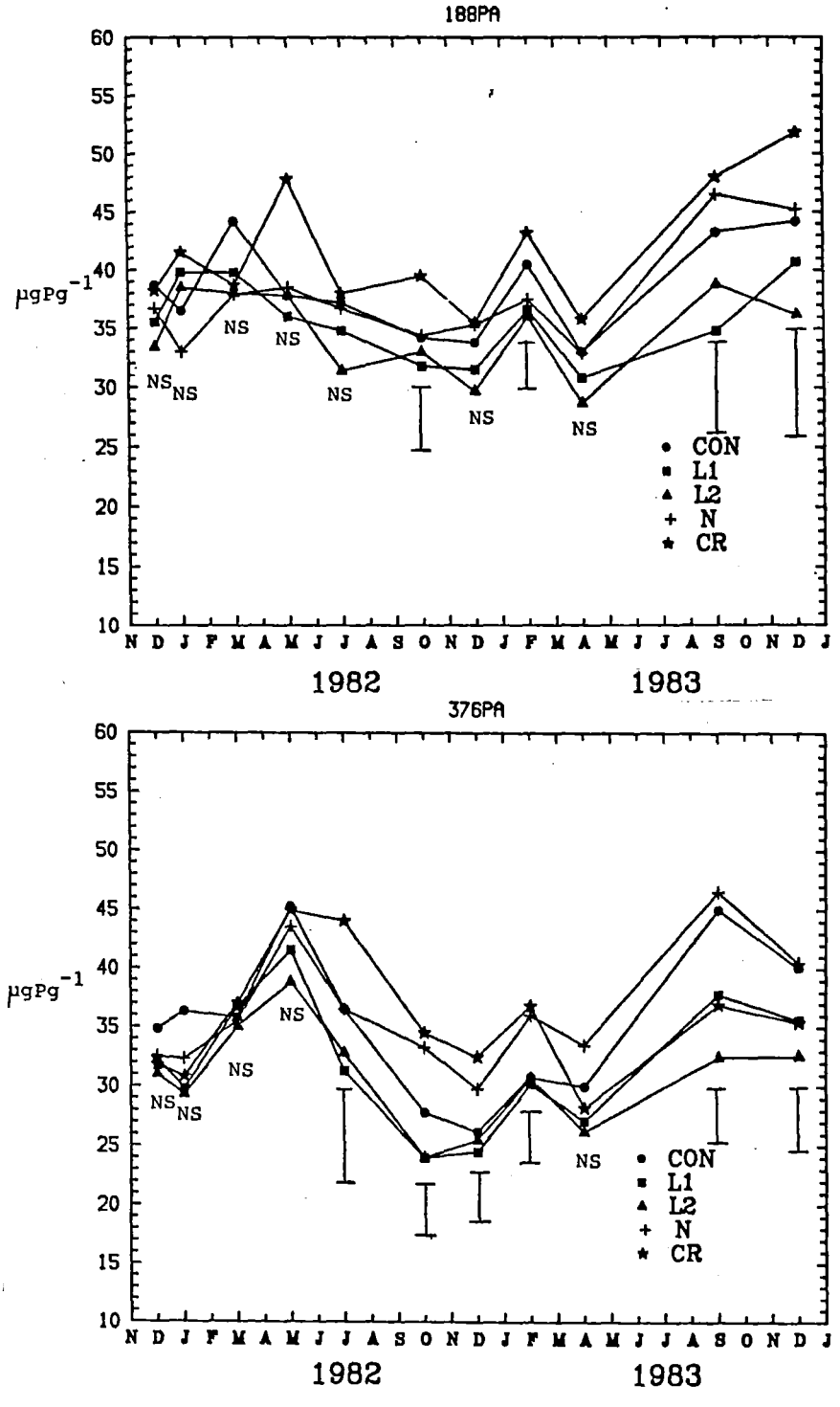
I =Tukey's honestly significant difference(HSD) at 5% level.
NS=no significant differences between treatment means.

Figure 6.3.3.5. Amounts (µg P g^{-1}) of $NaHCO_3$ organic P in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.



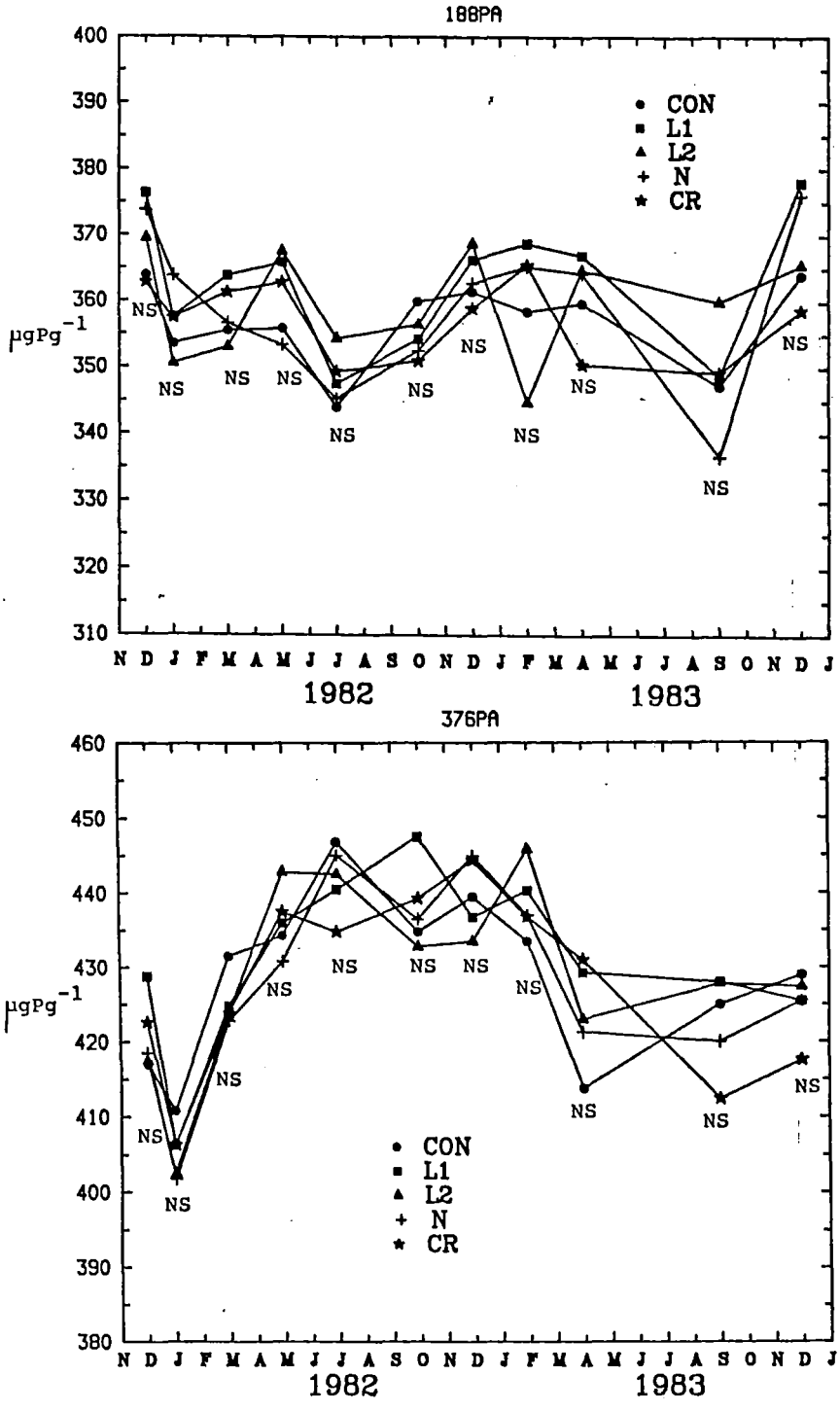
I =Tukey's honestly significant difference(HSD) at 5% level.
NS=no significant differences between treatment means.

Figure 6.3.3.6. Amounts (µg P g^{-1}) of NaOH organic P in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.



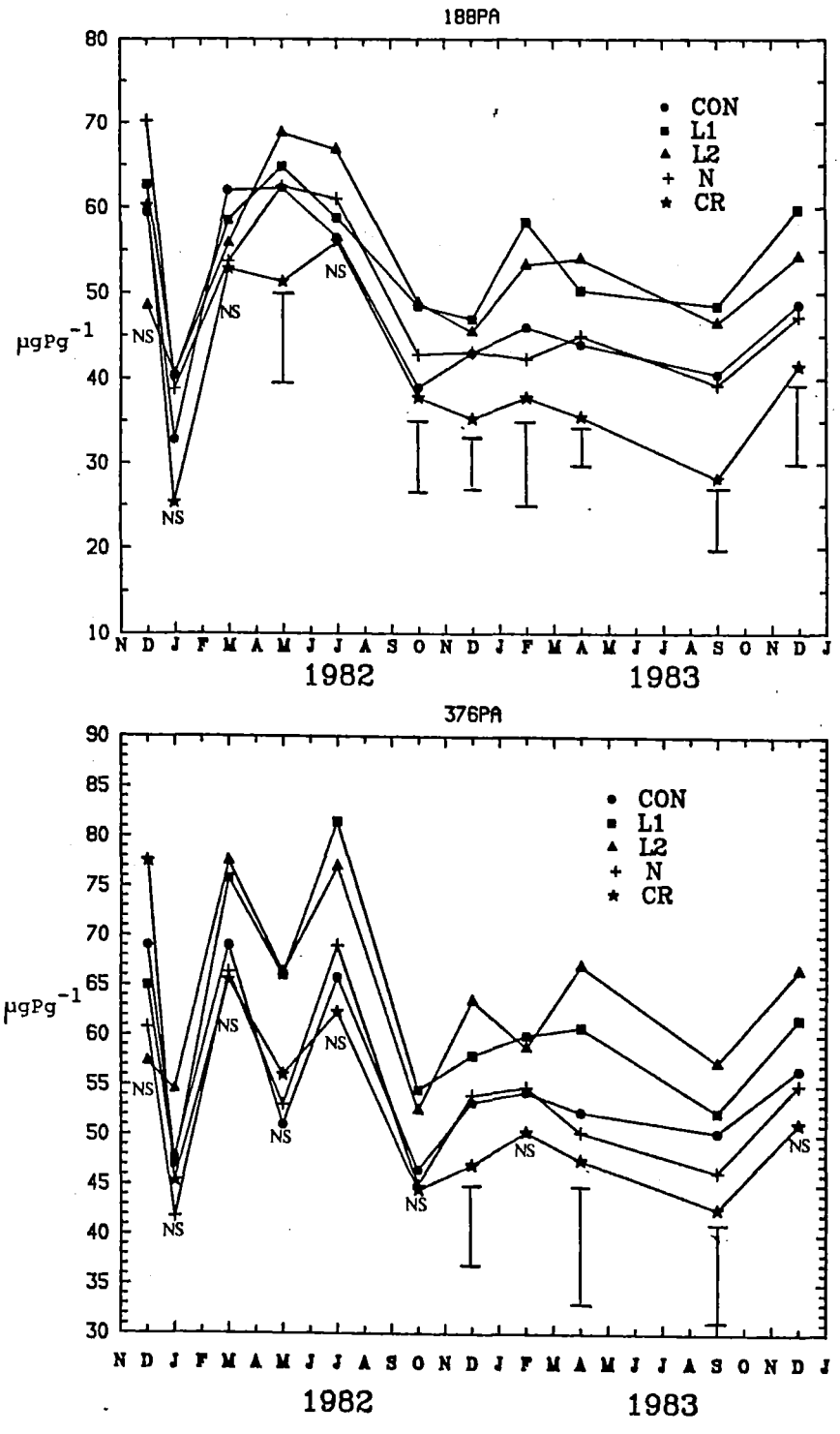
I =Tukey's honestly significant difference(HSD) at 5% level.
NS=no significant differences between treatment means.

Figure 6.3.3.7. Amounts (µg P g^{-1}) of sonicate-NaOH organic P in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.



NS=no significant differences between treatment means.

Figure 6.3.3.8. Amounts (µg P g^{-1}) of residual (non-extracted) P in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.



I =Tukey's honestly significant difference(HSD) at 5% level.
NS=no significant differences between treatment means.

Figure 6.3.3.9. Amounts (µg P g^{-1}) of microbial P in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.

particularly since in each fraction the magnitude of the changes in P levels which occurred between successive sampling dates were generally similar in all treatments (Figures 6.3.3.1 to 6.3.3.9). It is possible that variations in soil P may have been partly due to variations in the analytical procedures used in determination of the different soil P fractions on different sampling dates.

Some general trends in changes of P fractions with time were evident. For example, in the uncultivated treatments (i.e. control, L1, L2 and N) amounts of P in the $NaHCO_3$ inorganic P fraction decreased with time in both soils (Figure 6.3.3.1), while decreases in NaOH inorganic P occurred only in the 376PA soil (Figure 6.3.3.2). The depletion of these inorganic P fractions probably occurred mainly as a result of plant uptake of P and its subsequent removal by harvesting. This suggests that the $NaHCO_3$ and NaOH inorganic P fractions are the major sources of P for plants in these soils.

Overall decreases in $NaHCO_3$ inorganic P which occurred in the uncultivated treatments between December 1981 and December 1983 (Table 6.3.3.1.1) were higher in the 376PA soil (14-23 μg P g^{-1}) than those in the 188PA soil (7-13 μg P g^{-1}). This suggests that greater removal of P by plant uptake occurred in the 376PA soil, which is consistent with the results presented earlier (Table 6.3.1.3).

Table 6.3.3.1.1 Amounts (μg P g^{-1}) of $NaHCO_3$ inorganic P in soils from the uncultivated treatments in the 188PA and 376PA soils sampled in December 1981 and December 1983.

Treatment	188PA Soil			376PA Soil		
	Dec. 1981	Dec. 1983	Difference 1983-1981	Dec. 1981	Dec. 1983	Difference 1983-1981
Control	22.0	15.2	6.8	41.3	27.4	13.9
L1	24.7	15.2	9.5	41.6	23.4	18.2
L2	27.8	15.2	12.6	51.8	29.0	22.8
N	22.8	14.7	8.1	42.5	24.6	13.5

6.3.3.2 Treatment Effects on Soil Phosphorus Fractions. Significant treatment effects on P fractions were observed in the $NaHCO_3$ inorganic P (Figure 6.3.3.1), NaOH inorganic P (Figure 6.3.3.2), sonicate-NaOH inorganic P (Figure 6.3.3.3), NaOH organic P (Figure 6.3.3.6) and microbial P (Figure 6.3.3.9) fractions. In general, NaOH organic P decreased significantly as a result of lime addition, while cultivation significantly increased amounts of $NaHCO_3$, NaOH and sonicate-NaOH inorganic P in the soil. In order to give an indication of the magnitude of changes in the inorganic, organic and microbial P fractions, amounts of P in these fractions obtained in soils sampled in December 1982 and December 1983 are shown in Tables 6.3.3.2.1 and 6.3.3.2.2.

6.3.3.2.1 Lime Effects. In both soils, the addition of lime decreased the amounts of NaOH organic P, although this was not significant in the 376PA soil sampled in December 1983 (Tables 6.3.3.2.1 and 6.3.3.2.2). The magnitude of this liming effect was greater in the 188PA soil than that in the 376PA soil. For example, in December 1982 decreases in NaOH organic P which occurred in the limed treatments compared with those in the control were 18-21 $\mu g\ P\ g^{-1}$ and 10-13 $\mu g\ P\ g^{-1}$ in the 188PA and 376PA soils respectively (Table 6.3.3.2.1). These differences in the 188PA soil were slightly greater in December 1983 than in December 1982, while the corresponding

Table 6.3.3.2.1 Amounts (μg P g^{-1}) of P in different P fractions in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled in December 1982. (IP = inorganic P; OP = organic P).

	188PA Soil P Fractions					376PA Soil P Fractions				
	$NaHCO_3$ IP	NaOH IP	S-NaOH IP	NaOH OP	Microbial P	$NaHCO_3$ IP	NaOH IP	S-NaOH IP	NaOH OP	Microbial P
Control	21.0	121.5	31.0	230.0	43.0	34.5	158.5	33.2	225.3	53.3
L1	23.3	116.4	25.6	212.3	47.0	40.3	166.8	31.6	212.5	58.0
L2	22.7	109.0	28.0	208.8	45.5	40.5	167.5	28.2	214.8	63.5
N	21.2	125.5	29.0	237.5	43.1	31.8	159.0	35.7	227.8	54.0
CR	31.5	150.6	33.2	238.3	35.3	63.8	196.7	40.0	226.8	47.0
HSD	4.2	7.2	NS	17.5	6.0	8.2	21.2	2.4	9.2	8.3
CV%	10.4	3.4	11.0	4.6	8.3	11.5	7.4	4.1	2.5	9.0

HSD = Tukey's honestly significant difference at 5% level.
CV% = coefficient of variation.
NS = no significant differences between treatment means.

Table 6.3.3.2.2 Amounts (μg P g^{-1}) of P in different P fractions in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled in December 1983 (IP = inorganic P; OP = organic P).

Treatment	188PA Soil P Fractions					376PA Soil P Fractions				
	$NaHCO_3$ IP	NaOH IP	S-NaOH IP	NaOH OP	Microbial P	$NaHCO_3$ IP	NaOH IP	S-NaOH IP	NaOH OP	Microbial P
Control	15.2	94.1	30.5	240.5	48.7	27.4	120.2	31.7	229.3	56.6
L1	15.2	86.1	30.0	219.0	59.8	23.4	115.7	29.8	223.8	61.6
L2	15.2	86.6	29.0	207.0	54.3	29.0	123.7	27.2	217.8	66.6
N	14.7	103.1	30.1	246.6	47.3	24.6	126.7	34.2	237.7	55.1
CR	25.3	116.2	32.5	261.8	41.5	38.0	168.8	41.3	235.3	51.2
HSD	6.5	5.6	NS	19.9	9.4	8.7	25.4	5.1	NS	NS
CV%	22.5	3.4	5.0	5.0	11.1	18.2	11.6	9.3	6.5	12.1

HSD = Tukey's honestly significant difference at 5% level.
CV% = coefficient of variation.
NS = no significant differences between treatment means.

results for the 376PA soil were similar (cf. Table 6.3.3.2.1 and Table 6.3.3.2.2).

The liming effect on the NaOH organic P fraction may be due to net mineralisation of soil organic P. Liming may have increased microbial activity in the soil which, in turn, increased organic P mineralisation (Halstead et al., 1963).

Greater decreases in NaOH organic P observed in the 188PA soil compared with those in the 376PA soil (Tables 6.3.3.2.1 and 6.3.3.2.2) could be due to the presence of a lower level of labile $NaHCO_3$ inorganic P in the former soil (Table 6.3.3.2.1). This could have enhanced mineralisation of organic P to meet increased microbial demand for P. The present results support the conceptual model of soil organic P turnover (i.e. immobilisation-mineralisation) as proposed by McGill and Cole (1981). According to this model, the extent of soil organic P mineralisation is largely determined by the amounts of readily available inorganic P present in the soil (see section 2.3.4). Increased microbial activity and P uptake as a result of lime addition are confirmed to some extent by the significant increases in amounts of soil microbial P found in the limed treatments, particularly in the 188PA soil sampled in December 1983 and the 376PA soil sampled in December 1982 (Tables 6.3.3.2.1 and 6.3.3.2.2).

It is also possible that part of the observed

effect of liming on organic P mineralisation was due to increased solubility of organic P species such as inositol-hexaphosphate (IHP) held on soil colloid surfaces (see section 3.3.2.2.2).

The observed effect of liming on the NaOH organic P fraction was similar to that reported for the same soils (i.e. 188PA, 376PA) following liming of the long-term field trial in 1972 (section 3.3.2.2.2). However, the magnitude of decreases in the NaOH organic P fraction (i.e. NaOH I) which occurred in the 1972 lime addition was much greater than that found in the present study (cf. Figure 3.3.2.2.2 and Figure 6.3.3.1).

In the 1972 liming trial decreases in the NaOH I organic P fraction were largely counterbalanced by concomitant increases in the NaOH II organic P fraction (i.e. organic P extracted from the soil by alkali following acid treatment) (see section 3.3.2.2.2). This suggested that liming caused a change in the form (vis-a-vis extractability) of the soil organic P. The NaOH II fraction was not determined in the present study and most of the P in this fraction would have been included in the residual (non-extracted) P fraction. Although liming did not appear to have any significant effect on the residual P fraction (Figure 6.3.3.8), it is nonetheless possible that at least part of the small decreases in NaOH organic P which occurred in the

limed soils may have been due to its conversion to residual (non-extracted) forms.

Additions of lime significantly decreased the amounts of NaOH inorganic P in the 188PA soil, but not in the 376PA soil (Tables 6.3.3.2.1 and 6.3.3.2.2). This may have been due to increased desorption of inorganic P as a result of increased soil pH due to liming (Haynes, 1984), but similar increases in soil pH were obtained for both soils following liming (Figure 6.3.2.1).

6.3.3.2.2 Nitrogen Fertiliser Effects. Additions of nitrogen fertiliser did not appear to have any significant effect on amounts of P in the various soil P fractions in the 188PA and 376PA soils compared with those in the controls (Tables 6.3.3.2.1 and 6.3.3.2.2). These results are surprising, particularly in view of the significant increases in plant P uptake which occurred as a result of nitrogen fertiliser addition, especially in the 188PA soil (Table 6.3.1). It is possible that the absence of a significant nitrogen effect was due to increased root proliferation and P uptake from soil below the 0-7.5cm layer examined in the present study (Olsen and Kurtz, 1982).

6.3.3.2.3 Cultivation Effects. Cultivation and the subsequent 18 month fallow period resulted in significant increases in amounts of soil inorganic P in the $NaHCO_3$ and NaOH P fractions

compared with those in the control (Tables 6.3.3.2.1 and 6.3.3.2.2). In addition, cultivation significantly increased the amount of inorganic P in the sonicate-NaOH P fraction in the 376PA soil (Tables 6.3.3.2.1 and 6.3.3.2.2). The respective increases in $NaHCO_3$ and NaOH inorganic P were greater in the 376PA soil than those in the 188PA soil, while in both soils increases in NaOH inorganic P were greater than those in the $NaHCO_3$ inorganic P fraction. For example, samples taken in December 1982 show that increases in NaOH inorganic P which occurred as a result of cultivation were 29 μg P g^{-1} and 38 μg P g^{-1} in the 188PA and 376PA soils respectively, while the corresponding increases in $NaHCO_3$ inorganic P were 10 and 29 μg P g^{-1} (Table 6.3.3.2.3.1).

Increases in $NaHCO_3$ and NaOH inorganic P which occurred in the cultivated soils were probably mainly due to P released as a result of microbial decomposition of plant residues. The concentration of P in plant residues is an important factor in determining whether P is released during their decomposition in the soil. According to Alexander (1977), as a general rule P is released during the decomposition of plant residues containing greater than 0.2% P, while immobilisation of soil inorganic P occurs during the decomposition of residues containing less than 0.2%P. The

Table 6.3.3.2.3.1 Amounts (μg P g^{-1}) of $NaHCO_3$ and NaOH inorganic P in the control and cultivated (CR) treatments in the 188PA and 376PA soils sampled in December 1982 and December 1983.

	December 1982				December 1983			
	188PA		376PA		188PA		376PA	
Treatment	$NaHCO_3$	NaOH	$NaHCO_3$	NaOH	$NaHCO_3$	NaOH	$NaHCO_3$	NaOH
CR	31.5	150.6	63.8	196.7	25.3	116.2	38.0	168.8
Control	21.0	121.5	34.4	158.6	15.2	94.1	27.4	120.2
Difference	10.5	29.1	29.4	38.1	10.1	22.1	10.6	48.6

concentrations of P in the above-ground herbage found in the present study were greater than 0.2% in both soils (Tables 6.3.1.1 and 6.3.1.2) which may account for the increase in inorganic P observed in the cultivated soils.

Greater increases of $NaHCO_3$ and NaOH inorganic P in the 376PA soil compared with that in the 188PA soil (Table 6.3.3.2.3.1) was possibly due to the greater concentration of P in the plant residues in the 376PA plots. The concentration of P in the above ground herbage was found to be higher in the 376PA soil than that in the 188PA soil (cf.Table 6.3.1.1 and Table 6.3.1.2), even though both soils yielded similar levels of pasture production over the previous 30 years (Figure 3.3.2.1.6). This explanation assumes that similar amounts of plant residue were removed by sieving prior to P analysis (section 6.2.3).

Despite the fact that plant residues contain both inorganic and organic forms of P (section 2.2.5), cultivation and the subsequent decomposition of plant residues did not appear to significantly affect the amounts of organic P in the 188PA and 376PA soils (Tables 6.3.3.2.1 and 6.3.3.2.2). This suggests that either: (i) all of the organic P in the residues was rapidly mineralised following cultivation, or (ii) any increase in soil organic P originating from plant residues was counterbalanced by a decrease

in soil organic P due to enhanced mineralisation resulting from the physical effects of mechanical cultivation (see section 2.3.3.1.4). The latter explanation is probably more likely since it is known that some of the organic P components present in plant residues (e.g. inositol phosphates) are mineralised very slowly in the soil (Dalal, 1977; Anderson, 1980).

As described previously (section 6.3.3.1) amounts of $NaHCO_3$ and NaOH inorganic P in the uncultivated treatments (i.e. control, L1, L2, N) decreased with time as a result of plant uptake of P. Accordingly, differences in $NaHCO_3$ and NaOH inorganic P observed between cultivated and uncultivated treatments (Tables 6.3.3.2.1 and 6.3.3.2.2) could be partly due to the absence of continued plant removal of P in the cultivated soils during the fallow period. Amounts of soil microbial P were significantly lower in the cultivated treatments than those in the uncultivated treatments (i.e. control, L1, L2, N) of the 188PA soil, but not in those of the 376PA soil (Tables 6.3.3.2.1 and 6.3.3.2.2). These results suggest that while increases in microbial activity may have occurred during initial decomposition of plant residues in the cultivated soils, rapid exhaustion of the energy-rich carbonaceous substrates in the residues resulted in subsequent decreases in microbial P (Birch, 1961;

Hayman, 1975). The higher level of microbial P observed in the uncultivated soils could be due to additions of energy-rich carbonaceous substrates from root exudates, dead roots and above ground plant litter. Such additions are absent in the cultivated and fallow soils.

Differences between amounts of $NaHCO_3$ and NaOH inorganic P in the cultivated and control soils were greater in soils sampled in December 1982 than those sampled in December 1983 (Table 6.3.3.2.3.1). This depletion of inorganic P was probably caused by plant uptake following the sowing of perennial ryegrass in the cultivated plots in May 1983.

6.4 GENERAL DISCUSSION

Results of the present study showed that liming caused significant decreases in the NaOH organic P fraction in the 188PA (18-21 μg P g^{-1}) and 376PA (10-13 μg P g^{-1}) soils (Table 6.3.3.2.1). This was probably due to increases in soil organic P mineralisation as a result of lime addition which, in turn, may have been due to increased microbial activity in the soil. The latter is supported to some extent by the small though significant increases in soil microbial P which occurred in the limed treatments compared with those in the corresponding control treatments (Tables 6.3.3.2.1 and 6.3.3.2.2).

These present results support the earlier findings from the long-term field trials (Chapter 3) which showed that lime addition in 1972 caused significant increases in soil organic P mineralisation.

Increased mineralisation of organic P in limed soils may contribute directly to increased plant yield and P uptake. However, only small increases in dry matter yield and P uptake were found in the limed treatments probably due to small increases in soil pH after liming (0.5-0.8). ~~compared with those of the controls (Table 6.3.1.3)~~.

Mechanical cultivation and the subsequent fallow period significantly increased amounts of inorganic P in the $NaHCO_3$ and NaOH P fractions in both the soils studied. For example, in the cultivated treatments $NaHCO_3$ and NaOH inorganic P were considerably higher than those in the control treatments. In samples taken in December 1982, differences in combined $NaHCO_3$ and NaOH inorganic P were 40 μg P g^{-1} and 68 μg P g^{-1} in the 188PA and 376PA soils respectively (Table 6.3.3.2.1). This was probably due to P released from pasture plant residues as a result of microbial decomposition following cultivation. Thus, increases in soil inorganic P from plant residue decomposition may contribute to the immediate P fertiliser requirements of crops grown on pasture soils which had received P fertiliser additions for several years.

Overall, levels of microbial P in the cultivated treatments were significantly lower than those in the uncultivated treatments, particularly in the 188PA soil (Tables 6.3.3.2.1 and 6.3.3.2.2). This was probably due to the absence of plants in the cultivated soils since the presence of organic exudates and root detritus in the rhizosphere of plant roots is known to support higher levels of microbial activity than in non-rhizosphere soil (Hayman, 1975; Tinker, 1980b).

Although the application of nitrogen fertiliser (urea) significantly increased dry matter production and P uptake in both the soils studied (Table 6.3.1.3) this did not significantly affect amounts of P in the different soil P fractions (Tables 6.3.3.2.1 and 6.3.3.2.2). It was expected that, by increasing plant growth and P uptake, the addition of urea would have enhanced the depletion of soil P. These results illustrate the difficulties associated with attempting to relate changes in pasture growth and P uptake with changes in P fractions in the topsoil (0-7.5cm) under field conditions over a relatively short time period.

In the uncultivated treatments (i.e. control, N, L1, L2) decreases in amounts of inorganic P in the $NaHCO_3$ fraction occurred with time in both soils (Table 6.3.3.1.1). Decreases in NaOH inorganic P fraction were evident only in the 376PA soil (Figure 6.3.3.2). This suggests that $NaHCO_3$ and NaOH inorganic P fractions were the major sources of P

depleted by plants in these soils. These results are in agreement with those of the exhaustive pot trial (Chapter 5) which showed that $NaHCO_3$ and NaOH I inorganic P fractions represented the most readily available forms of P to plants in the annually fertilised Winchmore soils.

CHAPTER 7

GENERAL SUMMARY

The various aspects of soil P relevant to the present study were reviewed. These included forms of P in the soil, sources of P for plants in soil, the fate of applied fertiliser P in the soil-plant system and the chemical nature and turnover of soil organic P. This review showed that further studies were required to determine the forms and plant availability of residual fertiliser P in the soil and the chemical nature of soil organic P.

Soil P fractionation was used to examine the changes in soil P which occurred as a result of long-term P fertiliser additions to irrigated pasture in a field trial at Winchmore in Canterbury. The soil P fractionation scheme adapted for use in this study involved sequential extractions of the soil with 0.5M $NaHCO_3$ @ pH 8.5 ($NaHCO_3$ P), 0.1M NaOH (NaOH I P), 1M HCl (HCl P) and 0.1M NaOH (NaOH II P). This P fractionation scheme was similar to that developed by Stewart *et al.* (1980) except that a second 0.1M NaOH extract (NaOH II) was included following the acid extraction so as to increase the amounts of P extracted.

Soil samples collected from the long-term field trial at Winchmore were used to follow changes in soil P with time. This trial was initiated in

1952 and comprised 5 different treatments: control (no P applied since 1952), 376R (376 kg superphosphate ha^{-1} yr^{-1} 1952-1957, none since 1957), 564R (564kg superphosphate ha^{-1} yr^{-1} 1952-57, none since 1957), 188PA (188kg superphosphate ha^{-1} yr^{-1} since 1952), 376PA (376kg superphosphate ha^{-1} yr^{-1} since 1952). Only topsoil (0-7.5cm) samples from these different treatments in 1958, 1961, 1965, 1968, 1971, 1974 and 1977 were used in this study.

The results obtained showed that long-term P fertiliser additions increased levels of inorganic P in soils. In the annually fertilised treatments (188PA, 376PA) significant increases in soil inorganic P occurred between 1958 and 1977. As expected from the respective rates of P fertiliser applied, the overall increase in total soil inorganic P between 1958 and 1977 was greater in the 376PA treatment (159 μg P g^{-1}) than that in the 188PA treatment (37 μg P g^{-1}). In the 188PA treatment, the average annual rate of total soil inorganic P accumulation was markedly greater between 1971 and 1977 (4.6 μg P g^{-1}) than between 1958 and 1971 (0.7 μg P g^{-1}). This was related to the decreasing rate of soil organic P accumulation in the 188PA treatment with time.

The chemical nature of the soil inorganic P which accumulated in the annually fertilised treatments changed with time. Before 1971, most of the increases in soil inorganic P in the annually fertilised treatments occurred in the $NaHCO_3$ and

and NaOH I P fractions whereas after 1971 increases in soil inorganic P in these treatments were found mainly in the HCl and NaOH II P fractions. This was attributed to the combined effects of lime addition in 1972 and the presence of increased quantities of sparingly soluble apatite P and iron-aluminium P in the single superphosphate used after 1970.

Significant decreases in all of the inorganic P fractions studied (i.e. $NaHCO_3$ P, NaOH I P, HCl P, NaOH II P) were observed in the residual fertiliser treatments (376R, 564R) between 1958 and 1977. In spite of the cessation of P fertiliser inputs in 1957 in the residual fertiliser treatments, levels of these inorganic soil P fractions did not decrease to those found in the control treatment. This result was attributed to the accumulation of inorganic P from P fertiliser applied between 1952 and 1957 in forms which were stable and only became slowly available to plants.

Long-term P fertiliser additions also affected levels of organic P in soils. The results obtained showed that significant increases in soil organic P occurred in all treatments between 1958 and 1971 probably due to biological conversion (immobilisation) of native and fertiliser inorganic P to organic P via plant, animal and microbial residues. Overall net increases in soil organic P between 1958 and 1971

were greater in the annually fertilised (188PA, 376PA) treatments (70-86 μg P g^{-1}) than in the residual fertiliser (376R, 564R) treatments (55-64 μg P g^{-1}) or the control (34 μg P g^{-1}). This was attributed to the greater levels of pasture production in the annually fertilised treatments than those in the residual fertiliser or control treatments. In all treatments, most increases in soil organic P between 1958 and 1971 occurred in the NaOH I P fraction which represented the largest organic P fraction in the Winchmore soils.

In all treatments, rates of soil organic P accumulation between 1958 and 1971 decreased with time. For example, the average annual changes in total soil organic P in the control, residual fertiliser (376R, 564R) and annually fertilised (188PA, 376PA) treatments between 1958 and 1965 were +5.7, +7.3 and +7.1 μg P g^{-1} yr^{-1} respectively, compared with -1.0, +1.4 and +4.7 μg P g^{-1} yr^{-1} between 1965 and 1971. This was attributed to a steady-state condition with regard to soil organic P accumulation being reached in each treatment.

In the residual fertiliser treatments (376R, 564R), in which P fertiliser additions were stopped in 1957, soil organic P continued to increase between 1958 and 1971 while levels of soil inorganic P and pasture production declined. These results were attributed to the organic P which accumulated in the Winchmore pasture soils from P fertiliser additions

being more stable and less available to plants than inorganic forms of P.

The addition of lime (4 t ha^{-1}) in 1972 was found to affect the levels and chemical nature of soil organic P. Between 1971 and 1974, significant decreases in soil organic P occurred in all treatments. Overall decreases in total soil organic P due to liming were greater in the control (20 µg P g^{-1}) and the residual fertiliser treatments (23-38 µg P g^{-1}) than in the annually fertilised treatments (10-14 µg P g^{-1}). These decreases in soil organic P were attributed to increased mineralisation as a result of lime addition and probably accounted for the observed cessation of further net soil organic P accumulations in the different treatments after 1971.

Lime addition in 1972 also affected the chemical nature of soil organic P. This was shown by large decreases in the NaOH I organic P fraction (78-88 µg P g^{-1}) and the concomitant though smaller increases in the NaOH II organic P fraction (53-65 µg P g^{-1}) in all treatments which occurred between 1971 and 1974.

The chemical nature of soil organic P in the Winchmore long-term trial was examined. This involved quantitative extraction of organic P from the soil using sequential treatments with 0.1M NaOH, 0.5M HCl, 0.2M acetylacetone @ pH 8.3, 0.5M HCl and 0.5M NaOH. Topsoil (0-7.5cm) samples taken from the control and 376PA annually fertilised treatments of the

long-term trial in 1958, 1971 and 1983 were used for this study.

Preliminary results showed that no organic P was found in the HCl extracts and consequently these extracts were discarded. The amount of total organic P extracted by the sequential extraction procedure accounted for 80-87% (mean 84%) of the total soil organic P as determined by the ignition method.

The component alkali extracts (i.e. 0.1M NaOH, acetylacetone, 0.5M NaOH) from the control and 376PA soils sampled in 1958, 1971 and 1983 were combined for examining the chemical nature of extracted soil organic P. In the 376PA soil sampled in 1983 individual component alkali extracts were also examined separately.

Prior to examining the chemical nature of the extracted organic P by ^{31}P nuclear magnetic resonance (NMR) spectroscopy and gel filtration chromatography it was necessary to concentrate the extracts. This was achieved by the ultrafiltration technique using a membrane with a low molecular weight exclusion limit of 500. The results showed that only a small proportion (3-6%) of the total organic P in the soil extracts was lost during ultrafiltration and the technique increased total P concentrations in the extracts from 6-23 μg P g^{-1} mL to 230-820 μg P ml^{-1}.

The major forms of organic P identified in the concentrated soil extracts by ^{31}P NMR analysis

were orthophosphate monoesters ($RO.PO_4^{2-}$) and orthophosphate diesters ($R\ O.RO.PO_2^{-}$), although some of the pyrophosphate P ($P_2O_7^{4-}$) detected may have been present as organic ester forms in the soil which were hydrolysed during alkali extraction. In addition, choline phosphate ($(CH_3)_3N^+CH_2CH_2OPO_3^{2-}$) was resolved within the orthophosphate monoester P fraction of the acetylacetone and 0.5M NaOH extracts of the 376PA soil sampled in 1983 and accounted for 17-25% of the total organic P present in these extracts.

Quantitative ^{31}P NMR analysis of the combined alkali extracts showed that most (88-94%) of the total organic P in the Winchmore soils was present in the orthophosphate monoester P fraction, while the orthophosphate diester P and pyrophosphate P fractions made up only 3-7% and 3-5% of the total organic P respectively. Furthermore comparison between the control and 376PA soils sampled in 1971 showed that orthophosphate monoester P accounted for almost all (99%) of the increases in soil organic P which occurred as a result of continued annual applications of P fertiliser between 1952 and 1971. The predominance of orthophosphate monoester P, which are known to include P species such as inositol polyphosphates which mineralise very slowly in the soil, showed that organic P in the Winchmore soils was very stable.

The molecular weight distribution of organic matter and organic P in the concentrated soil extracts was examined using Sephadex G-100 gel which has

a nominal fractionation range of 1,000-100,000. The molecular weight distribution of organic components in all the extracts studied (i.e. 1958-control, 1958-376PA, 1971-control, 1971-376PA, 1983-control, 1983-376PA) was similar. Thus, organic matter and organic P were resolved in the gel in similar elution volumes and each showed 2 distinct peaks. These peaks were: (i) a peak eluted from the column in the void volume (kd = 0) which represented high molecular weight material (>100,000MW), and (ii) a peak eluted between the void and total column volumes (kd 0.81-0.92) representing low molecular weight material (<100,000MW). Results obtained showed that most (61-83%) of total organic P in the Winchmore soils was present in the low molecular weight fraction (<100,000MW), while the high molecular weight forms (>100,000MW) accounted for 17-39% of the total organic P. However, the proportion of high molecular weight organic P in the control and annually fertilised soils increased with time from 17-19% in 1958 to 38-39% in 1983. This shift towards high molecular weight forms of organic P in the soil with time was possibly due to microbial humification processes resulting in the formation of stable organic macromolecules.

The difference in organic P between the control and annually fertilised soils was assumed to represent the portion of organic P derived from fertiliser P and as such the results showed that in soils sampled in 1971 and 1983 this organic P was evenly distributed

between high and low molecular weight forms.

An exhaustive pot trial was conducted to examine the relative availability to plants of different forms of inorganic and organic P present in long-term fertilised soils under pasture. Three Lismore silt loam (Udic Ustochrept) soils (0-7.5cm) which had received different amounts of P fertiliser over the previous 30-80 years were used in this study. Two of these soils were taken from the annually fertilised treatments in the Winchmore long-term trial, namely the 188PA (188 kg superphosphate ha^{-1} yr^{-1} 1952-1982) and 376PA (376 kg superphosphate ha^{-1} yr^{-1} 1952-1982) treatments. The third soil (Fairton) was taken from a pasture adjacent to a meatworks near Winchmore which had been irrigated with works effluent (65 kg P ha^{-1} yr^{-1}) for over 80 years.

The three treatments used in the pot trial were control, N100 and N200. The N100 and N200 treatments consisted of adding nutrient solutions containing N, K, S, Ca, Mg, Cu and Zn with equivalent rates of 100 and 200 kg N ha^{-1} on an area basis for the N100 and N200 treatments respectively. Three successive crops of perennial ryegrass (Lolium perenne) were taken. Each crop consisted of 3 successive harvests followed by root harvest before the soil was mixed and ryegrass seeds were resown. Thus, a total of 9 tops harvests and 3 root harvests were taken.

The soil P fractionation scheme used in this experiment was the same as that used in the Winchmore

long-term trial study ~~and~~ i.e. sequential extractions of the soil with 0.5M $NaHCO_3$ @ pH 8.5 ($NaHCO_3$ P), 0.1M NaOH (NaOH I P), 1M HCl (HCl P) and 0.1M NaOH (NaOH II P).

As expected, within each treatment (control, N100, N200), dry matter yields and P uptake were greater in the high P status Fairton soil than in the lower P status Winchmore soils (188PA, 376PA). Furthermore, within each soil (188PA, 376PA, Fairton), dry matter yields and P uptake were significantly greater in the N100 and N200 treatments than those in the control.

Determination of amounts of P in the different inorganic P fractions in the original (uncropped) soils and those after the third cropping showed that significant decreases occurred in the $NaHCO_3$ P and NaOH I P fractions. In the control, N100 and N200 treatments in the 3 soils studied the average relative decreases in the $NaHCO_3$ and NaOH I inorganic fractions were 34% (18-47%) and 16% (10-23%) respectively. Corresponding decreases in HCl P and NaOH II inorganic P were generally small (<10%) and not significant, except in the N200 treatment in the Fairton soil where a significant decrease (16%) in HCl P occurred. The latter was attributed to the significantly low pH of the Fairton N200 soil (5.1) after cropping compared with that of the original soil (6.2) or those of the control (5.7) and N100 (5.5) treatments.

In general, the removal of P from the soil by 3 successive crops of ryegrass had little significant effect on amounts of P in the different organic P fractions in all the soils studied. Thus, net soil organic P mineralisation did not appear to contribute significantly to plant P uptake in the short-term, even under the P deficient conditions observed in the N100 and N200 treatments in the Winchmore soils.

The effects of different soil management practices on the availability to plants of different forms of P present in long-term fertilised pasture soils were also examined in a short-term field trial. The trial was conducted using existing plots of the long-term Winchmore experiment which had received annual additions of P fertiliser. In each of these long-term fertiliser treatments, 20 small plots (each 1 x 5m) were subjected to 5 treatments consisting of control, 2 rates of lime (2 and 4 t ha^{-1}), urea fertiliser (400 kg N ha^{-1}) and cultivation. The latter treatment involved mechanical cultivation of the topsoil (0-7.5cm) following which the plots were kept fallow for 18 months. The small plot trial areas were ungrazed and received no P fertiliser over the 2 year duration of the experiment. The above-ground herbage in the uncultivated treatments (control, lime, nitrogen) was harvested on 11 occasions between December 1981 and December 1983 and at each harvest topsoil samples (0-7.5cm) were taken from the uncultivated and cultivated plots for P analysis.

The soil P fractionation used involved sequential extractions of field moist soil with 0.5M $NaHCO_3$ @ pH 8.5 ($NaHCO_3$ P), 0.1M NaOH (NaOH P), ultrasonification in 0.1M NaOH (sonicate-NaOH) and 1M HCl (HCl P). In addition, soil microbial P was determined on separate samples using a chloroform-bicarbonate technique.

Results of soil P analysis showed that liming caused significant decreases in amounts of organic P in the NaOH P fraction in both the long-term fertilised main plots. Decreases in NaOH organic P due to liming were greater in the 188PA treatment soil (18-21 μg P g^{-1}) than those in the 376PA treatment soil (10-13 μg P g^{-1}). These decreases were attributed to increased microbial mineralisation of organic P in the limed soils. The results were supported by small significant increases in soil microbial P in the limed soils. However, this increased soil organic P mineralisation led to small but insignificant increases in dry matter yield and P uptake.

Significant increases in dry matter yield and P uptake occurred in the nitrogen treatments compared with those in the controls. However, this did not significantly affect the amounts of P in the different soil P fractions.

Mechanical cultivation of the soil and the subsequent fallow period significantly increased amounts of inorganic P in the $NaHCO_3$ and NaOH fractions

compared with those in the control treatments. In samples taken in December 1982, 1 year after cultivation, when the amounts of inorganic P in the $NaHCO_3$ and NaOH P fractions were added together for each treatment, differences between the cultivated and control treatments were 40 μg P g^{-1} and 68 μg P g^{-1} in the 188PA and 376PA treatments respectively. These higher levels of inorganic P in the cultivated soils were attributed mainly to P released from microbial decomposition of plant residues. Levels of soil microbial P were significantly lower in the cultivated treatments than those of the uncultivated treatments. This difference was attributed to the absence of roots and their exudates and debris containing energy-rich carbonaceous substrates.

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APPENDIX 3.1. Amounts (μgPg^{-1}) of P in the $NaHCO_3$ P fraction in soils (0-7.5cm) from the different treatments in the Winchmore long-term trial sampled in 1958, 1961, 1965, 1968, 1971, 1974 and 1977.

	YEAR													
	1958		1961		1965		1968		1971		1974		1977	
Treatment	IP	OP	IP	OP	IP	OP	IP	OP	IP	OP	IP	OP	IP	OP
Control	16.6	41.8	17.8	46.0	19.5	50.0	18.5	43.5	15.6	42.5	20.0	43.5	20.5	41.2
	(1.1)	(1.6)	(0.8)	(1.9)	(0.5)	(2.2)	(1.1)	(1.1)	(1.7)	(1.1)	(1.2)	(4.7)	(2.3)	(3.0)
188PA	19.5	48.2	24.5	53.8	26.0	60.2	26.8	58.6	27.2	56.0	24.5	59.0	26.5	59.0
	(1.1)	(2.3)	(0.5)	(1.8)	(1.9)	(0.8)	(1.2)	(2.2)	(1.5)	(1.0)	(1.6)	(3.2)	(1.8)	(2.5)
376PA	25.2	47.7	38.0	53.8	43.8	61.6	47.5	56.0	49.0	56.0	45.8	64.8	47.5	71.2
	(1.9)	(3.4)	(2.9)	(1.1)	(1.5)	(3.9)	(5.5)	(0.2)	(3.4)	(1.2)	(8.5)	(4.3)	(5.8)	(2.9)
376R	26.0	51.0	21.5	51.0	22.2	56.0	21.2	49.9	20.0	49.4	22.0	49.8	22.8	50.8
	(0.7)	(1.1)	(1.5)	(4.2)	(0.8)	(3.7)	(1.7)	(0.6)	(0)	(1.5)	(1.0)	(3.1)	(2.2)	(4.0)
564R	35.5	49.5	28.8	58.0	27.0	64.4	24.5	54.3	23.0	53.5	23.5	50.3	21.0	54.9
	(3.6)	(2.4)	(0.8)	(6.1)	(1.2)	(3.3)	(2.3)	(2.8)	(1.2)	(5.0)	(1.1)	(4.1)	(1.6)	(3.1)

Figures in parenthesis are standard deviations on the mean.

IP = inorganic P ; OP = organic P.

APPENDIX 3.2 Amounts (μgPg^{-1}) of P in the NaOH I P fraction in soils (0-7.5cm) from the different treatments in the Winchmore long-term trial sampled in 1958, 1961, 1965, 1968, 1971, 1974 and 1977.

	YEAR													
	1958		1961		1965		1968		1971		1974		1977	
Treatment	IP	OP	IP	OP	IP	OP	IP	OP	IP	OP	IP	OP	IP	OP
Control	110.8	207.9	107.5	209.0	107.8	229.0	98.0	226.8	94.6	236.9	87.8	151.0	89.0	160.5
	(3.9)	(4.3)	(2.3)	(4.6)	(3.9)	(17.1)	(2.4)	(9.3)	(3.5)	(6.1)	(3.8)	(3.7)	(5.3)	(8.4)
188PA	127.5	223.5	129.2	228.8	130.3	227.0	131.5	264.6	129.0	261.8	119.3	183.8	128.8	202.5
	(7.6)	(4.0)	(5.0)	(11.4)	(6.8)	(9.4)	(3.8)	(8.8)	(5.6)	(5.4)	(7.0)	(10.8)	(6.5)	(11.3)
376PA	140.5	210.3	161.2	212.3	173.5	234.0	189.0	249.5	194.8	263.0	177.0	183.8	189.3	197.8
	(7.2)	(9.3)	(8.7)	(9.3)	(3.4)	(3.7)	(14.6)	(4.4)	(9.8)	(5.3)	(14.2)	(13.3)	(15.2)	(11.3)
376R	142.5	209.3	123.0	229.0	120.5	239.0	113.6	253.3	108.5	257.3	106.3	175.5	105.3	182.5
	(5.4)	(18.2)	(9.2)	(12.0)	(3.6)	(14.1)	(4.7)	(5.4)	(5.0)	(15.8)	(9.9)	(10.4)	(8.4)	(11.1)
564R	171.5	211.5	147.1	223.3	136.7	241.7	122.2	255.8	113.5	263.8	110.5	175.8	112.8	187.8
	(18.1)	(10.4)	(13.1)	(16.5)	(7.6)	(8.8)	(14.9)	(11.2)	(10.6)	(11.0)	(18.0)	(11.0)	(9.4)	(9.5)

Figures in parenthesis are standard deviations on the mean.

IP = inorganic P ; OP = organic P.

APPENDIX 3.3 Amounts (μgPg^{-1}) of inorganic P in the HCl P fraction from soils (0-7.5cm) in the different treatments in the Winchmore long-term trial sampled in 1958, 1961, 1965, 1968, 1971, 1974 and 1977.

	YEAR						
Treatment	1958	1961	1965	1968	1971	1974	1977
Control	29.6	24.8	23.3	26.0	22.7	32.5	31.0
	(1.1)	(1.5)	(0.4)	(1.9)	(2.0)	(2.1)	(1.9)
188PA	37.8	31.0	32.0	36.7	42.5	54.0	58.0
	(1.5)	(2.3)	(3.9)	(1.7)	(5.0)	(4.2)	(2.9)
376PA	40.8	45.5	49.0	57.5	63.0	85.0	103.0
	(1.9)	(5.7)	(4.4)	(5.0)	(2.4)	(6.0)	(10.7)
376R	45.2	31.2	32.3	30.7	29.8	36.8	36.0
	(1.3)	(1.3)	(3.1)	(2.2)	(1.8)	(0.8)	(1.9)
564R	54.0	42.3	39.3	35.5	33.5	43.8	40.0
	(7.3)	(5.3)	(4.8)	(5.4)	(4.3)	(6.4)	(5.7)

Figures in parenthesis are standard deviations on the mean.

APPENDIX 3.4 Amounts ($\mu g P g^{-1}$) of P in the NaOH II P fraction in soils (0-7.5cm) from the different treatments in the Winchmore long-term trial sampled in 1958, 1961, 1965, 1968, 1971, 1974 and 1977.

	YEAR													
	1958		1961		1965		1968		1971		1974		1977	
Treatment	IP	OP	IP	OP	IP	OP	IP	OP	IP	OP	IP	OP	IP	OP
Control	93.0	97.5	80.5	104.0	81.0	108.0	83.0	102.5	79.9	101.3	90.5	165.8	84.3	159.3
	(3.3)	(4.2)	(2.1)	(5.1)	(0.7)	(7.5)	(3.7)	(6.8)	(1.4)	(2.8)	(1.1)	(6.5)	(1.9)	(5.6)
188PA	98.5	99.0	89.8	111.5	87.5	119.0	95.3	118.8	93.8	122.8	103.3	183.5	107.0	178.0
	(5.2)	(7.6)	(6.7)	(6.2)	(3.8)	(7.0)	(4.6)	(5.9)	(1.5)	(2.9)	(3.4)	(2.5)	(3.4)	(8.4)
376PA	96.2	101.5	91.5	121.5	98.2	130.3	104.5	119.5	110.2	128.3	114.5	188.5	122.3	180.3
	(3.1)	(4.2)	(3.2)	(9.3)	(1.8)	(11.6)	(4.0)	(10.2)	(3.2)	(5.4)	(5.9)	(11.0)	(7.0)	(7.2)
376R	105.0	111.3	95.4	118.8	91.0	117.8	86.9	117.5	85.8	116.3	96.3	174.5	97.3	167.5
	(4.1)	(7.3)	(3.1)	(6.2)	(3.5)	(6.2)	(3.8)	(5.7)	(2.9)	(3.3)	(4.6)	(9.8)	(5.5)	(17.4)
564R	105.8	103.3	96.0	117.8	92.4	117.4	90.2	110.3	89.0	112.8	98.8	165.5	95.3	167.8
	(8.2)	(9.4)	(3.6)	(5.7)	(4.2)	(6.6)	(7.3)	(7.9)	(5.4)	(6.0)	(6.8)	(11.2)	(8.7)	(10.8)

Figures in parenthesis are standard deviations on the mean.

IP = inorganic P ; OP = organic P

APPENDIX 3.5 Amounts (μgPg^{-1}) of total extracted inorganic and organic P in soils (0-7.5cm) from the different treatments in the Winchmore long-term trial sampled in 1958, 1961, 1965, 1968, 1071, 1974 and 1977.

	YEAR													
	1958		1961		1965		1968		1971		1974		1977	
Treatment	IP	OP	IP	OP	IP	OP	IP	OP	IP	OP	IP	OP	IP	OP
Control	250.0	347.1	230.8	359.1	231.5	387.0	225.5	372.8	214.0	380.7	230.8	360.3	224.8	361.0
	(2.7)	(7.5)	(5.9)	(15.4)	(3.5)	(26.0)	(7.2)	(14.4)	(7.0)	(5.8)	(5.4)	(8.7)	(6.5)	(9.5)
188PA	283.3	370.8	274.5	394.0	275.0	406.1	290.3	442.0	289.2	440.5	298.3	426.3	319.8	439.6
	(14.4)	(7.2)	(13.5)	(19.7)	(14.6)	(10.4)	(10.0)	(16.1)	(9.5)	(5.5)	(10.3)	(16.3)	(12.3)	(18.0)
376PA	302.8	361.3	340.8	387.5	361.3	425.9	398.5	425.3	417.0	447.3	422.3	437.0	462.0	449.3
	(10.8)	(9.9)	(11.1)	(5.4)	(6.0)	(7.0)	(26.6)	(11.8)	(13.3)	(9.0)	(28.3)	(28.3)	(35.5)	(20.1)
376R	318.8	368.3	266.3	396.5	265.0	412.8	252.3	420.7	244.0	423.0	261.3	399.8	261.3	400.8
	(10.8)	(20.0)	(12.3)	(21.6)	(7.7)	(18.7)	(10.7)	(11.1)	(7.6)	(15.1)	(14.4)	(21.4)	(15.7)	(26.7)
564R	366.8	365.8	313.7	399.3	295.8	423.7	272.4	420.6	259.0	430.1	276.3	391.5	269.0	410.3
	(36.3)	(12.4)	(22.5)	(6.3)	(16.5)	(16.3)	(29.6)	(20.5)	(20.9)	(18.7)	(29.3)	(18.1)	(24.4)	(14.5)

Figures in parenthesis are standard deviations on the mean.

IP = inorganic P ; OP = organic P

APPENDIX 3.6 Amounts (μgPg^{-1}) of residual (non-extracted) P in soils (0-7.5cm) from the different treatments in the Winchmore long-term trial sampled in 1958, 1961, 1965, 1968, 1971, 1974 and 1977.

	YEAR						
Treatment	1958	1961	1965	1968	1971	1974	1977
Control	65.7 (10.2)	71.8 (8.3)	62.3 (9.7)	62.5 (11.0)	72.3 (7.1)	63.0 (9.9)	63.9 (13.7)
188PA	76.2 (6.9)	74.4 (5.3)	67.9 (10.1)	73.5 (8.9)	84.0 (9.2)	77.6 (8.1)	70.8 (6.9)
376PA	72.3 (7.1)	76.6 (11.1)	72.3 (4.9)	78.7 (11.0)	79.0 (8.3)	71.8 (6.5)	73.4 (11.1)
376R	67.2 (9.4)	70.1 (7.3)	76.4 (9.8)	67.9 (7.7)	68.8 (16.0)	64.6 (7.4)	66.5 (12.4)
564R	76.7 (10.6)	74.3 (6.5)	70.8 (10.4)	74.8 (9.4)	73.9 (8.2)	69.1 (10.3)	54.4 (5.1)

Figures in parenthesis are standard deviations on the mean.

APPENDIX 5.1 Amounts (μgPg^{-1}) of P in the different P fractions in the original (uncropped) 188PA, 376PA and Fairton soils and those after the 3rd cropping in the Control, N100 and N200 treatments in the exhaustive pot trial.

		SOIL P FRACTION										
		$NaHCO_3$		NaOH I		HCl	NaOH II		Residual	Total Extracted		total
Soil	Treatment	IP	OP	IP	OP		IP	OP		IP	OP	
188PA	Original	29.5 (1.1)	43.8 (2.6)	122.8 (10.3)	226.0 (6.3)	83.2 (9.2)	110.5 (2.6)	175.8 (3.3)	82.3 (3.7)	346.1 (16.8)	445.6 (6.5)	874.0 (19.7)
	Control	23.5 (1.5)	43.7 (2.3)	108.7 (2.2)	214.3 (2.7)	85.3 (13.0	108.0 (3.4)	188.5 (1.5)	86.3 (5.9)	325.3 (17.8)	446.7 (5.0)	858.3 (22.0)
	N100	20.2 (1.1)	44.0 (5.0)	103.0 (5.6)	225.8 (8.8)	74.7 (7.0)	104.6 (3.1)	181.3 (4.5)	83.5 (4.5)	303.6 (6.8)	453.4 (7.9)	840.5 (13.1)
	N200	21.5 (1.1)	50.5 (5.6)	108.7 (6.9)	223.5 (2.2)	66.6 (16.0)	102.8 (3.3)	173.8 (5.7)	84.5 (4.2)	299.9 (11.2)	447.8 (10.6)	832.2 (6.4)
376PA	Original	54.0 (0.7)	44.2 (1.5)	188.3 (3.3)	236.0 (10.3)	151.5 (23.3)	127.5 (4.3)	180.8 (10.0)	96.5 (2.9)	521.3 (26.4)	461.1 (1.2)	1078.9 (24.9)
	Control	39.2 (1.5)	43.8 (1.5)	158.7 (3.3)	223.4 (9.2)	142.2 (4.8)	130.5 (1.3)	195.9 (3.7)	92.8 (3.3)	476.6 (6.5)	463.2 (11.4)	1032.6 (11.0
	N100	31.6 (1.1)	44.7 (3.3)	154.5 (6.1)	224.5 (6.7)	144.4 (20.5)	125.6 (4.0)	190.8 (4.4)	95.5 (7.1)	455.6 (27.0)	459.9 (6.6)	1011.0 (29.1)
	N200	30.8 (1.3)	47.0 (2.4)	144.5 (6.4)	237.0 (11.1)	146.8 (28.6)	125.0 2.3)	180.5 (6.8)	96.0 (2.9)	449.6 (28.0)	464.5 (18.0)	1010.1 (34.6)
Fairton	Original	243.5 (2.7)	49.3 (2.5)	573.8 (5.8)	241.9 (5.5)	254.5 (8.9)	172.0 (3.6)	192.2 (4.3)	174.4 (4.4)	1243.6 (16.8)	483.4 (9.6)	1901.7 (21.6)
	Control	170.0 (9.0)	57.3 (3.5)	517.6 (4.5)	254.0 (9.2)	238.8 (12.8)	176.8 (4.4)	208.8 (6.3)	168.3 (6.8)	1103.4 (22.1)	520.5 (16.0)	1792.2 (10.2)
	N100	137.0 (8.5)	53.4 (5.3)	475.8 (18.8)	252.5 (12.2)	239.0 (11.2)	169.3 (4.4)	209.8 (6.4)	176.0 (10.6)	1021.3 (25.1)	515.8 (20.0)	1713.1 (32.4)
	N200	129.3 (6.8)	51.5 (2.6)	464.7 (12.3)	262.3 (6.6)	214.5 (5.7)	166.7 (2.4)	205.8 (12.4)	172.8 (11.6)	975.3 (20.9)	519.6 (20.7)	1667.7 (127.5)

Figures in parenthesis are standard deviations on the mean.

IP = inorganic P ; OP = organic P.

APPENDIX 6.1 Amounts (μgPg^{-1}) of P in the $NaCO_3$ inorganic P fraction in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.

		SAMPLE DATE										
Soil	Treatment	12/81	1/82	3/82	5/82	7/82	10/82	12/82	2/83	4/83	9/83	12/83
188PA	Control	22.1 (1.1)	22.8 (1.9)	15.5 (0.9)	19.5 (1.1)	19.7 (0.8)	15.0 (0.7)	21.0 (0.7)	17.0 (1.2)	20.2 (0.8)	16.0 (2.3)	15.2 (2.2)
	L1	24.7 (2.3)	24.0 (3.2)	16.3 (0.8)	19.0 (1.9)	20.8 (1.9)	16.3 (1.1)	23.3 (1.2)	18.7 (1.3)	20.8 (2.6)	14.2 (1.5)	15.2 (2.1)
	L2	27.5 (2.7)	21.0 (1.9)	16.7 (0.4)	19.2 (0.4)	20.5 (0.9)	16.5 (1.1)	22.7 (0.8)	18.3 (0.4)	20.3 (1.5)	14.8 (0.8)	15.2 (1.8)
	N	22.8 (1.4)	20.8 (2.0)	16.2 (1.1)	18.5 (2.3)	19.0 (1.4)	15.8 (1.3)	21.1 (2.2)	16.5 (2.2)	18.6 (0.5)	12.4 (0.9(	14.7 (1.7)
	CR	24.2 (3.7)	26.3 (1.8)	21.5 (1.5)	25.8 (2.3)	30.5 (1.8)	25.5 (6.3)	31.5 (5.0)	28.5 (2.5)	28.7 (2.2)	25.2 (1.9)	25.3 (3.7)
376PA	Control	41.3 (2.9)	47.8 (8.4)	32.0 (1.6)	37.5 (2.9)	35.5 (1.5)	27.2 (2.3)	34.5 (2.1)	27.5 (0.5)	30.5 (4.0)	20.0 (0.7)	27.4 (3.4)
	L1	41.5 (5.0)	45.0 (5.2)	36.5 (2.1)	41.8 (5.9)	47.5 (6.8)	32.8 (1.9)	40.3 (3.8)	31.0 (1.9)	39.8 (5.4)	25.5 (5.5)	23.4 (4.1)
	L2	51.8 (5.1)	50.0 (11.2)	40.0 (10.0)	41.0 (7.6)	45.3 (7.3)	32.0 (4.5)	40.5 (7.5)	33.0 (7.5)	38.0 (6.1)	26.0 (7.4)	29.0 (3.4)
	N	42.5 (7.7)	46.5 (2.1)	32.5 (3.9)	33.0 (2.7)	38.0 (5.5)	25.5 (0.9)	31.8 (2.0)	26.3 (2.3)	26.4 (1.8)	19.3 (0.4)	24.6 (3.7)
	CR	37.8 (5.4)	50.5 (4.4)	44.1 (4.9)	50.8 (4.6)	50.9 (4.2)	51.5 (4.5)	63.8 (1.8)	57.8 (4.3)	58.0 (2.5)	44.9 (5.5)	38.0 (5.4)

Figures in parenthesis are standard deviations on the mean.

APPENDIX 6.2 Amounts (μgPg^{-1}) of P in the NaOH inorganic P fraction in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.

		SAMPLE DATE										
Soil	Treatment	12/81	1/82	3/82	5/82	7/82	10/82	12/82	2/83	4/83	9/83	12/83
188PA	Control	95.1 (8.1)	111.0 (8.0)	103.3 (7.2)	96.6 (5.1)	102.2 (6.5)	110.8 (7.9)	121.5 (7.2)	103.2 (6.2)	103.5 (5.3)	104.3 (2.5)	94.1 (5.0)
	L1	99.5 (10.5)	112.5 (10.7)	97.3 (8.9)	94.0 (10.1)	100.0 (7.8)	104.0 (10.4)	116.4 (9.4)	97.3 (7.8)	98.3 (6.0)	90.5 (5.2)	86.1 (5.6)
	L2	105.3 (8.3)	107.3 (9.7)	99.8 (4.9)	92.3 (6.4)	95.8 (4.9)	104.8 (1.9)	109.0 (5.5)	93.7 (7.8)	89.5 (3.2)	88.0 (6.2)	81.6 (3.2)
	N	100.3 (9.5)	112.0 (6.7)	101. 8 (7.4)	92.5 (11.1)	102.7 (4.1)	109.8 (7.2)	125.5 (9.4)	105.8 (5.4)	102.8 (9.4)	99.0 (1.6)	103.1 (5.6)
	CR	94.7 (11.4)	111.3 (6.2)	107.8 (4.9)	107.8 (5.4)	122.5 (4.7)	132.9 (9.1)	150.6 (7.8)	130.8 (5.0)	125.3 (5.5)	116.8 (9.7)	116.3 (5.1)
376PA	Control	149.8 (4.4)	167.5 (15.9)	144.0 (3.7)	140.5 (6.3)	140.0 (4.3)	146.0 (4.6)	158.6 (11.4)	135.3 (2.2)	142.0 (11.4)	127.0 (9.8)	120.2 (7.8)
	L1	145.8 (14.0)	165.3 (3.3)	158.3 (19.2)	145.8 (9.0)	156.0 (6.5)	154.8 (7.0)	166.8 (10.2)	139.8 (9.1)	146.9 (10.2)	141.2 (14.8)	115.7 (8.2)
	L2	160.8 (12.0)	171.5 (20.5)	162.0 (24.4)	145.5 (19.8)	149.0 (7.2)	156.3 (11.3)	167.5 (12.5)	144.0 (17.0)	151.0 (19.5)	139.5 (26.9)	123.7 (8.2)
	N	152.3 (16.2)	173.0 (4.4)	148.0 (8.7)	133.8 (5.5)	148.5 (9.7)	152.3 (3.5)	159.0 (7.8)	139.3 (6.6)	139.0 (4.0)	134.6 (4.2)	126.7 (3.5)
	CR	138.3 (9.2)	161.3 (9.2)	155.0 (14.0)	159.3 (6.9)	174.9 (8.6)	184.3 (4.6)	196.8 (9.2)	188.5 (9.2)	186.0 (6.7)	176.1 (8.7)	168.9 (9.7)

Figures in parenthesis are standard deviations on the mean.

APPENDIX 6.3 Amounts ($\mu g P g^{-1}$) of P in the sonicate NaOH inorganic P in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.

		SAMPLE DATE										
Soil	Treatment	12/81	1/82	3/82	5/82	7/82	10/82	12/82	2/83	4/83	9/83	12/83
188PA	Control	25.6 (1.3)	28.5 (1.1)	34.8 (2.6)	33.4 1.6)	32.5 (3.5)	28.2 (1.3)	31.0 (1.2)	35.3 (1.8)	29.3 (1.1)	33.2 (1.9)	30.5 (1.1)
	L1	23.5 (1.5)	26.7 (2.7)	32.5 (2.3)	32.8 (2.2)	28.5 (1.5)	24.5 (2.7)	25.6 (1.3)	31.8 (1.8)	24.8 (1.9)	32.3 (0.8)	30.0 (0.9)
	L2	23.3 (2.1)	26.2 (4.7)	32.0 (4.5)	26.0 (2.2)	29.0 (1.2)	25.8 (1.9)	28.0 (4.1)	29.4 (1.6)	25.0 (1.6)	32.5 (2.6)	29.0 (0.9)
	N	24.0 (1.6)	27.7 (1.8)	32.0 (2.7)	32.2 (1.5)	29.8 (1.3)	24.5 (2.1)	29.0 (3.6)	32.2 (3.9)	26.7 (2.4)	31.8 (0.4)	30.1 (2.0
	CR	23.5 (2.3)	29.8 (1.3)	33.5 (1.1)	35.0 (2.1)	31.3 (1.3)	29.3 (1.6)	33.2 (3.1)	36.5 (2.3)	30.8 (2.2)	35.2 (3.1)	32.5 (1.1)
376PA	Control	32.2 (1.5)	33.7 (1.5)	35.0 (0.7)	36.5 (0.9)	33.5 (2.2)	28.7 (1.1)	33.2 (1.3)	38.5 (1.1)	32.8 (2.4)	38.5 (1.8)	31.7 (1.3)
	L1	32.0 (1.4)	31.2 (1.5)	35.3 (1.3)	34.0 (2.8)	34.5 (2.3)	27.8 (1.5)	31.6 (1.9)	36.0 (1.4)	32.4 (1.3)	38.7 (3.1)	29.8 (0.9)
	L2	30.5 (2.3)	29.0 (1.2)	35.0 (3.0)	33.2 (2.2)	33.0 (1.2)	26.5 (0.9)	28.2 (1.1)	36.8 (2.2)	33.0 (3.5)	36.7 (2.7)	27.2 (1.07)
	N	32.5 (2.5)	33.5 (1.5)	36.2 (0.8)	35.5 (2.3)	35.6 (0.9)	30.7 (1.1)	37.8 (1.3)	40.3 (1.6)	34.2 (2.9)	39.5 (2.3)	34.2 (2.3)
	CR	32.5 (1.6)	33.6 (1.6)	38.4 (1.1)	37.8 (1.8)	39.0 (1.2)	34.6 (1.8)	40.0 (1.0)	46.2 (1.8)	38.3 (0.4)	42.9 (3.0)	41.3 (2.2)

Figures in parenthesis are standard deviations on the mean.

APPENDIX 6.4 Amounts ($\mu g P g^{-1}$) of P in the HCl P fraction in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.

		SAMPLE DATE										
Soil	Treatment	12/81	1/82	3/82	5/82	7/82	10/82	12/82	2/83	4/83	9/83	12/83
188PA	Control	70.8 (10.1)	55.0 (13.5)	58.4 (8.5)	61.5 (5.6)	61.3 (5.0)	55.0 (2.8)	62.3 (6.2)	54.0 (6.7)	61.3 (5.1)	67.5 (5.0)	70.5 (4.0)
	L1	65.8 (7.8)	61.0 (8.7)	59.0 (8.6)	60.8 (9.5)	67.0 (7.7)	60.0 (10.8)	68.0 (7.6)	55.5 (2.2)	65.0 (8.9)	67.3 (8.8)	77.0 (4.1)
	L2	67.2 (10.4)	55.0 (3.7)	60.8 (9.1)	62.0 (8.1)	68.3 (2.5)	63.3 (1.9)	64.8 (3.8)	61.3 (4.4)	67.3 (2.9)	72.3 (1.8)	74.5 (4.8)
	N	66.2 (10.8)	55.3 (4.6)	61.5 (6.1)	58.3 (2.7)	68.0 (11.2)	60.5 (11.4)	62.0 (4.4)	58.0 (4.8)	60.8 (2.6)	64.8 (7.6)	62.0 (4.3)
	CR	66.0 (12.7)	60.5 (4.2)	58.3 (10.3)	59.5 (6.9)	65.0 (10.3)	58.3 (7.7)	60.8 (7.6)	55.6 (4.5)	62.0 (1.2)	69.8 (11.2)	65.2 (4.6)
376PA	Control	125.5 (11.4)	119.0 (24.9)	111.0 (10.1)	120.3 (8.6)	122.0 (5.2)	113.8 (8.6)	119.5 (8.5)	116.5 (9.4)	119.8 (8.4)	120.8 (3.7)	125.7 (7.2)
	L1	129.0 (6.7)	119.0 (11.1)	122.0 (8.2)	118.0 (9.8)	122.0 (8.3)	118.9 (11.4)	123.3 (10.8)	118.0 (9.3)	115.3 (9.4)	127.5 (9.6)	131.2 (9.9)
	L2	134.0 (8.0)	124.8 (17.6)	125.6 (25.1)	119.5 (20.9)	126.0 (10.8)	121.0 (15.6)	126.3 (10.6)	117.0 (9.4)	120.8 (14.7)	129.5 (14.8)	128.7 (8.7)
	N	136.8 (24.0)	124.3 (7.0)	117.9 (9.3)	120.3 (3.3)	128.9 (9.5)	116.8 (8.8)	115.9 (5.8)	116.5 (15.8)	113.6 (11.2)	124.3 (11.9)	121.7 (9.2)
	CR	117.3 (14.9)	122.8 (14.5)	121.3 (6.7)	113.6 (12.6)	134.0 (13.5)	112.8 (8.4)	120.4 (6.1)	120.6 (10.3)	116.8 (5.5)	128.2 (13.0)	124.9 (7.2)

Figures in parenthesis are standard deviations on the mean.

APPENDIX 6.5 Amounts (μgPg^{-1}) of P in the $NaHCO_3$ organic P fraction in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.

		SAMPLE DATE										
Soil	Treatment	12/81	1/82	3/82	5/82	7/82	10/82	12/82	2/83	4/83	9/83	12/83
188PA	Control	38.2 (5.4)	52.3 (4.3)	31.8 (1.1)	32.9 (0.7)	39.5 (1.7)	35.0 (1.6)	38.0 (2.3)	41.8 (1.5)	39.5 (1.5)	32.8 (2.7)	38.1 (3.0)
	L1	40.0 (5.4))	48.8 (3.6)	33.7 (3.9)	32.7 (1.6)	39.3 (1.1)	33.7 (1.9)	36.8 (1.5)	39.0 (1.2)	34.5 (2.9)	30.3 (1.3)	37.1 (2.7)
	L2	43.6 (1.3)	47.3 (4.4)	31.5 (2.6)	33.0 (0.7)	40.5 (0.9)	35.0 (2.2)	36.5 (0.9)	36.7 (1.3)	33.9 (2.5)	29.0 (0.7)	34.6 (2.6)
	N	44.3 (1.5)	47.0 (7.2)	31.2 (1.1)	33.5 (2.3)	40.2 (0.8)	33.2 (0.8)	33.7 (6.8)	40.4 (2.2)	37.3 (4.3)	30.8 (0.8)	39.1 (2.7)
	CR	40.3 (2.4)	44.5 (3.4)	30.7 (1.1)	34.5 (2.6)	40.5 (0.9)	35.3 (2.9)	40.7 (2.4)	42.9 (4.1)	42.0 (1.4)	32.2 (2.5)	36.5 (5.3)
376PA	Control	42.0 (7.7)	43.8 (1.3)	32.0 (0.7)	30.5 (2.3)	40.7 (0.8)	33.8 (1.1)	40.0 (1.2)	41.0 (1.7)	37.5 (5.1)	33.2 (2.4)	43.6 (2.8)
	L1	46.5 (2.1)	46.7 (1.6)	33.8 (2.5)	33.0 (1.9)	38.0 (4.5)	31.3 (3.1)	38.0 (2.1)	42.0 (1.2)	36.2 (4.4)	33.0 (1.2)	43.1 (1.6)
	L2	46.5 (2.9)	49.4 (2.5)	30.3 (1.9)	33.0 (0.8)	39.0 (2.2)	35.5 (2.1)	39.0 (2.1)	40.5 (1.6)	37.0 (1.0)	29.2 (1.8)	39.6 (1.7)
	N	47.3 (4.4)	50.0 (3.4)	35.5 (3.6)	31.6 (1.5)	38.3 (3.3)	34.0 (2.2)	40.8 (2.3)	42.6 (1.1)	42.3 (1.9)	31.8 (1.3)	43.1 (1.4)
	CR	46.5 (3.4)	48.5 (4.8)	29.8 (1.9)	29.3 (2.5)	36.8 (0.8)	29.1 (3.7)	40.0 (0.7)	44.0 (1.2)	32.8 (6.1)	33.6 (0.9)	45.0 (2.0)

Figures in parenthesis are standard deviations on the mean.

APPENDIX 6.6 Amounts (μgPg^{-1}) of P in the NaOH organic P fraction in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled netween December 1981 and December 1983.

Soil	Treatment	SAMPLE DATE 12/81	1/82	3/82	5/82	7/82	10/82	12/82	2/83	4/83	9/83	12/83
188PA	Control	230.4 (13.4)	228.5 (6.0)	237.5 (15.1)	243.3 (10.4)	224.0 (19.4)	249.5 (13.9)	230.0 (5.5)	232.3 (8.6)	234.0 (14.0)	225.8 (13.2)	240.5 (10.1)
	L1	216.5 (18.7)	216.3 (10.8)	231.8 (21.5)	225.8 (15.5)	204.8 (9.8)	230.8 (11.5)	212.3 (10.8)	211.8 (10.6)	216.8 (6.3)	192.0 (15.3)	219.0 (7.8)
	L2	225.8 (15.6)	219.3 (15.3)	217.3 (13.0)	223.3 (6.6)	203.0 (6.4)	227.5 (3.4)	208.8 (7.6)	194.8 (6.5)	200.5 (2.5)	188.6 (12.9)	207.0 (3.9)
	N	222.1 (12.1)	220.8 (12.1)	226.3 (11.2)	234.5 (7.3)	222.3 (9.6)	248.3 (11.2)	237.5 (15.8)	229.8 (9.0)	235.5 (9.6)	226.0 (5.1)	246.6 (4.8)
	CR	216.8 (16.3)	214.8 (6.4)	209.5 (14.2)	222.5 (3.8)	221.5 (9.9)	260.8 (13.9)	238.3 (6.6)	238.3 (9.5)	226.4 (6.4)	241.3 (13.1)	261.8 (7.5)
376PA	Control	225.5 (5.7)	225.0 (7.6)	224.0 (5.2)	241.0 (10.0)	225.0 (3.7)	236.8 (1.9)	225.3 (6.1)	228.0 (6.7)	226.5 (10.2)	221.8 (13.2)	229.3 (9.7)
	L1	228.0 (14.5)	215.3 (7.0)	228.3 (8.2)	237.8 (4.8)	221.8 (3.3)	230.0 (7.8)	212.6 (1.8)	216.0 (6.6)	218.6 (6.0)	216.3 (8.4)	223.8 (5.4)
	L2	220.8 (12.0)	225.8 (14.0)	222.8 (8.3)	239.5 (11.9)	219.8 (2.2)	235.3 (3.3)	214.8 (5.9)	217.0 (6.8)	211.3 (4.9)	218.9 (2.0)	217.8 (5.4)
	N	224.3 (13.8)	221.6 (8.2)	224.3 (8.4)	238.0 (12.2)	231.8 (9.5)	246.8 (10.3)	227.9 (8.6)	232.8 (3.8)	239.5 (8.4)	237.3 (10.4)	237.7 (19.3)
	CR	216.5 (8.1)	211.9 (12.8)	221.0 (7.5)	231.3 (14.2)	228.9 (4.1)	241.3 (5.1)	226.8 (1.3)	228.9 (11.8)	234.5 (8.7)	229.3 (6.0)	235.3 (14.2)

Figures in parenthesis are standard deviations on the mean.

APPENDIX 6.7 Amounts (μgPg^{-1}) of P in the sonicate NaOH organic P fraction in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.

		SAMPLE DATE										
Soil	Treatment	12/81	1/82	3/82	5/82	7/82	10/82	12/82	2/83	4/83	9/83	12/83
188PA	Control	38.7 (2.4)	36.5 (2.1)	44.2 (6.4)	37.8 (11.3)	37.2 (4.1)	34.2 (2.9)	33.8 (4.8)	40.5 (3.5)	33.0 (5.8)	43.3 (7.1)	44.2 (4.8)
	L1	35.3 (2.3)	39.8 (4.3)	39.8 (2.9)	36.0 (4.2)	34.8 (1,3)	31.8 (2.9)	31.5 (2.7)	36.6 (2.7)	30.8 (3.9)	34.8 (2.9)	40.7 (7.0)
	L2	33.4 (4.3)	38.5 (1.1)	38.0 (2.1)	37.8 (4.8)	31.4 (2.3)	33.0 (1.0)	29.7 (1.8)	36.0 (1.2)	28.7 (1.1)	38.8 (3.0)	36.2 (4.6)
	N	36.7 (2.8)	33.0 (4.5)	37.9 (2.4)	38.5 (2.3)	36.7 (3.5)	34.4 (2.5)	35.3 (2.6)	37.5 (1.8)	32.9 (1.3)	46.5 (8.5)	45.2 (4.6)
	CR	38.2 (4.4)	41.5 (7.4)	38.7 (5.1)	47.8 (1.8)	38.0 (6.1)	39.5 (2.9)	35.5 (2.5)	43.2 (3.4)	35.8 (3.3)	48.0 (1.6)	51.8 (4.8)
376PA	Control	34.8 (1.3)	36.3 (6.5)	35.8 (1.9)	45.2 (3.7)	36.5 (2.7)	27.8 (1.6)	26.2 (0.8)	30.8 (2.4)	30.0 (3.2)	45.0 (3.1)	40.1 (1.6)
	L1	32.3 (1.6)	29.8 (4.6)	36.7 (1.9)	41.5 (3.0)	31.3 (6.3)	24.0 (1.0)	24.5 (3.9)	30.2 (3.3)	27.1 (1.8)	37.8 (2.9)	35.6 (2.2)
	L2	31.0 (1.6)	29.3 (2.9)	35.0 (2.2)	38.8 (2.7)	32.8 (1.3)	24.0 (3.2)	25.5 (2.1)	30.7 (1.3)	26.2 (2.2)	32.5 (2.3)	32.6 (3.5)
	N	32.5 (1.1)	32.3 (3.7)	35.5 (3.5)	43.5 (2.3)	36.5 (7.0)	33.3 (4.1)	29.8 (2.4)	36.0 (3.0)	33.5 (5.4)	46.5 (2.3)	40.5 (2.2)
	CR	31.8 (1.1)	30.8 (4.4)	37.0 (3.0)	44.9 (4.8)	44.0 (3.1)	34.5 (2.7)	32.5 (1.6)	36.8 (3.6)	28.2 (2.4)	36.9 (4.2)	35.4 (1.5)

Figures in parenthesis are standard deviations on the mean.

APPENDIX 6.8 Amounts ($\mu g P g^{-1}$) of P in the residual (non-extracted) P fraction in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.

		SAMPLE DATE										
Soil	Treatment	12/81	1/82	3/82	5/82	7/82	10/82	12/82	2/83	4/83	9/83	12/83
188PA	Control	363.9 (7.8)	353.5 (15.2)	355.5 (9.6)	355.8 (4.3)	344.0 (4.3)	360.0 (12.7)	361.5 (10.3)	358.5 (11.4)	359.8 (18.0)	347.3 (6.0)	364.1 (11.5)
	L1	376.3 (6.4)	357.5 (10.5)	363.8 (4.4)	365.8 (12.1)	347.5 (13.1)	354.3 (14.9)	366.3 (10.9)	368.8 (9.5)	367.0 (7.0)	348.8 (11.4)	378.1 (10.8)
	L2	369.5 (9.6)	350.5 (8.4)	353.0 (13.4)	367.5 (11.9)	354.3 (16.5)	356.5 (12.6)	368.8 (8.9)	344.8 (9.8)	364.8 (15.9)	360.0 (14.8)	365.6 (14.2)
	N	373.8 (9.6)	363.8 (10.3)	356.6 (6.1)	353.3 (11.2)	345.3 (18.5)	352.5 (14.7)	362.8 (19.4)	365.3 (12.4)	364.3 (13.4)	336.7 (17.0)	376.1 (12.0)
	CR	362.8 (12.9)	357.4 (11.1)	361.3 (6.8)	362.8 (12.3)	349.3 (12.4)	351.0 (13.2)	359.0 (7.9)	365.5 (12.0)	350.5 (12.6)	349.3 (10.2)	358.8 (13.8)
376PA	Control	417.0 (11.1)	410.8 (7.1)	431.5 (10.1)	434.3 (7.2)	446.8 (11.0)	434.8 (13.1)	439.5 14.0)	433.5 (6.3)	413.8 (9.3)	425.0 (8.7)	429.0 (3.8)
	L1	428.8 (8.4)	406.3 (15.6)	424.8 (5.3)	436.0 (14.3)	440.5 (12.3)	447.5 (9.8)	436.8 (8.2)	440.3 (12.7)	429.3 (6.6)	428.0 (7.0)	425.5 (5.1)
	L2	417.5 (9.2)	402.5 (7.2)	423.5 (5.0)	442.8 (6.5)	442.5 (9.2)	432.8 (12.7)	433.5 (10.0)	445.8 (21.0)	423.0 (10.1)	427.8 (11.7)	427.4 (3.6)
	N	418.5 (6.8)	401.8 (13.7)	422.8 (4.1)	430.8 (5.8)	445.0 (5.4)	436.5 (6.2)	444.9 (11.0)	437.0 (18.4)	421.3 (10.7)	420.0 (12.5)	425.5 (9.6)
	CR	422.6 (11.8)	406.3 (9.1)	423.8 (11.0)	437.5 (13.3)	434.8 (6.8)	439.3 (13.1)	444.3 (5.9)	436.8 (13.2)	431.0 (11.4)	412.4 (6.4)	417.6 (7.4)

Figures in parenthesis are standard deviations on the mean.

APPENDIX 6.9 Amounts ($\mu g P g^{-1}$) of microbial P in soils (0-7.5cm) from the different treatments in the 188PA and 376PA soils sampled between December 1981 and December 1983.

Soil	Treatment	SAMPLE DATE 12/81	1/82	3/82	5/82	7/82	10/82	12/82	2/83	4/83	9/83	12/83
188PA	Control	59.4 (7.6)	32.8 (8.1)	62.0 (8.4)	62.3 (2.3)	56.5 (5.9)	39.0 (3.6)	43.0 (4.6)	46.0 (3.6)	44.0 (4.6)	40.5 (2.2)	48.7 (1.1)
	L1	62.7 (4.8)	40.3 (16.6)	58.5 (8.6)	64.8 (9.2)	58.8 (6.8)	48.5 (4.2)	47.0 (1.2)	58.3 (7.9)	50.3 (4.5)	48.5 (2.9)	59.8 (3.9)
	L2	48.5 (10.6)	40.5 (7.8)	55.8 (5.5)	68.8 (6.4)	66.8 (4.4)	48.9 (6.4)	45.5 (6.3)	58.3 (3.3)	54.0 (2.8)	46.5 (5.8)	54.3 (2.5)
	N	70.2 (8.3)	38.8 (8.7)	53.7 (6.8)	62.5 (3.9)	61.0 (5.7)	42.8 (7.9)	43.1 (1.6)	42.3 (5.4)	45.0 (1.6)	39.3 (3.4)	47.3 (2.8)
	CR	60.2 (17.4)	25.3 (8.5)	52.8 (7.9)	51.3 (2.2)	56.0 (8.6)	37.8 (3.9)	35.3 (2.6)	37.8 (2.0)	35.5 (2.1)	28.2 (1.5)	41.5 (1.9)
376PA	Control	69.0 (13.1)	47.8 (13.3)	69.0 (2.2)	51.0 (17.8)	65.8 (6.9)	46.5 (7.4)	53.3 (4.9)	54.3 (3.7)	52.3 (6.3)	50.3 (3.2)	56.6 (1.4)
	L1	65.0 (28.1)	47.0 (11.6)	75.8 (10.5)	66.0 (23.3)	81.5 (6.8)	54.5 (6.0)	58.0 (3.4)	60.0 (9.4)	60.8 (3.8)	52.3 (2.9)	61.7 (6.8)
	L2	57.3 (22.8)	54.5 (23.3)	77.8 (11.9)	66.3 (18.2)	77.0 (6.8)	52.3 (9.6)	63.5 (2.3)	58.8 (9.1)	67.0 (4.4)	57.3 (3.9)	66.7 (1.4)
	N	60.8 (18.4)	41.8 (3.5)	66.3 (4.1)	53.0 (17.1)	69.0 (9.0)	45.0 (6.0)	54.0 (6.8)	54.8 (5.0)	50.3 (6.2)	46.3 (8.9)	55.1 (1.5)
	CR	77.5 (12.5)	45.3 (13.8)	65.5 (13.9)	56.0 (8.7)	62.3 (7.8)	44.5 (9.8)	47.0 (4.9)	50.3 (8.0)	47.5 (8.6)	42.6 (1.5)	51.2 (3.1)

Figures in parenthesis are standard deviations on the mean.