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Polyphosphates and microbial uptake of phosphorus: Studies with soil and solution culture

A thesis presented in fulfilment of the requirements for the Degree of Master of Agricultural Science, Faculty of Agricultural and Natural Resource Sciences, The University of Adelaide.

by

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Summary

Micro-organisms play a major role in phosphorus (P) cycling in most soils. They are involved in the concurrent processes of mineralization and immobilization of P in soil. Micro-organisms mineralize organic P in plant tissues and other soil organic matter to orthophosphate. This orthophosphate is then available for uptake by plants or micro-organisms or reaction with other components of the soil system. However, micro-organisms may also compete with plants for added P in fertilized soil.

Currently there is no measure of the potential P uptake of the soil biomass. The presence of polyphosphates was investigated as an indicator of the the phosphorus status and potential P uptake of some micro-organisms. Micro-organisms can grow without accumulating polyphosphates, but this accumulation is a result of adequate or luxury P uptake. Polyphosphate is a P- and energy-storage compound which accumulates in most micro-organisms when phosphorus does not limit microbial growth.

The aim of this project was to investigate microbial P uptake and polyphosphate content with a view to using microbial polyphosphate content as a measure of potential P uptake by micro-organisms. Three experiments were conducted with the following objectives:

i) to determine if the addition of wheat or annual medic root material to soil had differing effects on soil microbial C and P;

ii) to investigate the effect of previous P nutrition on P uptake of two bacteria and two fungi in single solution culture and relate this to the presence or absence of polyphosphates in these micro-organisms; and

iii) to investigate the presence of polyphosphates in field soil over a range of P status as estimated by extractable P concentration and to determine whether these polyphosphates are present in the microbial biomass.

Annual medic (*Medicago trunculata* cv Paraggio) and wheat (*Triticum aestivum* cv Molineux) plants were grown in a glasshouse and their finely chopped roots mixed with soil at similar rates of carbon addition. Soil was incubated at 20°C and 60% water holding capacity for 21 days and microbial carbon (C) and P measured at the time of peak carbon dioxide evolution (determined in a preliminary experiment). Soil microbial respiration was measured by addition of glucose solution measurement of the initial peak of CO_2 evolution. Glucose is an immediately metabolisable microbial substrate and the (immediate) maximum CO_2 evolution of soil treated with glucose solution is considered to be a measure of the size of the initial soil biomass. Microbial P was measured by fumigation with hexanol and extraction with 0.5 M sodium bicarbonate solution.

Medic roots mixed with soil at a rate of 43 mg C g^{-1} soil and 0.40 mg P g^{-1} soil stimulated peak soil respiration of 3.0 μg CO_2 g^{-1} soil h^{-1} and a soil microbial P increase of 8.3 μg P g^{-1} soil. Wheat roots mixed with soil at a rate of 37 mg C g^{-1} soil and 0.27 mg P g^{-1} soil stimulated peak soil respiration of 2.7 μg CO_2 g^{-1} soil h^{-1} and a soil microbial P increase of 0.6 μg P g^{-1} soil. Medic roots stimulated a larger relative increase in microbial P than wheat roots for a given rate of addition. This implied that, for a similar quantity of biomass, the P content of micro-organisms stimulated by medic roots was about three times that of micro-organisms stimulated by wheat roots. It was concluded that plant species affects the chemical composition of the soil biomass.

The effect of previous P nutrition on orthophosphate uptake and polyphosphate accumulation by two species of bacteria (*Aerobacter aerogenes* and *Enterobacter sp*) and two species of fungi (*Mucor racemosis* and *Thanatophorus cucumeris*) was measured.

Bacteria and fungi were grown in nutrient solution of low or high P concentration (0.15 and 0.70 mM P respectively for bacteria, and 0.40 and 0.94 mM P for fungi). P uptake from solution of intermediate P concentration was measured and subsamples of cultures were analysed for polyphosphates. Bacterial polyphosphate content was measured by extraction with sodium hypochlorite and hydrolysis for 10 minutes with 1N HCl. Fungal polyphosphate content was measured by extraction with ethanol and water and analysis by ^{31}P n.m.r. All micro-organisms pretreated with high P took up less orthophosphate from solution than those pretreated with low P. Polyphosphates were found in bacteria pretreated with high P (*A. aerogenes* 6.3 mg polyphosphate g^{-1} dry weight and *Enterobacter sp* 2.3 mg polyphosphate g^{-1} dry weight), but not in fungal hyphae.

Field soil of low, medium or high P status (2.8, 5.6, and 14.0 µg $NaHCO_3$-extractable P g^{-1} soil) from under wheat or medic plants was analyzed for polyphosphates. Samples were extracted with perchloric acid and activated charcoal. Extracts were hydrolyzed to determine polyphosphate content. Soil polyphosphate content ranged from 0 to 13.9 µg g^{-1} soil. Polyphosphates were present in all soil under medic, and in soil of medium and high P under wheat. Soil polyphosphate content increased with extractable P. Soil containing polyphosphates was sequentially fractionated with 0.5 M sodium bicarbonate, 0.1 M NaOH, ultrasonic dispersion, extraction again with 0.1 M NaOH and with 1 M HCl. Soil polyphosphates (up to 13.0 µg polyphosphates g^{-1} soil) were present in the microbial biomass. No polyphosphates were present in other soil extracts. Changes in soil effected by wheat growth appear to be less suitable for the synthesis and accumulation of soil polyphosphates than those effected by medic growth.

Micro-organisms containing polyphosphates took up less P from solution than those not containing polyphosphates. The polyphosphate concentration of field soil increased with extractable soil P. The presence, quantity and persistence of soil polyphosphates differered between soil under wheat and under medic plants.

All objectives of this project were achieved. Amendment of soil with medic or wheat roots had different effects on microbial C and P. Pretreatment of micro-organisms with low P solution increased subsequent P uptake. Pretreatment of micro-organisms with high P solution decreased subsequent P uptake. This was accompanied by the presence of polyphosphates in bacteria pretreated with high P, but not in fungi. Polyphosphates in soil were shown to be present in the soil microbial biomass, and to increase with extractable soil P.

Polyphosphates can be used as a measure of microbial P uptake in solution. It remains to be seen if polyphosphates can be used as a measure of P uptake by the microbial biomass.

Statement

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university. To the best of the author's knowledge and belief, this thesis contains no material previously published or written by another person, excepts where due reference is made in the text of the thesis.

I consent to this thesis being made available for photocopying and loan.

Nigel Kevin Fleming

February, 1992

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CHAPTER ONE: INTRODUCTION

In most unfertilized soils most phosphorus (P) available to plants cycles through the microbial biomass (Tate 1984) during the breakdown of plant tissues, decomposing micro-organisms, and other soil organic matter. Micro-organisms also play a major role in P cycling in fertilized soils. The microbial biomass mineralizes organic P in plant tissues and other soil organic matter to orthophosphate, which is then available for uptake by plants and other micro-organisms or for reaction with soil components. Micro-organisms are actively involved in the concurrent processes of P immobilization and mineralization, and may compete with plants for added P in fertilized soil.

It is not known what proportion of P fertilizer added to soil will be taken up by the soil biomass. A predictor of microbial P uptake may improve the efficiency of P use by showing how strongly the soil biomass will compete with plants for added P fertilizer. McLaughlin et al (1987) found wheat plants took up 12% and micro-organisms 14% of added fertilizer P over a 22 day period, showing that micro-organisms can take up a considerable proportion of added fertilizer P.

An indication of the potential P uptake of soil micro-organisms may improve understanding of fertilizer uptake and allow prediction of the likely fate of fertilizer added to soil. Currently there is no technique available to predict P uptake by the soil biomass. Metabolic activities of soil micro-organisms may be measured by their adenylate energy charge - the proportion of high energy phosphates present as adenosine triphosphate (Atkinson 1977). This gives a reasonable indication of the energy state of soil micro-organisms and can discriminate between a small, active microbial biomass and a large, dormant one. There is no analogous technique for likely microbial P uptake. It is not known how strongly a given soil biomass will compete with plants for soil P. It would seem logical that a large microbial biomass would require more P than a small one, and would compete more strongly with plants for this P. However, if the large biomass had previously been able to take up luxury amounts of P, and the smaller biomass was P-limited, the reverse may be true. The population of micro-organisms which comprise the soil biomass is exceedingly complex and many parts interact with each other. Of necessity, the investigations reported here have been confined to the behavior of individual micro-organisms, and relating this behavior to the biomass as a whole system.

Measuring the C:P ratio of the biomass may be one way to determine the P status of the soil biomass, but in order to use this value to predict P uptake by the microbial biomass it is necessary to define a "critical" C:P ratio. This will depend on the age, activity and dominant

microbial species of the soil biomass. The soil contains thousands of microbial species which vary in competitive ability (and thus dominance) with changes in environmental conditions (Alexander 1977). Techniques which measure soil microbial biomass are not able to identify specific micro-organisms and the proportion they comprise of the total biomass.

Polyphosphate may be a suitable indicator of the P status of micro-organisms comprising the soil biomass. Polyphosphate is a P- and energy-storage compound which accumulates in most micro-organisms under conditions when P is not limiting (Harold 1966, Kulaev 1979, Beever and Burns 1980). Accumulation of polyphosphates is not essential for microbial growth. Polyphosphates are not always found in micro-organisms growing in conditions of adequate P, but are never found in P-deficient micro-organisms. Thus, polyphosphates may provide an indicator of microbial P status.

Various indicators of the microbial biomass change with plant species, e.g. biomass C:P ratios(Brookes et al 1984) and fungal:bacterial ratios (Clark and Paul 1970). Little is known, however, of the way biomass P nutrition varies with plant species. This project investigates the effect of adding wheat (*Triticum aestivum*) and medic (*Medicago trunculata*) root to soil on microbial C and P. It is known that plants may influence the type of microbial population present in their vicinity, so it is likely that plant type will affect the P status of the soil biomass in the vicinity of plant roots. Samples of the same soil from under wheat and medic at a range of extractable soil P concentrations, were examined to determine biomass P status and the presence or absence of polyphosphates. Wheat and medic crops were used in the study as these two crops are agronomically significant in southern Australia.

The previous P nutrition of micro-organisms is known to affect their subsequent P uptake and is also known to affect the synthesis and accumulation of polyphosphates. These parameters have not, however, previously been linked, to the author's knowledge. This project investigates the P uptake of micro-organisms following pretreatment at different P concentrations and relates this uptake to the presence or absence of polyphosphates.

The objectives of this study were:

1) to determine whether microbial P uptake could be related to polyphosphate in micro-organisms, and

2) to examine differences in the P status of the microbial biomass in soil under wheat and medic plants.

The project was carried out in three stages. The first determined whether soil biomass was affected by plant type - soil was incubated with different types of plant root and microbial C and P were measured (Chapter 3).

The second stage related the P uptake of selected micro-organisms to both prior P nutrition and the presence or absence of polyphosphates in these micro-organisms (Chapter 4).

The third stage determined the effect of soil P status and plant type on the polyphosphate content of field soil (Chapter 5).

CHAPTER TWO: LITERATURE REVIEW

Phosphorus in microbial cells

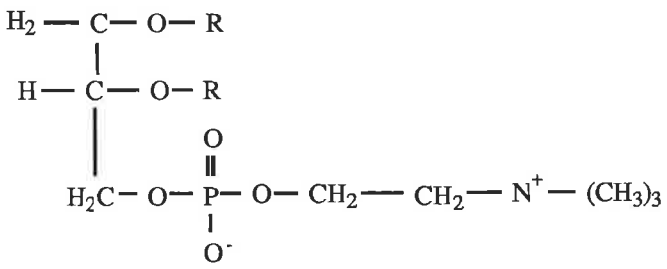
Organic phosphates

Phosphorus (P) is found in soil, plants and micro-organisms in a number of organic and inorganic compounds, as a nutrient required by plants and micro-organisms. Its main physiological role is in the accumulation and release of energy.

P compounds present in micro-organisms include nucleic acids, phospholipids, techoic acids, orthophosphate, polyphosphates and many co-enzymes and adenosine phosphates (Alexander 1977).

The proportion of P found in different compounds varies with microbial P status, stage of growth and type of micro-organism. Ribonucleic acid makes up the bulk of P in the bacterial cells, usually one third to one half of the total P (Alexander 1977). Deoxyribonucleic acid comprises 5-10% of total P.

Phospholipids (Figure 1) are found almost exclusively in the cell membranes. The membrane consists mainly of phospholipids in a lipid bilayer with interdispersed proteins. This presents one monolayer surface to the cytoplasm and another to the external medium (Finnerty 1978)

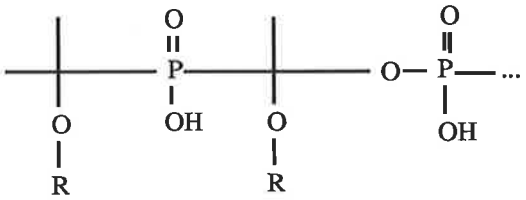


Lecithin-type compound (phospholipid)
(R) = fatty acid.

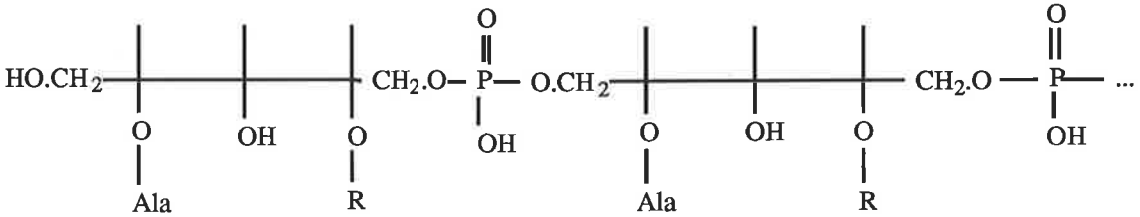
Figure 1. A phospholipid compound (from Alexander 1977).

The concentration of phospholipids in bacteria varies with species and age, but is generally less than 10% of cell P.

Techoic acids (Figure 2) are anionic polymers which contain either glycerol or ribitol, usually combined with sugar and D-alanine, and in which sugar residues may form an integral part of the chain (Berkeley 1979).



Structure of 1,3-polyglycerol phosphate teichoic acid.
(R) = H- or D-alanine or sugar residues.



Structure of 1,5-polyribitol phosphate teichoic acid
(R) = sugar residues.

Figure 2. Techoic acid compounds (from Berkeley 1979)

Techoic acid compounds occur in almost all Gram positive bacteria, are located exclusively in the outer layers of the cell (membrane, cell wall and capsule), and can account for more than 10% of their dry weight (Archibald 1974).

Polyphosphates

Inorganic polyphosphates are linear polymers in which orthophosphate residues are linked by energy-rich phospho-anhydride bonds (Harold 1966). They are polymers which have the general formula $M_{(n+2)}P_nO_{(3n+1)}$ where M is a metal cation. Polyphosphates consist of chains of orthophosphate residues in which each P atom is linked to its neighbors through two oxygen atoms, forming a linear structure as shown in Figure 3. The chain length of polyphosphates found in living organisms can vary from two (pyrophosphate) to several

thousand (Kulaev 1979). The structure and properties of polyphosphates have been comprehensively reviewed in a monograph by Kulaev (1979).

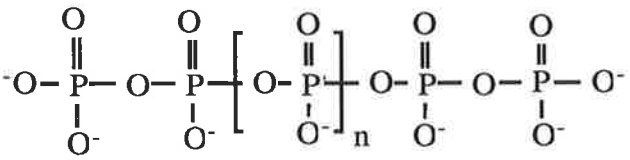


Figure 3. A polyphosphate compound of chain length n+4.

Polyphosphates can be found at certain stages of development and under certain conditions in most living organisms. They may occur in large amounts in micro-organisms (e.g. bacteria, fungi and algae), but in quantities only barely detectable in higher plants and animals (Kulaev 1979).

Polyphosphates in cells of fungi and other micro-organisms can be separated into several fractions by sequential extraction. These fractions differ in chain length and extractability (Kulaev 1975), and have been arbitrarily named $polyP_1$ - $polyP_5$. The smallest polyphosphates comprise the $polyP_1$ fraction and have an average chain length of 2-10 units. These compounds can be extracted from cells by cold dilute acid. The $polyP_2$ fraction (average chain length of 20-40 units) can be extracted by cold saturated sodium perchlorate. The most highly polymerized polyphosphates ($polyP_3$ - $polyP_5$) with average chain lengths of 300-500 units can be extracted by cold dilute alkali or hot perchloric acid.

$PolyP_1$ compounds may be considered the most readily mobilised reserve of activated P used by the cell when P deficient (Kulaev 1979). Short-chain polyphosphates exist in solution and are found inside the cell in the cytoplasm, vacuoles and nucleus.

Highly polymerized fungal polyphosphates ($polyP_3$ - $polyP_5$) can be considered specialised reserves of P and energy, being used for specific biochemical and physiological processes. They usually exist complexed with cellular components such as nucleic acids, proteins or polysaccharides, and can be very difficult to separate from their complexing molecules. These polyphosphates are found close to, or outside the cell wall (Kulaev and Vagabov 1983).

Bacterial polyphosphates occur as granules which may be found in any part of the cell. Often polyphosphate granules in bacterial cells are formed by gradual deposition of polyphosphates

on strands of cytoplasmic material (Kulaev 1979). Fungal polyphosphates may also occur as granules (Lapeyrie et al 1984)

Kulaev and Vagabov (1983) considered polyphosphates important to microbial metabolism because micro-organisms do not have a well-developed system of hormonal and nervous regulation, and depend very much on environmental conditions. This is a result of direct contact between cells and the surrounding medium. A non-functioning regulatory system must lead, in some circumstances, to the demise of the cell. Therefore micro-organisms need "metabolic traps" such as high molecular weight polyphosphates to maintain intracellular homeostasis. The availability of endogenous pools such as polyphosphates makes micro-organisms both less dependent on external conditions and capable of quickly initiating growth and reproduction without a significant lag period.

Hydrolysis of the P-O-P bond in linear polyphosphates releases approximately the same amount of energy as the terminal phosphate bond in ATP. Polyphosphates can thus be considered a reserve of "activated" P, accumulated under conditions of restricted growth, but ready to release P and energy when required.

The conditions which favour polyphosphate accumulation and breakdown have been investigated for several micro-organisms. A common feature is the great variation in polyphosphate content of micro-organisms studied. The polyphosphate content is generally low during rapid growth but increases when a nutrient imbalance inhibits growth, although there are some exceptions (Kulaev 1979).

Harold (1966) showed two patterns of polyphosphate accumulation with the bacteria *Aerobacter aerogenes*. One pattern involved the cessation of nucleic acid synthesis through shortage of an essential nutrient. When growth and nucleic acid synthesis stopped, P was still taken up by bacteria. This caused a gradual accumulation of polyphosphates. When the limiting nutrient was supplied, growth resumed and polyphosphates were rapidly degraded and incorporated into nucleic acids. The other pattern occurred when phosphate was added to bacteria previously starved of P. A rapid and massive polyphosphate accumulation resulted - this was referred to as "polyphosphate overplus". When normal growth resumed, polyphosphates were gradually degraded and converted to nucleic acids.

These polyphosphate accumulation patterns also occur in fungi. Polyphosphate overplus is especially marked in yeast, where polyphosphates can account for up to 20% of dry weight (Liss and Langen 1962).

Polyphosphate content is low during rapid growth and accumulates under conditions of nutrient imbalance. When growth is restricted, therefore, the presence of polyphosphate rules out P deficiency as the growth-limiting factor.

Polyphosphates in soil

The role that polyphosphates play in the soil P cycle is not well known. Previous study of polyphosphates in soil has consisted of occasional observation of polyphosphates in nmr spectra (e.g. Tate and Newman 1982), inducement of their synthesis by addition of large amounts of orthophosphate (Ghonsikar and Miller 1973, Pepper et al 1976) and the use of manufactured polyphosphates as fertilizer.

Polyphosphates are suspected to be the major form in which P is translocated by mycorrhiza to host plants (Callow et al 1978, Cox et al 1980). Although ^{31}P nmr has been used to identify and roughly quantify polyphosphates in mycorrhiza (Martin et al 1985) the actual quantities of mycorrhizal polyphosphates in soil are not known. It is possible, therefore, that not all polyphosphates detected in soil come from micro-organisms competing against plants for available soil P. Some polyphosphates may be from mycorrhiza which, in fact, assist the P uptake of infected plants.

Most research on polyphosphates in soil has been concerned with their use as P fertilizers. Advantages of polyphosphate fertilizers include high P concentration (low freight costs), water solubility and mobility in soil (Kudeyarova and Kvaratskheliya 1984). Polyphosphates may behave as slow-release P fertilizers, as they must be hydrolysed to orthophosphate before uptake by plants or micro-organisms (Ansiaux 1978).

Dick and Tabatabai (1987a) investigated the effectiveness of a range of polyphosphate fertilizers and orthophosphate in a glasshouse experiment using annual ryegrass and maize. They found all P fertilizers were equally effective in terms of plant uptake per unit P added, and in dry matter increases per unit P taken up. Hydrolysis of polyphosphates was rapid enough not to limit P uptake, although polyphosphates were not considered superior to orthophosphate. Dick and Tabatabai (1987b) modelled polyphosphate hydrolysis in soil. Polyphosphate hydrolysis was positively correlated with pH and water-soluble calcium, but negatively correlated with pyrophosphatase activity.

Aseeva et al (1981) added long-chain polyphosphates to soil and followed their hydrolysis. By calculating rate constants they proposed that the same enzyme hydrolysed many long-chain polyphosphates (average chain length 47-180). Pyrophosphate and tripolyphosphate

were hydrolysed at a different rate, implying that this was via the action of different enzymes. Polyphosphate hydrolysis in soil following enzyme deactivation by heat or 0.01 M NaF was only 20-40% of that in soil stored at 0-4°C. Hydrolysis of soil polyphosphates, whether originating from micro-organisms or fertilizer, was due to both biological and chemical action.

The above work concerned the fate of polyphosphates added to soil. Ghonsikar and Miller (1973) investigated polyphosphate accumulation *in situ*. They extracted eight field soils with 0.5 M perchloric acid (PCA) and found acid-labile inorganic phosphate compounds in four of them. These compounds were identified as polyphosphates by comparison of their elution patterns through Sephadex gel with those of standard polyphosphates. Polyphosphate accumulation was induced in seven of the eight soils by incubation firstly with glucose (2% solution), then large amounts of orthophosphate (1 000 $\mu g\ P\ g^{-1}$ soil). The glucose stimulated microbial growth and induced P deficiency which was rectified by orthophosphate addition. These extreme changes in P nutrition allowed polyphosphates to be synthesized by a "polyphosphate-overplus" mechanism, that of rapid and extensive accumulation of polyphosphates following P starvation (Harold 1966). The authors speculated that polyphosphates could form a biological reserve of P which is protected from soil adsorption reactions and yet may become available for uptake by higher plants. Pepper et al (1976) followed up these experiments and induced polyphosphate synthesis in soil with as little as 100 $\mu g\ P\ g^{-1}$ soil.

Soil texture and structure are known to affect the size and persistence of the soil microbial biomass, but there is no published work on their effect on soil polyphosphate concentrations.

All work reviewed by the author on polyphosphates in soil has been concerned with the presence of polyphosphates either as a source of P for plants, or as components of P transfer in mycorrhizal fungi. As far as the author is aware, no work has been published on the use of polyphosphates as indicators of likely microbial uptake of added P, or even on the normal concentrations of polyphosphates themselves in soil.

Micro-organisms in soil

Types of micro-organisms in soil

Micro-organisms in soil may be divided into five major groups - bacteria, actinomycetes, fungi, algae and protozoa. For the purposes of agricultural research, these comprise the soil

microbial biomass - the living part of soil organic matter, excluding plant roots and soil organisms larger than about 5×10^3 μm^3 (Jenkinson and Ladd 1981). Bacteria and fungi are the major microbial contributors to nutrient turnover in soil and will be discussed here.

In well aerated soil, bacteria and fungi dominate, but bacteria account for almost all activity in anaerobic conditions. Winogradsky (1925) broadly categorized soil bacteria into zymogenous (opportunistic) and autochthonous (indigenous) species. Numbers of autochthonous bacteria do not fluctuate markedly. Their nutrient supply is derived from the soil organic matter and they are less affected by external nutrients or energy sources than zymogenous bacteria. Zymogenous bacteria are most active in chemical transformations. They are usually scarce, but flourish when nutrients are added. Zymogenous bacteria respond to soil amendment, become and remain numerous as long as nutrients are available, then decline once their food source is depleted. Bacterial cells are more numerous than fungal hyphae in soil, although their small size means that in most well-aerated, cultivated soils, fungi have a greater microbial biomass. This is because of the large diameter and the extensive network of fungal hyphae in soil (Alexander 1977).

Mycorrhizal fungi infect the roots of a wide range of plants (Francis et al 1986) and enhance their ability to grow and absorb P from soils which are P deficient (Mosse 1973, Tinker 1980), as are most Australian soils (Blair 1982). A discussion of the role of mycorrhiza is, however, outside the scope of this review. For further information see Hayman (1978), Bowen (1980), Smith (1980) and Harley and Smith (1983). Bolan et al (1984) summarised possible mechanisms to explain the mycorrhizal response as:

1) greater exploitation of soil through hyphae extending beyond the zone of depletion of non-mycorrhizal roots;

2) higher P uptake rates and possibly a lower threshold of P uptake from solution by mycorrhizal plants;

3) exudates (e.g. oxalate) from mycorrhizal plants which may increase the availability of P; and

4) differences in the absorption of cations and anions by mycorrhizal and non-mycorrhizal plants which may affect rhizosphere pH and change the availability of P.

Although mycorrhizal mycelia may form a large proportion of the soil microbial biomass (Hayman 1978), present techniques for measuring biomass are unable to differentiate between

mycorrhizal and free-living fungal mycelia. Mycorrhizal biomass must be determined by microscopy (e.g. Bethlenfalvay et al 1981, Kucey and Paul 1982), which is very time-consuming, and there has been little progress in finding the proportion of soil fungal biomass which is mycorrhizal.

Table 1. Microbial C:P under various systems of soil management

Cropping History	Microbial C ($\mu g\ C\ g^{-1}$ soil)	Microbial P ($\mu g\ P\ g^{-1}$ soil)	Microbial C:P ratio
continuous wheat	158	6.0	26.3
continuous wheat	190	5.3	35.9
continuous wheat	342	28.9	11.8
deciduous woodland	715	67.2	10.6
unmanured - permanent grass	1627	72.3	22.5
permanent grass	1379	106.0	13.0
permanent grass	847	61.7	13.7
arable rotation	305	21.0	14.5
grassland	1112	87.6	12.7
grass/clover ley	300	15.0	20.0
arable rotation	99	7.0	14.1
8-year grass ley	148	12.0	12.3
grass ley	635	24.8	25.6
permanent grass	569	48.6	11.7
barley/grass-clover root crop rotation	492	27.5	17.9

Adapted from Brookes et al (1984)

Until a viable method is found to determine mycorrhizal biomass, it would appear the best that can be done is to keep in mind that a certain proportion of fungal biomass is not competing with the plant for P.

The P content of soil organisms grown *in vitro* varies widely with P supply (Chauhan et al 1981; Hedley and Stewart 1982), C supply (Anderson and Domsch 1980), culture age (Hedley and Stewart 1982) and water stress (van Veen and Paul 1979). P generally comprises 5-10 mg g^{-1} dwt of fungal mycelia and 10-30 mg g^{-1} of the dry weight of bacteria and probably of actinomycetes (Hayman 1975; Alexander 1977). However, micro-organisms can show luxury consumption in culture media, so these values are probably higher than those found under natural conditions. Little is known about the P concentration or C:P ratio of the microbial biomass *in situ*, or how this ratio varies between soils, due to the difficulty of extracting whole, undamaged, uncontaminated cells from soil. The C:P ratio can be estimated by chemical methods, although values obtained will be subject to errors and assumptions of techniques used in the determination of microbial C and P (discussed later). Table 1 shows some published values of microbial C:P ratios in soil:

Why soil biomass developed under different systems has such differences in P concentration is as yet unknown. There must be a theoretical limit below which the P content of the biomass cannot fall if a cell is to survive. However, it appears that the P concentration in the biomass is dependent on many soil factors, not just the level of available inorganic P (Brookes et al 1984). Factors limiting microbial growth (e.g. soil water, substrate availability) are likely to be important in determining the P concentration of the biomass.

As most micro-organisms are capable of synthesising polyphosphates (Kulaev 1979) and there is a wide variety of micro-organisms in soil (Alexander 1977) it follows that there is the potential for polyphosphate accumulation in most soils.

Distribution of micro-organisms in soil

It has long been known that in the soil around a root (rhizosphere) the abundance of bacteria and fungi is much greater than in soil further from the root (Newman 1985). The rhizosphere may be regarded as a series of micro-environments ranging from the growing apex to the oldest parts of the root (Darbyshire and Greaves 1973). The rhizosphere effect is caused primarily by soluble and insoluble compounds released from living and dead root cells (Rovira 1979), which may be substrates for fungal spore germination and bacterial growth

(Lynch 1982). Other factors which may stimulate microbial growth in the rhizosphere have been suggested, such as higher CO_2 concentration, lower O_2 concentration, changes in pH, or the partial desiccation of rhizosphere soil (Starkey 1929) but they are probably less important than rhizodeposition of root material (Darbyshire and Greaves 1973). For example, estimates of root input to the rhizosphere range from 17-40 % of total net ^{14}C fixed of cereal plants (Whipps 1984). This C is then vulnerable to uptake and utilization by micro-organisms.

Published data on bacterial growth rates in the rhizosphere or on the root surface (e.g. Turner and Newman 1984) show that bacterial growth is only rapid for the first few days of the existence of the root. It is likely that bacteria multiply rapidly until they reach a population density where competition for C substrate is intense. Thus it is a mistake to think of the rhizosphere as always a region of ample substrate supply and rapid microbial growth: that is so only for short periods, for any particular root (Newman 1985).

Fungi may cover either more or less of the root surface than bacteria. Newman et al (1981) found that when all essential nutrients were in ample supply bacteria exceeded fungi, but when either N or P was omitted, or no nutrients were supplied, fungi exceeded bacteria.

The ratio of fungi to bacteria in soil has been found by physiological methods to range from 1.5 to 9 in the rhizospheres of crop plants and from 0.13 to 1.5 in the rhizospheres of prairie grasses (Anderson and Domsch 1975; Vancura and Kunc 1977; Nakas and Klein 1980).

Fungi seem to exceed bacteria in bulk soil biomass, whereas either group may dominate biomass in the rhizosphere. Of fungi growing close to roots, many can degrade cellulose, pectin and starch. Newman (1985) suggested that these fungi are obtaining most of their C substrate from dead roots nearby, and from individual dying cells in roots which are still partly alive and functional. For such fungi their habitat would span from one root to another.

The habitat of a bacterium, however, extends only micrometres around it. Most bacteria living at the root surface or in the rhizosphere are unable to degrade cellulose or pectin (Newman 1985) and presumably are dependent on soluble soil organic materials from the root. The growth rate supported by soluble exudates is high only for short periods; bacteria soon become so abundant that competition is intense. The classical rhizosphere picture applies well to bacteria, occupying a habitat a fraction of a millimetre wide and dependent on soluble exudates from the root for their main substrate. In contrast, fungi, although they form a substantial part of the biomass within the rhizosphere and may well influence other organisms there, are probably obtaining most of their substrate from elsewhere (Newman 1985).

The magnitude of the rhizosphere response is influenced by physical, chemical and biological factors. Soil water, temperature, aeration and fertility can affect the rhizosphere population directly, or indirectly through plant growth (Katznelson 1965), as can pH, redox potential and soil structure. The physiological age and metabolic state of the plant are also important factors. The rhizosphere population begins to develop soon after germination of seeds and usually reaches a peak around the time of flowering and fruit formation. During plant senescence the size of the rhizosphere population decreases sharply (Darbyshire and Greaves 1973).

Plant type can influence the composition and magnitude of the rhizosphere bacterial population (Martin and Kemp 1980), which may in turn influence the availability of nutrients to the plant. Distribution of micro-organisms in the rhizosphere can be very important to the plant, as phosphate diffuses so slowly that a depletion zone may extend only a fraction of a millimetre in many days (Bhat and Nye 1973). Micro-organisms taking up P 1 mm from the root may not affect root P uptake because the P molecule may have never reached the root anyway. P uptake by micro-organisms nearer the root, however, could reduce the P supply to the plant (Newman and Watson 1977).

Bacterial and fungal biomass tends to decrease with depth in soil (Schnurer et al 1986). This is most likely to be due to decreasing organic matter content (except in peat soils) and hence substrate supply down the profile. The decrease in bacterial biomass is probably due to smaller root biomass in lower layers. Biomass distribution may also be affected by soil use and cultivation method (Lynch and Panting 1980b).

Soil structure affects the spatial distribution of biomass by the formation of micro-environments. Improved water relations in the interior of soil aggregates enhances the survival of desiccation-sensitive bacteria, while spore-forming bacteria, actinomycetes and fungi dominate the outer portion of partly-dried aggregates (Marshall 1975).

Soil texture affects the distribution of macro- and micro-pores in soil. This in turn affects the relative efficiency of substrate utilization by bacteria and fungi. Adu and Oades (1978b) distributed labeled glucose or starch in macro-, or macro- and micro-pores of aggregates. Where substrate was in macropores, bacteria were as active as fungi in releasing $^{14}CO_2$. When in micropores as well, the fungi showed more efficient utilization. This was not the case in a self-mulching clay. The advantage of fungi in small pores was attributed to bacterial dependence on water films and large pores for adequate movement, and access, to substrate.

Quantity of micro-organisms in soil

The measurement and turnover of microbial biomass in soil has been reviewed by Jenkinson and Ladd (1981). Generally, 2-3% of organic C in soil was present in the biomass, although biomass C comprised only 0.8% of the soil organic C in a sandy semi-arid Australian soil (Jenkinson and Oades 1979) while it made up 4% of the soil organic C in a Nigerian forest soil (Ayanaba et al 1976).

Situations favouring accumulation of organic matter generally increase both the amount of biomass and its proportion in the soil organic matter. Sarathchandra et al (1984) measured biomass and microbial P in 21 soils from high-producing New Zealand pastures. Biomass C ranged from 540 to 1890 $\mu g\ C\ g^{-1}$ soil (mean = 1240), being 1-3.9% of total organic C. Biomass P was 20-88 $\mu g\ P\ g^{-1}$ soil (mean = 52). This gave biomass C:P ratios from 15 to 63. The wide variation in C:P ratio was considered to be due to differences in C and P availability.

Plants are the main sources of substrate for micro-organisms in soils, both from rhizodeposition (Newman 1985) and as a source of root, stem and leaf litter. Biomass tends to increase over the growing season and decrease when plants are growing less actively (Lynch and Panting 1980a). This is most likely to be mediated through C inputs from roots, although unfavorable conditions (e.g. dry periods) during the growing season may disrupt this pattern and delay the use of potentially available C (Schnurer et al 1986). Bacteria may be at a competititve disadvantage when substrate is limiting as fungi can exploit new sites of nutrient accumulation by translocating cytoplasm and leaving empty hyphae behind (Schnurer et al 1985).

Cultivation can promote microbial activity (after the initial mortality caused by physical rupture) by increasing contact between soil and decomposing plant material (Brown and Dickey 1970) or exposing fresh organic matter surfaces to microbial attack (Powlson and Jenkinson 1981). This may bring non-motile organisms into contact with fresh nutrient sources, causing a peak in microbial numbers and biomass after cultivation as noted by Warcup (1957). Microbial activity may also be stimulated by improved aeration in cultivated soil (Smith et al 1974).

Biomass samples taken during summer and winter from the same soil can vary in their reaction to soil treatment. Lynch and Panting (1981) proposed that different microbial populations occurred in summer and winter to explain the variable effect of sieving (7 mm) on the biomass of clay soils. Sieving reduced the biomass of soil sample in winter by an

average of 61%, but increased the biomass of soil sampled in summer by an average of 39%. In winter, root activity is minimal and the major source of energy to the biomass is plant residues from preceding crop plants. Cellulolytic fungi are probably the dominant colonists, and most of these have resistance to poor aeration found in heavy soils in winter. By sieving soil, the organisms and substrate were parted on the sieve and organisms were exposed to lethally high oxygen potentials. This killed a proportion and sieving reduced soil respiration.

In spring and summer (during active plant growth) it was proposed that the main active components of biomass are aerobic rhizosphere bacteria. When samples were sieved, roots were broken up and provided substrate in the form of easily-available root cell contents. This substrate was mixed with the microflora, and sieving increased respiration of summer samples.

The type of farming system can affect both the size of the biomass and the fungal:bacterial ratio. Fungi dominate total biomass in most soils (Clark and Paul 1970), but fungal:bacterial ratios can vary with soil type and season (Clark and Paul 1970, Shields et al 1973, Faegri et al 1977). Fungal:bacterial ratios vary with soil use; from 3.9 to 4.4 in arable soils and 1.0 to 8.0 in grassland soils (West 1986), but tend to be higher in arable soils than in pasture (West 1986, Coupland 1979, Shields et al 1973).

Total biomass is generally higher in grassland or ley soil than in arable crops - Lynch and Panting (1980b) found the biomass in a 9-year grassland soil was three times greater than the same soil which had been cultivated for 4 years. Powlson and Jenkinson (1976) ranked biomass in the same soil under different crops as:

grassland > cropped arable > bare fallow

Schnurer et al (1986) considered ley crops would support larger microbial biomass than annual crops because of their more extensive root growth over a long growing season (more C input) and the absence of soil management in perennial leys.

Within a cropping system, differences in biomass can occur between conventional cultivation and direct drilling. Direct drilled fields tend to have a larger biomass in surface soil through their large organic matter and plant root concentration at the surface. Lynch and Panting (1980a) found the top 5 cm of direct-drilled soil had an average of 30% more biomass than that of ploughed soil. A sharp decrease with depth, however, makes total biomass comparable to that more uniformly distributed through the plough layer of a cultivated field (Powlson and Jenkinson 1981). There may, however, be differences in the seasonal pattern of

P turnover between these cultivation systems, caused by differences in soil water and temperature relationships (Elliott et al 1984).

Biomass declines more rapidly after substrate exhaustion in a sandy than clay soil. Ladd et al (unpubl.; cited Jenkinson and Ladd 1981) found biomass declined about two-thirds as quickly in a clay soil as it did in a sandy soil. Material released from cells following death in clay soil may be mainly retained in the vicinity of surviving organisms, forming a nearly-closed system from which only a small proportion of readily-metabolizable substrates can leak away (van Veen et al 1985). This would increase efficiency of substrate utilization. However, clay minerals have been shown to protect microbial metabolites during degradation of labeled substrates (Ladd et al 1981) so clay may slow degradation of organic substrate.

Survival of micro-organisms in soil

Microbial grazing

Microbial grazers (e.g. protozoa, nematodes) which feed on soil bacteria and fungi have long been known to stimulate microbial activity (Cutler and Crump 1929). Ingham et al (1985) outlined several mechanisms which could be responsible for nematodes stimulating bacterial populations:

i) incomplete assimilation of bacteria, with surviving bacteria having enhanced growth,
ii) transportation of bacteria (internally or externally) to an unexploited substrate (Anderson et al 1982), and
iii) stimulation of bacterial growth by waste products of nematodes, which become locally available, e.g. nematodes secrete significant amounts of amino acids (Anderson et al 1983).

Clarholm (1981) observed field population dynamics of microbial grazers. Peaks in numbers and biomass of bacteria appeared after rain, then a peak of naked amoebae followed. Of the three protozoan groups (ciliates, flagellates and amoebae) only amoebae showed populations large enough, and which fluctuated in a way that showed them to be bacterial regulators. Bacterial increases were transient and amoebae alone were calculated as causing 60% of the bacterial decrease. Protozoa will feed on bacteria and fungi and an inverse relationship between bacterial biomass and amoebae in soil has been noted (Schnurer et al 1986).

Effect of Water Stress

Soil water is an important factor in variations in microbial flora. Orchard and Cooke (1983) found a 10% reduction in soil respiration when soil water content was reduced from -10 to -20 kPa, which is a water content not generally considered limiting in soil. A similar effect was noted by Ross and Tate (1984). In the field, biological activity may be temporarily synchronized by a large rainfall (Clarholm 1981).

As soils dry, the relative contribution of bacteria and fungi to soil respiration changes (Griffin 1981). The relative competitive advantage between bacteria and fungi moves toward fungi as soil water decreases. Soil water affects bacterial activity by restricting both movement and metabolism. Bacterial movement is restricted to water films present in soil at -20 kPa to -100 kPa (Wong and Griffin 1976), metabolic activity drops sharply from -50 kPa to -300 kPa, and is negligible at -1500 kPa.

Fungi, however, are more resistant to water stress. Wilson and Griffin (1975) found total soil respiration declined little when soil water content was reduced from -800 to -3000 kPa. Most of this respiration was probably due to fungi, as hyphal extension can occur at much lower potentials than this, and allow fungi to bridge air-filled pores and actively explore for nutrients (Griffin 1981).

In countries with marked dry seasons the death of the biomass can cause a substantial release of organic nutrients such as P. On rewetting a dry soil there is a burst of activity as organisms consume substrate made available by the dry conditions. The microbial activity in soil after drying and wetting can be considered to proceed in waves, where the two bacterial populations (zymogenous and autochthonous) and fungi have different growth and activity patterns. The overall effect is an increase in the mineralization rate, where easily available nutrients from dead micro-organisms are used first, but which finally ends with the mineralization of humic substances. This is why fluctuating water activity in soil may result in a more rapid decomposition and mineralization than constant water under optimal conditions (Lund and Goksoyr 1980)

Sparling et al (1985) measured microbial C and P and $NaHCO_3$-extractable P in soil before and after air-drying. The increase in extractable P caused by air-drying agreed closely with decreases in microbial C and P. This shows that a major change in soil microbial population can release significant amounts of extractable P in soil.

Drying causes the physical disruption of aggregates and rearrangement of soil components, which may be important factors in causing a flush of microbial activity after a soil has been rewetted (Birch 1960, Adu and Oades 1978a).

Improved water relations in the interior of aggregates enhance the survival of desiccation-sensitive bacteria compared with those on the aggregate surface (Marshall 1975). Spore-forming bacteria, actinomycetes and fungi dominate the outer portion of partly dried aggregates, but on rewetting, the numbers of Gram negative, desiccation-sensitive bacteria increase until they become dominant (Marshall 1975).

Bacterial numbers peak a few days after rewetting dry soil (Cutler and Crump 1929, Clarholm and Rosswall 1980). Fungi and actinomycetes respond more slowly (Lund and Goksoyr 1980, Campbell and Biederbeck 1976). Patterns of water stress can influence the characteristics of soil microbiota. Sparling et al (1987) found microbial populations from soils with high (> 400 mm) annual water deficits were more resistant to air drying, and released less extractable P_i, than those of wetter soils.

As water stress is a major factor in microbial survival in soil, changes in the amount of P held in the microbial biomass will depend on the soil population's resistance to desiccation.

Effect of Soil Structure

The type, content and structure of clay is a major factor affecting the dynamics of soil organic matter (Tisdall and Oades 1982). Much substrate in soil is physically protected from microbial attack. Most polysaccharide deposits in soil are enclosed in pores < 1 μm wide, too narrow for micro-organisms to enter and therefore physically protected from them (Foster 1985). Adu and Oades (1978a) amended soil with starch or glucose, the soil was then aggregated. The starch in amended soil was protected by aggregates from microbial breakdown. Glucose, however, diffused from the aggregates and was consumed. Disruption of aggregates greatly increased starch breakdown.

Micro-organisms and the soil P cycle

Uptake of P by soil micro-organisms

Plants and soil micro-organisms may compete for orthophosphate from the soil. Estimates of the comparative rates of P uptake by plants and micro-organisms (Beever and Burns 1977)

show that micro-organisms may have more efficient uptake mechanisms than plant roots (Table 2).

Table 2. Potential inorganic P (P_i) uptake rate of the soil flora (from Beever and Burns 1977).

	Estimated fresh weight biomass (1) (g)	Calculated P_i uptake rate in 10 µM P_i (2) (µM P min^{-1})	Ratio of calculated uptake relative to plant roots
Bacteria	103	359	9.0
Fungi	260	71	1.8
Plant roots	5 000	40	1

(1) Biomass per 1m x 1m block of soil, 10 cm deep. Bacterial and fungal figures based on Clark and Paul (1970) assuming a d.w./f.w. ratio of 0.2. Root figure based on value of 50 cm cm^{-3} for root length per unit volume given by Barley (1970) and assuming a conversion factor of 1 mg f.w./cm root length.

(2) Calculated from kinetic parameters assuming all fungi behave like *A. nidulans*, bacteria like *E. coli*, and plant roots like millet.

Bacteria, with their large specific surface area, can absorb P effectively from the low concentrations (about 0.03 mM P depending on pH) at which P compounds are in equilibrium in soil solution (Nye and Tinker 1977). Rosenberg et al (1977) showed *E. coli* could take up orthophosphate (P_i) by two kinetically different systems. The higher affinity system (low k_m), apparently involved a phosphate-binding protein, and was activated only at low internal P concentrations. Beever and Burns (1980) outlined a similar system of P uptake for fungi, whereby two separate uptake systems operated in parallel. The first system had a high affinity for P (low k_m). The second system had a low affinity for P (high k_m). Total uptake was due to simultaneous operation of both systems.

Micro-organisms are also ubiquitous in soil (Beever and Burns 1977). At a given point in soil there are likely to be more micro-organisms in close proximity than plant roots. Micro-organisms are likely to be in a better position to take advantage of localised nutrient sources

than roots, which need to grow towards nutrient sources. This confers a competitive advantage on micro-organisms over plants for uptake of nutrients in soil.

Immobilization of P by soil micro-organisms

Immobilization of a nutrient occurs when C substrate is available to the microflora in proportionally larger amounts than nutrient elements (St John and Coleman 1983). At low concentrations of P, plants may suffer a deficiency induced by microbial P uptake (Barber and Loughman 1967, Benians and Barber 1974, Bowen and Rovira 1969). Elliott et al (1979) found more P was immobilized by bacteria with increasing amendments of glucose than in unamended soil. Soil micro-organisms may take up as much P from solution as a crop (Hayman 1975). McLaughlin et al (1987) found wheat plants took up 12% and micro-organisms 14% of added fertilizer P over a 22 day period.

It is difficult to predict the importance of microbial uptake of phosphate in the field on plant growth. Much depends on the rate of arrival of phosphate at the root surface, continuity of micro-organisms around the root, and their capacity to absorb and store phosphate (Bowen and Rovira 1969). If the bacterial population is rapidly growing, limiting elements released by death of a cell will be immediately taken up by nearby cells - a given ion may move no further than the first cell in its diffusion pathway (St John and Coleman 1983).

When micro-organisms are adequately supplied with P, they do not seem to affect plant growth or plant P uptake adversely (Barber 1966, Benians and Barber 1974) and may increase P uptake in young seedlings (Campbell 1977). Barber et al (1976) concluded this effect was due to the production of plant growth substances by microflora, with plant susceptibility to stimulation decreasing with age.

Microbial uptake of inorganic P and conversion to organic compounds (immobilization) may benefit plants and grazing animals in itself - Bromfield and Jones (1972) found microbial uptake decreased the leaching of P from senesced pasture plants.

Loss of P from plants after death is considered to be the main route by which plant P returns to the soil. Birch (1961) noted that a large proportion of plant P was inorganic. Martin and Cunningham (1973) found root P could be released into solution by autolysis before an extensive microbial population had developed and was not necessarily incorporated into micro-organisms decomposing the roots. They suggested this was due to the autolytic activity of plant enzymes. Dalal (1977a) thought it was the inorganic fraction in plant residues which then supported the metabolic and synthetic process of micro-organisms in

initial stages of decomposition. During decomposition of plant residues there is an increase in the microbial population and hence a greater demand for phosphate (Hayman 1975). The rate of residue decomposition is related both to its own total nutrient concentration and that proportion degradable by micro-organisms (McGill et al 1981). The importance of organisms during decomposition of plant residues appears to be linked more to their assimilation of P_i released from plant material than the hydrolysis and assimilation of organic P (P_o) (McLaughlin 1987).

Mineralization of soil P by micro-organisms

Mineralization is the release of P_i from organic compounds. McGill and Christie (1983) proposed two types of nutrient mineralization - biological and biochemical mineralization. Biological mineralization is the release of elements as waste products during the oxidation of C compounds to provide energy. It occurs within the cell membrane and releases elements directly associated with carbon (N and C-bonded sulphur). Biochemical mineralization is the release of elements by extracellular enzymes in response to demand for a particular element. It occurs outside the cell and involves elements found as esters and related structures (e.g. P released by phosphatase enzymes).

In the atmosphere, the C, N, sulphur (S) and P cycles are linked by chemical reactions. In soils they are primarily linked by growth processes of soil organisms. The soil P cycle is altered by the cycles of C, N and S primarily through alterations of soil microbial and plant environments (by N and S) and demand for P (regulated by C and N).

Factors affecting P mineralization and immobilization in soil have been covered in a review by Tate (1985). Organic P is mineralized in soil largely through the activities of soil micro-organisms and free enzymes (phosphatases) present in soil; both extracellularly, and intracellularly (released by lysis of microbial cells) (Dalal 1977a).

Mineralization and immobilization of plant nutrients are concurrent processes in soil. Adding organic material to soil provides a source of energy and nutrients - nutrients which may be released as the organisms release and assimilate C (McLaughlin 1987). The same processes may also occur with "native" soil organic matter. P_o contributes to the nutrition of plants primarily after being mineralized into P_i (Dalal 1977b). In many laboratory studies where microbial activity has been stimulated by adding a C source, changes in soil P distribution have been observed (Hedley et al 1982), showing withdrawal of P from the plant-available pool (immobilization) or addition to it (mineralization). Many studies have tried to relate net mineralization or immobilization to the C:P ratio of residues being decomposed. Critical C:P

ratios collated by McLaughlin (1987) ranged from 55 to over 500. This variation reflects the heterogeneity of the soil biomass, not only in species but in the age and metabolic status of micro-organisms.

The change from net immobilization to net mineralization depends on the C:P ratio of the material actually being used by organisms, not that of the material being added (White 1981). Care must also be taken with nutrient contents as the form of nutrient in the plant may change with age, e.g. a young plant may have the same absolute nutrient concentration as an older one, but the nutrient could be in a form easier to break down (Till et al 1982). Net immobilization can occur during the early stages of plant decomposition, followed by net mineralization as the C:P ratio of the residue is lowered by CO_2 evolution (Chang 1939, Alexander 1977).

The rate of mineralization of soil P_o, rather than the amount of P_o in soil is the main factor that determines the availability of P_o to plants.

The spatial distribution of biodegradable P_o (e.g. whether physically protected as aggregates) may determine the rate of mineralization. Tarafdar and Claasen (1988) suggested that availability of hydrolysable P_o was the limiting factor on plant utilization of organic P. Rates of P_o mineralization are normally measured by chemical determination of the net change of P_o or P_i with time. This can involve errors such as physical and chemical variation within and between soil samples, the use of strong acid extractants (which can hydrolyze some P_o) and the difficulty of accurately recovering P_o and P_i from soils (Harrison 1982a).

The balance between mineralization and immobilization of P in soil is strongly influenced by temperature, which can cause large seasonal fluctuations in net immobilization or mineralization (Tate 1985). Dormaar (1972) found the P_o concentration of irrigated soil under lucerne pasture varied from 102 to 20 $\mu g\ P\ g^{-1}$ soil during a six-month period. This fluctuation was linked to temperature and seasonal effects. Floate (1970) found P_o mineralization increased with temperature from 5 to 30°C. The effect of temperature on P mineralization means that in temperate soils P_o is mineralized more slowly by cultivation than C,N, or S. This results in an increased proportion of P in soil organic matter. In tropical soils, however, P_o is mineralized at about the same rate as C, N and S (Dalal 1977a). At temperatures below 30°C, immobilization of P by soil micro-organisms is increasingly favoured (Tate 1985).

Mineralization and immobilization of P presumably affects soil polyphosphate content. There is, however, no published work on temporal variations in soil polyphosphate concentration.

Adding P_i to soil may cause net immobilization (Chauhan et al 1981, Hedley et al 1982), mineralization (Enwezor 1966) or have no effect (Dalal 1977a, Ghoshal 1975) depending on whether or not uptake of added P_i is followed by enhanced mineralization of native soil P_o. This in turn depends on the availability of energy sources to micro-organisms. Chauhan et al 1981 found P_i accumulated in soil when added without an energy source (cellulose or glucose). When added with an energy source, P_i was immobilized in various P_o forms.

The presence of plant roots affects the use of soil P, through factors such as the reduction of P concentration in soil solution, release of phosphatase enzymes, stimulation of microbial growth, and pH changes in the rhizosphere. P_o competes with P_i for sites on soil surfaces. This keeps P_o in a form less vulnerable to mineralization (Tate 1985). In the absence of plants, adding P_i can release P_o from exchange sites and cause mineralization.

In the presence of plants, P_i addition can stimulate immobilization by microbial response to increased plant growth. McLaughlin and Alston (1986), using double-labelling techniques, found P_i addition to wheat plants increased the immobilization of P_i in the microbial biomass.

Death of the biomass

Seasonal fluctuations in extractable P concentrations in soil (Felleca et al 1983) may be related to microbial death and turnover. Water is a major factor determining microbial survival in soil and has been discussed previously. Following summer drying of soil, microbial P could contribute to the autumn flush of plant growth after rewetting of soil (Sparling et al 1985). Air drying and grinding prior to analysis is part of soil testing procedure. Many of the common soil P analyses may over-estimate available P due to release from the microbial biomass and other soil organic matter by soil treatments used in routine soil testing. Problems may arise where soils have the same extractable P concentration but differ in microbial P content. Although a soil test of each will give the same result, there may be large differences in the amount of P available to plants. Sparling et al (1987) considered the following factors on plant nutrition.

i) It is not known to what extent nutrients in micro-organisms are "plant-available". The P measured by a test for microbial P comes from a pool of P which may or may not become available to plants, depending, in an unpredictable manner, on many factors.

ii) The microbial biomass is a comparatively labile fraction of soil organic matter.

iii) The relatively slow turnover time of organic matter in Australian and British soils (1.25-2.5 years - Jenkinson and Ladd 1981) means that, under stable conditions, organic P from relatively stable soil organic matter can be expected to become available only slowly. On the other hand, because the biomass is comparatively labile, factors such as water or cultivation may cause a major reduction in biomass. On soils with a large microbial contribution to extractable P, the pools of plant-available P can change very rapidly.

Typically the biomass declines slowly when deprived of organic substrates, e.g. during soil storage (Ladd et al 1981, Marumoto et al 1982). Starvation is not likely to provide a large flush of available P as it is a gradual process, and surviving micro-organisms will have the opportunity to compete with plants for nutrients released from the biomass. To effect a significant release of P, an environmental factor must either kill a large proportion of the biomass, or continuously mineralize P_o to provide a longer-term source. Cultivation, for instance, reduced microbial C from 815 $\mu g\ C\ g^{-1}$ soil to 455 $\mu g\ C\ g^{-1}$ soil after four years (Sparling and Shepherd 1986).

Release of P from micro-organisms

Microbial cells contain a wide range of P compounds. On death of the cell, these compounds will be released into soil and will interact to varying degrees with soil colloids. Once mineralized, they will be available for further uptake by plants or micro-organisms. The rate of mineralization of these cells will depend on soil phosphatase activity as well as the type of P_o released (McKercher et al 1979, Tate and Newman 1982).

Plant uptake of P is generally as the inorganic orthophosphate ion. Although DNA may be taken up directly by barley plants (McKercher and Tollefson 1978), it only comprises 5-10% of microbial P (Stewart and McKercher 1982) , and nucleic acids are rapidly mineralized in soil in any case (Tate 1984). In general, mineralization of microbial cells must occur before plants can make use of the nutrient contained within them.

Researchers have generally investigated the decomposition of microbial cells added to soil under conditions which will encourage microbial growth, i.e. moist incubation (discussed later). These conditions may not necessarily reflect the situation found in field soil. An environmental stress which reduces the soil population will produce dead microbial material for decomposition by other micro-organisms. This reduces the diversity of the soil microbial population, through an inability of some organisms to compete under the new conditions (Alexander 1977). The microbial population of soil which has recently been subjected to

water stress will be different from that of a soil which has not. The rate of P_o mineralization depends largely upon the population as well as the activity of micro-organisms in soil (Dalal 1977a).

It would seem reasonable to assume that mineralization of microbial material would be affected by environmental stress, say, water stress, in a similar way as the decomposition of other soil organic matter, although Nakas and Klein (1979) found the susceptibility of bacterial and fungal cell walls inversely related to soil water content from 1% to 15% water w/w. They suggested that autolytic enzymes may be stimulated in dry soil, and that cell walls could be an important nutrient source in times of water stress. In wetter soil, however, Bumbieris and Lloyd (1967) found that fungal hyphae lysed faster at 25% w/w than at 13% w/w. Cell contents disappeared in all soils, but cell walls persisted in dry soil.

Three approaches have commonly been used to determine mineralization of microbial cells added to soil - addition of whole cells, separated cell walls and cytoplasm, or specific components.

Whole cells

Jenkinson (1976) compared the mineralization of C in two yeasts, two fungi, one actinomycete and one invertebrate species. He found no significant difference between bacterial and fungal mineralization and measured 50% mineralization of C in 10 days incubation. Anderson and Domsch (1978a) compared the mineralization of 12 species of bacteria and 15 species of fungi over 10 days. They found the average mineralization of bacteria was $33.3 \pm 9.9\%$ while that of fungi was 43.7 ± 5.3 %. The higher proportion of microbial C mineralized noted by Jenkinson (1976) may have been partly due to an incubation temperature 3°C higher than that of Anderson and Domsch (1978a). Jenkinson also used freeze-dried organisms of undefined age, whereas Anderson and Domsch used cells harvested late in the linear or logarithmic phase of growth which were mostly living and contained cytoplasm bounded by intact membranes.

Anderson and Domsch (1978a) attributed the differences in C mineralization between bacteria and fungi to a higher ratio of cell wall to cell contents in the bacteria. Since cell walls are generally more resistant to decomposition than cytoplasm, the group with the highest cell wall:cytoplasm ratio would be expected to be least mineralized.

Cole et al (1978) found the extractability of microbial P decreased with culture age, and attributed this to an increase in the proportion of P in cell walls and membranes which were

not extractable. Age of micro-organisms may be an important factor for the proportional recovery of microbial constituents, but one which has not been reported often in the literature, although Ross et al (1987) found proportions of C and N mineralized from fungal mycelia decreased with culture age. The effect of organism age would be very difficult to study in the field unless biological activity could be synchronized by, for example, the release of water stress (e.g. Clarholm 1981).

It would be useful to know the mineralization rates of polyphosphates added to soil within micro-organisms, but there has been no work published on the extractability of polyphosphate-rich micro-organisms added to soil.

Cytoplasm and cell walls

Researchers investigating the breakdown of cell wall and cytoplasm added separately to soil (e.g. Marumoto et al 1977, Hurst and Wagner 1969) have shown that cytoplasm generally mineralizes much faster than cell wall material. Cytoplasm leaves very little residue after decomposition, while cell wall leaves considerable residue due to the formation of resistant complexes with humus and clay minerals (Marumoto et al 1977). Wagner and Mutatkar (1968) analyzed the amino acid content of soil after 6 months incubation with ^{14}C glucose. They found the most highly labeled (resistant) group of amino acids were those found predominantly in cell walls.

As fungi form the major portion of soil biomass (Clark and Paul 1970) the decomposition of fungal hyphae in soil is of interest. The hyphae from non-pigmented (hyaline) fungi have been shown to be more susceptible to decomposition than those of pigmented (melanized) fungi (Kapoor and Haider 1982, Hurst and Wagner 1969, Linhares and Martin 1978). In melanized fungi, the melanins are mainly found in cell walls and inhibit mineralization of the cell contents and especially the walls themselves (Bloomfield and Alexander 1967, Bartnicki-Garcia and Nickerson 1962). On the other hand, Kapoor and Haider (1982) found the amount of $NaHCO_3$-extractable P from mycelial residues was relatively high and not correlated with melanization. This fraction consisted, however, mainly of P_o. Since the melanins themselves are not readily extracted by $NaHCO_3$, it seems most of this P is not specifically linked to melanins.

Mineralization of specific compounds

One approach to measurement of P mineralization in soils has been to follow the fate of specific P_o compounds added to soil. Bowman and Cole (1978) followed the mineralization

of various commercial P_o substrates in soil, during an 18 day incubation. Glycerophosphate and all tertiary nucleotide components of ribonucleic acid (RNA) were completely mineralized and accounted for in $NaHCO_3$ solution after three days. While RNA was completely degraded in 18 days, sodium inositol hexaphosphate (Na-phytate) was relatively unaffected by incubation and extraction. Harrison (1982a) used the mineralization of added ^{32}P-RNA as an indicator of rates of mineralization in woodland soils. Over 50 soils, mineralization of added RNA ranged from -3.7 to 24.4% (l.s.d. 1.3%) during 24 hours at 13°C. In a subsequent paper Harrison (1982b) related the rate of ^{32}P-RNA mineralization to soil physical and chemical properties. RNA mineralization increased with soil pH and extractable calcium content.

Availability of microbial phosphorus to plants

Extensive literature has been written on the inorganic reactions of P in soil, but little on the sorption or availability of organic P compounds. Maire (1984) determined the adsorption characteristics of ATP, which seemed to be adsorbed onto clay minerals. The amount of ATP adsorbed was related to soil composition, soil weight and the actual ATP content of the sample being analyzed. McKercher and Anderson (1989) investigated the adsorption of some P_o compounds in neutral and basic soil. They found adsorption increased greatly with the number of phosphate esters on the parent molecule. In order of increasing sorption:

monophosphate esters $\leq$ orthophosphate $<$ myo-inositol hexaphosphate.

The sorption of P_o compounds will depend on the amounts and nature of these compounds, which will depend on the microbial population in the soil, and the nature and properties of the soil itself. For example, Anderson (1980) considered P esters to be stabilized in soil by sorption with colloids found mainly in clay and silt fractions, but Campbell and Racz (1975) found P_i and P_o added as manure extract leached faster through a soil than an equivalent concentration of KH_2PO_4. This was thought to be due to coating of soil adsorption sites by organic compounds in the manure.

As well as affecting microbial populations, wetting and drying cycles can increase sorption of inorganic P (Haynes and Swift 1985). This has been attributed to the breakdown of aggregates and subsequent exposure of fresh P adsorption surfaces (Olsen and Court 1982).

To determine the availability of microbial P to plants, some workers have "fertilized" soil with microbial tissue. Kapoor and Haider (1982) added isotopically labelled hyaline and melanic fungal hyphae to soil to determine plant uptake of microbial P. Over five weeks,

wheat plants took up around 13% of hyaline and 7.5% of melanic hyphal ^{32}P. This compared with > 20% uptake of applied fertilizer phosphate. Sixty to seventy per cent of added hyphal P was extractable with $NaHCO_3$, mostly as inorganic phosphate. Stewart and Hedley (1980) added ^{33}P-labelled bacteria to soil-plant systems. In 21 days, 20% of the added ^{33}P was found in the resin-extractable ("available") form, of which 70% was taken up by the plant. Microbial P, although only 3% of the total soil P, played an important role in the distribution of P in soil. The ^{33}P labelled bacteria used, however, would probably have broken down faster than native soil bacteria, due to cell death caused by transfer, radiation damage or an exposed position in soil.

Soil treatment may have a considerable effect on biomass P. Sparling et al (1985) investigated the contribution of microbial P to the bicarbonate-extractable P of a range of New Zealand soils. Bicarbonate extraction of soil generally involves preliminary sieving and air-drying. By measuring the change in biomass C caused by sieving and air-drying, then applying this change to the initial microbial P content, Sparling et al (1985) estimated that between 4 and 76% of bicarbonate-extractable P originated from micro-organisms. This microbial component of soil P could be related to the variable response of many New Zealand grass-clover pastures to P fertilizer (Saunders and Metson 1971). Further work is needed to quantify the availability of microbial nutrients to plants and to determine those factors which control survival and mineralization (Sparling et al 1985). Allowance for microbial input to available P levels should give more reliable assessment of the fertility status of soils.

Measurement of microbial P

The first estimates of microbial P were made from measurements of microbial biomass and published values for the P contents of laboratory cultured micro-organisms (Anderson and Domsch 1980a, Halm et al 1972). P concentrations in laboratory-grown micro-organisms vary widely depending on growth conditions (van Veen and Paul 1979). Indirect estimates of P held in soil micro-organisms showed relatively large amounts could be held in the microbial biomass. For example, Anderson and Domsch (1980) reported that the microbial biomass could contain up to 83 kg P ha^{-1}. Direct estimates of microbial P became possible with the development of two variations on the fumigation procedure of Jenkinson (1976), allowing the extraction and measurement of microbial P in soils (Brookes et al 1982, Hedley and Stewart 1982).

The use of these methods has provided information not only on the size of the biomass P pool, but also on the variation in P content of the biomass (Brookes et al 1984). The methods

are based on the $CHCl_3$ fumigation procedure. P is lysed from microbial cells following fumigation of the soil with $CHCl_3$ and extracted from the soil with $NaHCO_3$, which is a relatively mild extractant and has little effect on bacterial numbers and viability (Sparling et al 1985). Some of the P released will be immediately fixed by soil colloids, so a correction factor is required. In one method (Brookes et al 1982), recovery of a spike of P_i is used to calculate this. Values obtained by the other method (Hedley and Stewart 1982) are calibrated for recovery of added microbial P for each soil type.

Microbial P is calculated from the difference in extractable P between fumigated and unfumigated soil, using a correction factor for the proportion of microbial P extracted after fumigation. Not all microbial P is extracted from soil. An experimentally determined recovery factor (k_p) is used to calculate total microbial P, i.e. microbial P extracted from soil plus than left in soil:

$$\text{microbial P} = \frac{\text{P from fumigated soil - P from unfumigated soil}}{k_p}$$

P in this "flush of fumigation" can be measured as P_i (Brookes et al 1982) or total P (P_t) (Hedley and Stewart 1982).

Brookes et al (1982) considered the microbial P flush was better measured as P_i for two reasons: Firstly, they found P_i formed the greater proportion (about 80-90%) of the soil microbial P flush. Secondly, recovery of P_i could be corrected for sorption by soil, whereas this was not possible for P_o. Hedley and Stewart (1982), however, added laboratory-grown micro-organisms to soil and found no relationship between microbial P added and microbial P_i recovered, but consistent recovery of microbial P_t from microbial P added. Choosing between these two methods would seem to be a matter of personal preference. The method of Brookes et al (1982) measures *in situ* microbial P. They found the greater proportion of this was recovered as P_i. While Hedley and Stewart (1982) found P_i was not a good indicator of microbial P recovery, they were adding laboratory-grown micro-organisms to soil. These micro-organisms may have differed from *in situ* ones in species, nutrient concentration, and placement in soil. Placement affects physical protection of micro-organisms (from biocides and extractants) and proximity to extracellular soil enzymes. Each method for measuring microbial P can be justified by the evidence given.

The range of P compounds in the soil, the diversity of P compounds in a microbial cell, and the the ease of extracting these compounds from soils with different P sorption capacities must be considered when the fumigation technique is used. The proportion of microbial P in

inorganic or organic forms within the cell depends on P concentration in the soil solution, P content of the cell, and the age of the cell. Because bacterial and fungal species differ in cell composition and P content, the proportion of microbial P measured in a fumigation extraction may differ from one species to another (Hedley and Stewart 1982). Differences in recovery within each group are also likely. The magnitude of variation in k_p from soil to soil, and from time to time in the same soil has not yet been determined or related to environmental variables. The range of published k_p values from 0.32 (Hedley et al 1982) to 0.57 (McLaughlin et al 1986) shows a need to investigate these changes.

Brookes et al (1982) and Hedley and Stewart (1982) found k_p factors of 0.37 and 0.47 respectively. Brookes et al (1982) used freeze-dried micro-organisms while Hedley and Stewart (1982) used fresh ones. The validity of these methods rests on how close the k_p values are to that of a native soil population, and this is unknown at the present time. A k_p of 0.4 has been provisionally recommended for use in determining soil microbial P (Brookes et al 1982, Hedley and Stewart 1982). Further modifications of this method have used hexanol as the biocide and 0.5 M $NaHCO_3$ as the extractant (McLaughlin et al 1986) In experiments conducted on three soils, k_p factors were determined by adding known amounts of fresh micro-organisms to soil. They found k_p factors between 0.33 and 0.57, and concluded that calibration was necessary for each soil.

Ross et al (1987) investigated the effect of culture age on recovery factors for C, N and P (k_c, k_n and k_p) of *Fusarium oxysporum* mycelia. K_c and k_n were lowest in the oldest culture, although k_p showed no consistent trends due to high variability caused by high background levels of extractable P (> 80 μg P g^{-1} soil) and high P sorption which resulted in less than 38% recovery.

A limitation of the fumigation technique used in soils with actively growing plants is interference of live root material. Fumigation may lyse root cells and release significant quantities of plant P (McLaughlin and Alston 1985, Sparling et al 1985). While most roots can be removed by hand, an undefined amount is left. This can result in an over-estimate of microbial P (McLaughlin et al 1987). Removal of roots also removes many micro-organisms in soil adhering to the root surface. In studies of rhizosphere soil, this poses a serious problem as bacteria and fungi are 20 to 50 times more abundant in the rhizosphere than in bulk soil (Rovira 1979). Competition for the small amount of P in the soil solution is most intense in the rhizosphere where substances from root exudates, sloughed-off root cells, tissues and mucigels sustain a larger and more active microbial population than in the bulk soil (Tate 1985). As roots withdraw P from the soil solution and may also exude or leak P back in the soil (McLaughlin et al 1987), the rhizosphere is a key site for P transformations.

The degree of extraction of microbial P from soil depends to some extent on the P sorption capacity of the soil. Hedley et al (1982) found recovery of bacterial P and fungal P was slightly decreased in soil with a higher sorption capacity, although Hedley and Stewart (1982) found no direct relationship between P_i sorption capacity and k_p factor. A k_p factor of 0.4 was suggested for soils with pH values ranging from 6.2 to 8.2. Sparling and Williams (1986) obtained anomalous values for microbial P in acid organic soils amended with glucose or cellulose. They suggested that standard k_p factors may not be applicable to these soils when recently amended.

A need exists for k_p factors to be investigated on a wider range of soils. More accurate determination of the size of the microbial P pool would aid P modelling in soil systems. Prediction of the amount of P_i likely to be mineralized from the soil biomass over summer and released into soil solution would allow more efficient fertilizer planning. If a plant response to phosphate fertilizer applied in autumn is not likely, then it may be more efficient to with-hold the fertilizer until spring. Whether or not a full response could be expected from autumn-applied phosphate fertilizer may depend on the amount of microbial P which has become available to plants. Sparling et al (1985) found that 4-76% of the bicarbonate-extractable P of a range of New Zealand soils originated from the microbial biomass.

The proportion of the soil microbial P flush recovered as P_o may vary with soil phosphatase activity and extraction conditions (temperature and duration of fumigation and extraction). If polyphosphates were present in soil micro-organisms, they would be found in fumigation extracts of soil as part of the P flush. These polyphosphates could be present in their original form; partly or fully hydrolyzed to orthophosphate by soil enzymes. Fumigation extracts of soil may contain P as organic (P_o) and inorganic (P_i) forms. The sum of these is defined as total P in the extract (P_t). Although polyphosphates are an inorganic form of P, colorimetric tests for P_i only measure orthophosphate and do no register the presence of polyphosphates (e.g. the phosphomolybdate complex formed by the technique of Murphy and Riley 1962). If present in a soil P extract, polyphosphates would have undergone an unknown degree of hydrolysis to orthophosphate. Intact polyphosphates would be included in the P_o fraction. If hydrolyzed to orthophosphate they would be indistinguishable from the rest of microbial or soil P_i.

Chemical extractions for soil polyphosphates (e.g. Ghonsikar and Miller 1973) have used an acid extractant (0.5 M perchloric acid) and low temperature (4°C) to minimize enzymatic hydrolysis of soil polyphosphates. Soil polyphosphates have also been found in 0.1 M NaOH

soil extracts (Tate and Newman 1982). Enzyme inactivation has occurred through extremes of pH and temperature.

Methods which have detected polyphosphates in soils have involved extractants which inactivate soil enzymes. Polyphosphates are susceptible to attack by soil enzymes (Aseeva et al 1981). The 0.5 M sodium bicarbonate extractant used for determination of soil microbial P is a mild chemical extractant and will not inactivate soil phosphatase enzymes (Speir and Ross 1978). Thus it is likely that soil polyphosphates undergo a degree of hydrolysis during fumigation and extraction for determination of soil microbial P.

Summary

Microbial P exists in a variety of cellular compounds - from orthophosphate to nucleic acids. After cell death, a proportion of these compounds will be hydrolysed to inorganic orthophosphate; available for uptake by plants or micro-organisms. Polyphosphates are P storage compounds which may accumulate in microbial cells when P is not limiting. Their accumulation and hydrolysis has been studied in solution culture and soil, but they do not seem to have been used as indicators of microbial P status. The amounts, distribution, temporal and spatial variation of polyphosphates in soil have not been determined.

Micro-organisms in soil include bacteria, actinomycetes, fungi and microfauna. Bacteria and fungi are recognized as leading participants in soil biochemical reactions, although microfauna are significant through the effect of their predation on the two major groups of organisms.

The size of the microbial biomass in soil is dependent on the supply of substrate, modified by environmental limitations such as temperature and water. In general, the more organic input to soil, the more microbial biomass is present (e.g. permanent pasture vs fallow). As the size of the microbial biomass increases, it forms an increasing proportion of soil organic C. Mycorrhiza play an important part in the soil P cycle but no accurate method has yet been developed to define what proportion of the microbial biomass they constitute, although microscopy may provide a crude estimate.

Microbial population changes in soil are very complex, resulting from the interplay of environmental conditions and the particular adaptive strengths of each species. As conditions

arise which are favorable for one species, that species will dominate. The soil has a large variety of micro-organisms awaiting their chance to flourish given the right conditions.

Uptake of P by micro-organisms is enhanced by conditions favouring microbial growth. Net immobilization of P by the biomass will occur where P is limiting microbial growth. Death of the biomass may release a flush of inorganic P, but microbial populations can recover and compete with the plant for available P. Micro-organisms are a moderately labile P source and the contents of microbial cells are rapidly mineralized in soil.

Fumigation/extraction of P is a neat method of directly measuring microbial P, although use of a standard k_p factor may cause error.

Questions which need to be addressed include the relationships between P nutrition, polyphosphates and subsequent P uptake by micro-organisms, and the occurrence and origin of polyphosphates in soil.

CHAPTER THREE: Effect of amendment of soil with root material on microbial biomass

Introduction

Microbial P is calculated from the difference in inorganic extractable P (Brookes et al 1982) or total extractable P (Hedley and Stewart 1982) between fumigated and unfumigated soil, using a correction factor (k_p) for the proportion of microbial P extracted after fumigation.

The magnitude of variation in k_p has not yet been determined or related to environmental variables. Published values range from 0.32 (Hedley and Stewart 1982) to 0.57 (McLaughlin et al 1986). K_p values are obtained by adding known amounts of laboratory-grown micro-organisms to soil, and measuring recovery of the added microbial P. Separate calibrations are required for each soil. At present, a k_p factor of 0.4 is generally used. Although changes in k_p cannot be measured *in situ*, due to the difficulty of isolating sufficient whole, unchanged micro-organisms from soil for analysis, changes in the soil microbial population can be qualitatively shown by the Gram positive:Gram negative ratio of soil bacteria.

One factor which may contribute to differences in microbial populations (and hence k_p) in the same soil is plant type. Medic (*Medicago trunculata* L.) and wheat (*Triticum aestivum* L.) provide the basis of cereal-livestock farming which is the principal farming system in much of southern Australia. Adding root material from medic and wheat plants to soil may produce differences in the microbial populations which are stimulated by the two types of substrate.

The aim of this experiment was to determine if one variable, namely substrate type, had a detectable, quantitative effect on microbial C and P, and the ratio of Gram negative:Gram positive bacteria in a single soil. Gram staining is correlated with major differences in the chemical composition and ultrastructure of bacterial cell walls (Stanier et al 1986) and is a measure of physiologically different species of bacteria. Changes in this ratio would show differences in microbial populations between soils in each treatment. Microbial C and P provide more information on the biomass in each soil. Significant differences between soils amended with different substrates would show that substrate type affects the composition of the soil biomass and that the practice of using standard k_p factor on the same soil under different crops may need to be carefully evaluated.

Materials and Methods

The soil used was the 0-100 mm layer of a solonised brown soil (of high P status) from Mallala, South Australia (Table 3).

Table 3. Description of soil.

Sand(%)	55
Silt(%)	24
Clay(%)	21
pH_w(1:5)	8.3
Gravimetric water content at -66 kPa (%)	23.8
Organic C (%)	1.50
Organic N (%)	0.17
Land Use	Cultivated wheat/pasture
Olsen P[1] ($\mu g\ P\ g^{-1}$ soil)	16.6
Soil Classification(USDA)	Calcixerollic xerochrept

[1]Olsen et al (1954)

Four substrates (glucose, cellulose, medic roots and wheat roots) were mixed into soil in separate treatments. Soil in two control treatments was disturbed in a similar manner at the same time, without the addition of substrate. The six treatments (glucose, cellulose, medic roots, wheat roots, control, control) were each replicated four times. Glucose and cellulose were of analytical grade.

The chopped fresh roots of young medic or wheat plants, glucose or cellulose powder were added to soil at equivalent rates of C. Microbial C, P and bacterial Gram staining were examined to determine differences in microbial populations caused by the substrates added.

Samples of air dry soil equivalent to 20 g oven-dry soil were wetted to 23.8 % gravimetric water content (60% water holding capacity) and incubated in 60 ml specimen jars (capped with plastic film) at 20°C for 21 days to allow microbial activity to stabilize. Samples were uncovered and watered to 60% w.h.c. every 2-3 days during incubation.

Young medic and wheat plants were grown to provide root substrates. Medic plants were grown in plastic trays (350 x 250 x 120 mm) containing 5 kg of sand packed to a bulk density

of 1300 kg m^{-3} into which a basal dressing of $CaCO_3$ (3 mg g^{-1} sand), $CaSO_4$ (0.2 mg g^{-1}) and $Ca(NO_3)_2.4H_2O$ (16.87μg g^{-1}) had been mixed. Sixty germinated medic (*Medicago trunculata* cv Paraggio) seeds were placed 2 cm below the surface of the sand. Sand was watered to 15% gravimetric water content (-50 kPa). Plants were grown for 21 days at 20°C (day), 15°C (night), photosynthetically active radiation was 500 μmol quanta m^{-2} s^{-1} during the 12 hour photoperiod. Sand was watered daily to -50 kPa. At 0 and 14 days, a solution containing 62.49 mg KH_2PO_4, 24.99 mg $MgSO_4.7H_2O$, 2.49 mg $FeSO_4.7H_2O$, 2.49 mg $MnSO_4.4H_2O$, 1.5 mg $ZnSO_4.7H_2O$, 1.0 mg $CuSO_4.5H_2O$, 1.0 mg H_3BO_3, 99.9 μg $CoSO_4.7H_2O$, and 100 μg H_2MoO_4 was added to each tray. Basal fertilizers and nutrient solutions were standard mixtures for these plant species.

Sixty germinated wheat seeds were grown under the same environmental conditions as medic. The basal fertilizer for wheat was 0.2 mg $CaSO_4$ g^{-1} and 16.87 μg $Ca(NO_3)_2$ g^{-1} sand . The combined nutrient solution for wheat was half strength that of medic solution, except for KH_2PO_4, which was the same. No basal nutrients were added used because the soil was taken from a site well supplied with nutrients.

After 21 days, plants were harvested, roots washed and cut into about 3 mm lengths. Soil was amended with cellulose or glucose (600 mg C kg^{-1} soil), medic roots (650 mg C and 6 mg P) or wheat roots (550 mg C and 4 mg P).

Time of peak CO_2 evolution was determined for each substrate in a preliminary experiment in which amended soils were incubated in 1 litre preserving jars with a vial containing 10 ml 1N NaOH to trap evolved CO_2. NaOH was changed daily (4-hourly for glucose). A vial containing 10 ml H_2O was included in the jar to prevent soil desiccation. CO_2 evolved was determined by titration with 0.1 N HCl using a Radiometer autotitrator. CO_2 evolution curves were fitted by eye to graphed data points.

Soils sampled at peak CO_2 evolution were tested for substrate induced respiration (S.I.R.) (Anderson and Domsch 1978b) using the modification of West and Sparling (1986) which involved adding glucose in solution. The CO_2 evolved from two until five hours after substrate addition was measured in a Series 225 infra-red Gas Analyzer (Analytical Development Co.); 1ml of sample being injected with a syringe. Values of CO_2 evolved are the average hourly rates over this time. Microbial C was not calculated as this alkaline soil had not previously been calibrated for this method. S.I.R. results are thus a relative indication of differences in microbial C between treatments.

Microbial P was determined by the fumigation-extraction method (Brookes et al 1982) using modifications by McLaughlin et al (1986) and a standard k_p factor of 0.4.

Gram-positive and gram-negative bacteria were determined by dispersing about 1.5 g soil in 60 ml of filtered 0.5% agar (70°C) with a Branson B12 sonifier, (full power for 2 minutes). Around 0.05 ml of dispersed suspension was placed onto a clean microscope slide. Slides were Gram stained, then the Gram ratio counted over 10 fields per slide, 2 slides per replicate, using a micrometer eyepiece and Gallileo binocular eyepiece microscope (1700x).

Results

Times of peak CO_2 evolution are shown in Table 4:

Table 4. Peak CO_2 evolution times of amended soils (estimated from CO_2 evolution curves).

Substrate	Time after substrate addition (h)
glucose	12
medic	200
wheat	200
cellulose	200

Due to the large differences in peak CO_2 evolution times between glucose and other substrates, two control treatments were used. One was analysed 12 hours after substrate addition, the other 200 hrs after substrate addition.

Glucose and cellulose amendments did not significantly increase soil CO_2 evolution (Figure 4). Medic and wheat amendments significantly increased CO_2 evolution but were not significantly different from each other.

Medic soil contained the most microbial P (Figure 5). There were no other significant effects of substrate amendment.

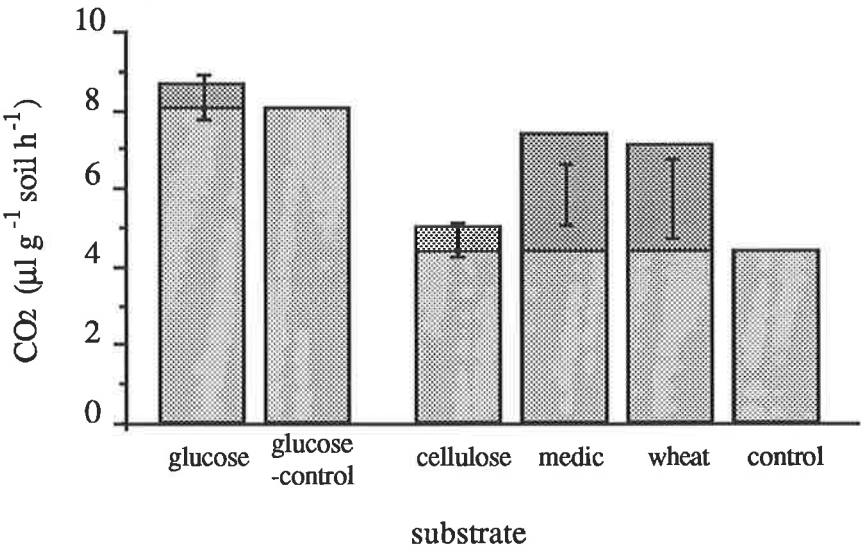


Figure 4. Substrate induced respiration of amended soil sampled at peak CO_2 evolution (average substrate induced respiration from two until five hours after substrate addition). Error bar = l.s.d.$_{(p=0.05)}$ relative to control treatments. Light shading = respiration of control sample. Dark shading = respiration greater than that of control samples.

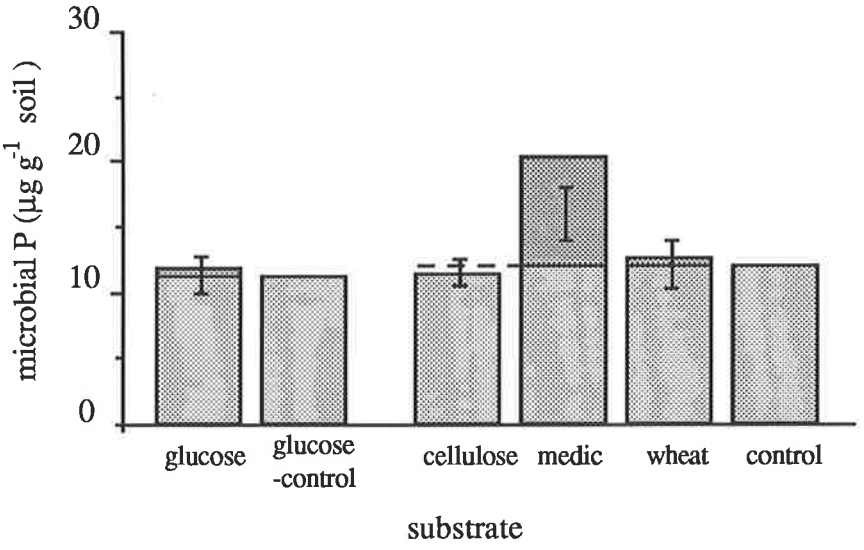


Figure 5. Microbial P of amended soil sampled at peak CO_2 evolution. Error bar = l.s.d.$_{(p=0.05)}$ relative to control treatment. Light shading = microbial P concentration of control sample. Dark shading = microbial P concentration greater than that of control samples.

Table 5. Gram positive:Gram negative ratios of bacteria in amended soil (Gram negative = 10).

Treatment	Gram positive: Gram negative ratio	Significance (relative to control)
glucose	9.7	n.s.
glucose-control	9.0	
medic	10.4	n.s.
wheat	9.4	n.s.
cellulose	14.6	*
control	10.1	

significance: n.s. = $p>0.05$
* = $p<0.05$

Cellulose amendment increased the Gram positive:Gram negative ratio of soil bacteria. There were no other significant differences.

Discussion

Medic and wheat amendment significantly increased substrate induced respiration (S.I.R.). Soil in the glucose-control had higher S.I.R. (8.05 µl CO_2 g^{-1} h^{-1}) than control soil (4.41 µl). This was probably due to physical disruption caused by mixing substrate with soil (Rovira and Greacen 1957, Adu and Oades 1978b), as the respiration of all treatments (including controls) increased, then tailed off immediately after mixing with substrate (data not shown). Peak CO_2 evolution from glucose addition occurred 12 h after substrate addition, much earlier than other substrates (200 h).

The small responses of the biomass to additions of glucose and cellulose may be due to the fact that these substrates supply carbon only. Microbial response to substrate can be limited by nutrients other than carbon (Stotzky and Norman 1961). The soil used had been incubated moist for several weeks and some available nutrients may have been at a low level.

Soil respiration results must be treated with caution, however, as Sparling and Williams (1986) found SIR to be unreliable on recently amended soil.

The microbial P values in this experiment are similar to those found by McLaughlin and Alston (1986) in a pot experiment with the same soil (19-25 $\mu g\ P\ g^{-1}$ soil). Medic amendment gave the largest value for microbial P; the addition of other substrates did not significantly increase microbial P. The higher P content of medic roots (25 mg $P\ g^{-1}$ root) than wheat roots (20 mg $P\ g^{-1}$ root) may have contributed to the high microbial P value for medic. The differences in root P content, however, are minor compared to the differences found in microbial P. These results imply that P may have been more available in medic than in wheat roots, although other nutrients may also have been involved.

Gram negative bacteria tend to dominate the rhizosphere (Rovira and McDougall 1967), with its greater abundance of readily-utilizable substrates, and I would have expected glucose to have stimulated these bacteria. Possibly the bacterial population did not have enough time to develop in the 12 hours between substrate addition and sampling. Varying lag periods in bacterial numbers after glucose addition have been reported in the literature. Although Paul and Johnson (1977) found bacterial numbers increased rapidly after 6 hours, Anderson et al (1978) found bacterial numbers did not increase significantly until 24 days after glucose amendment. Anderson and Domsch (1978b) based their S.I.R. biomass technique on the assumption that biomass would not increase significantly within 6 hours of glucose addition. Anaerobisis caused by high oxygen demand from rapid decomposition of glucose probably did not limit respiration, due to the small amount of glucose added.

Cellulose stimulated a population of bacteria with a high Gram positive:Gram negative ratio. This may have shown the presence of a different suite of bacterial species (able to utilize cellulose) from those consuming other substrates. Cellulose requires hydrolysis before assimilation and its carbon is not immediately available to all micro-organisms. In fact, a significant proportion of rhizosphere bacteria are unable to utilize cellulose (Newman 1985).

The differences found in biomass P between soil amended with wheat and medic roots suggest that the soil biomass is reacting differently to additions of these two substrates. As root material is a major source of substrate for the soil biomass (Newman and Watson 1977), this shows that in the same soil, biomass composition may vary with crop species.

Changes in the chemical composition of the biomass were caused by substrate type. This implies that substrate type may also affect k_p. To extend these findings to the field situation; the use of a single k_p factor across a range of crops on the same soil may be questionable.

Conclusions

The aim of this experiment was to compare the effects of amendment with medic or wheat roots on soil biomass P and C, and to compare these substrates in turn with cellulose and glucose. The latter two substrates are carbon sources of contrasting availability - glucose, which is immediately available to most soil micro-organisms, and cellulose, which is available primarily to those micro-organisms possessing cellulolytic enzymes. Glucose and cellulose had no effect on substrate induced respiration or microbial P in the soil, but cellulose increased the ratio of Gram positive:Gram negative bacteria. Although both wheat and medic roots increased substrate induced respiration, only medic significantly increased microbial P. The two plant substrates differed in their effect on soil microbial P.

Significant differences in P content have been shown between micro-organisms stimulated by these substrates. This implies a variation in growth and nutrition of micro-organisms associated with different species, and that the use of a single k_p factor may not provide an accurate value of microbial P in soil from under both wheat plants and medic plants.

Plant species (substrate type) may affect the cycling of phosphorus in the biomass and requires further investigation.

CHAPTER FOUR: Effect of P nutrition on P uptake and polyphosphate content

Introduction

In recent years much work has been done on quantifying P in the biomass (e.g. Brookes et al 1982; Hedley and Stewart 1982, Sparling et al 1987), but little is known about the degree of competition between micro-organisms and plants. Beever and Burns (1977) considered that micro-organisms may have several advantages over plants with respect to P uptake - they are ubiquitous, have a higher surface area:volume ratio, and a more effective P uptake system than plant roots, and may therefore be at an advantage in the acquisition of P_i in soil.

Some of the P in the biomass may be present as polyphosphates. Polyphosphates are accumulated by many micro-organisms as P and energy storage molecules (Harold 1966, Kulaev 1979). As polyphosphates are unlikely to be formed during times of P deficiency, their presence should indicate adequate P nutrition of micro-organisms.

It would seem logical that micro-organisms growing in conditions which allow luxury uptake of P, as shown by the presence of polyphosphates, should compete less strongly for a fresh supply of P than micro-organisms which had been grown under P-limited conditions and which contained little or no polyphosphate. Clipson et al (1987) found P uptake by a Basidiomycete fungus was reduced by changing pretreatment from 10 μM P to 10 mM P. Polyphosphates are known to accumulate only when P is not limiting growth, thus the presence of these polyphosphate compounds might indicate a reduced requirement for added P, compared with micro-organisms not containing polyphosphates.

A predictor of microbial P uptake may improve the efficiency of P fertilizer use by showing how strongly the soil biomass will compete with plants for added P fertilizer. McLaughlin et al (1987) found wheat plants took up 12% and micro-organisms 14% of added fertilizer P in a solonised brown soil over a 22 day period, showing that micro-organisms can take up a considerable proportion of added fertilizer P. An indication of the potential P uptake of soil micro-organisms may improve understanding of fertilizer uptake, and allow prediction of the likely fate of fertilizer added to soil. This may lead to more efficient tailoring of fertilizer rates to soil, plant and microbial requirements.

Most investigations of polyphosphates in bacteria and fungi have been made on micro-organisms grown in either P-free media or culture media with very high P concentrations (relative to soil conditions). Thus differences in polyphosphate synthesis have been demonstrated by drastic changes in P nutrition. It has been established that many micro-organisms synthesize polyphosphates in conditions of abundant available P and do not synthesize polyphosphates when microbial growth is limited by P (Kulaev 1979).

The aims of this experiment were two; firstly to see if polyphosphate synthesis could be initiated by the change from slightly P deficient nutrient solution to a nutrient solution adequate in P, using a relatively small (four-fold) increase in P concentration; secondly, to see if the presence of these polyphosphates could be used to indicate subsequent P uptake from solution by the micro-organisms concerned.

Methods and Materials

Experimental Design

Bacterial and fungal species were grown singly in solution culture of either low or high P concentration. Subsequent P uptake from nutrient solution of intermediate P concentration was measured and compared to the polyphosphate content of the bacteria and fungi.

Two species of bacteria and two species of fungi were used. The bacterium *Enterobacter sp* and the fungus *Thanatophorus cucumeris* were isolated from soil, while standard cultures of *Aerobacter aerogenes* and *Mucor racemosus* were used. *A. aerogenes* and *M. racemosus* were chosen because these micro-organisms are known to be capable of polyphosphate synthesis (Smith et al 1954; James and Casida 1964).

The organisms were grown in solutions of low or high P concentration until the late linear growth phase. They were then harvested under sterile conditions, washed and transferred to solution of intermediate P concentration. Micro-organisms were analyzed for P (including polyphosphates) when harvested from nutrient solution of low or high P concentration. This allowed the P composition of micro-organisms to be related to their subsequent P uptake from nutrient solution of intermediate P concentration. All solutions and growth vessels were autoclaved before use; harvesting and transfer of organisms was carried out under sterile conditions. Checks on the purity of cultures (clarity of fungal solutions, slides of bacterial media) showed no contamination.

For the ease of reference, micro-organisms which had been previously grown in nutrient solution of low P concentration are referred to as "low P" micro-organisms. Correspondingly, those previously grown in nutrient solution of high P concentration are referred to as "high P" micro-organisms

Bacteria

Bacterial inoculum was cultured on tryptic soy agar (Martin 1975) before being washed into 125 ml of nutrient solution of high P concentration. This was shaken at room temperature on a wrist-action shaker (200/min.) to produce inoculum for the main experiment. One ml of inoculum was then added to 500 ml of nutrient solution.

Bacterial nutrient solutions contained the following compounds (per litre H_2O):

2.50 g glucose, 1.32 g $(NH_4)_2SO_4$, 0.74 g KCl, 0.284 g Na_2SO_4, 0.384 g citric acid, 0.256 g $MgCl_2.6H_20$, 0.222 mg $CaCl_2$, 4 mg ZnO, 54 mg $FeCl_3$, 20 mg $MnCl_2$, 2 mg $CuCl_2$, 5 mg $CoCl_2$, 0.6 mg H_3PO_4, 0.1 mg Na_2MoO_4

Nutrient solutions also contained orthophosphate (KH_2PO_4 and K_2HPO_4) as shown in Table 6 to provide a range of P:C ratios giving P deficiency in solutions of low P concentration but not in solutions of high P concentration (Poindexter and Eley 1983). Preliminary experiments determined that P deficiency limited bacterial growth in solution of low P concentration.

Table 6. Phosphorus and carbon analyses of bacterial nutrient solutions.

Solution	NaH_2PO_4 (mg l^{-1})	P:C ratio (mg P g^{-1} C)	P concentration (mM P)
low P	22	4	0.15
intermediate P	51	9	0.33
high P	103	19	0.70

Growth of bacteria was determined by relating the measured light absorbance of the culture (660 nm) to calibration curves prepared by measuring the dry matter content of bacterial cultures over a range of absorbances. Cultures of bacteria grown in solutions of low and high P concentration were calibrated separately.

Bacteria were harvested by centrifugation (10 000 g for 20 min.), washed with cold distilled water and re-centrifuged twice before suspension in 500 ml intermediate P solution. This solution was sampled hourly for five hours for determination of growth and solution P concentration. Three replicates of all treatments were used.

Fungi

Fungi were cultured on NDY/6 agar containing the following compounds (per litre H_2O): 1.6 mg $FeSO_4$, 10 mg Difco yeast extract, 10 mg $MgSO_4.7H_2O$, 10 mg KCl, 160 mg KH_2PO_4, 33 mg $NaNO_3$, 5.0 g sucrose and 15 g agar. Fungi were then grown in 125 ml of solution of high P concentration to provide inoculum. Inoculum was homogenized (Sorvall Omni-mixer, 1 minute in ice bath) before use to allow delivery by volume (1 ml per sample). The finely chopped hyphae provided a greater number of regrowth points than entire hyphae; this aided uniform distribution of inoculum between samples

Nutrient solutions for the fungi were adapted from de Beer's rhizoctonia solution (de Beer, 1965) by varying the P content. Note that fungal growth was limited by P deficiency in the low P nutrient solution. Solutions contained the following ingredients (per litre of H_2O):

Low P solution: 18.5 g sucrose, 5 g peptone (2.2 mg P g^{-1}), 133 mg $MgSO_4.7H_2O$.

Intermediate P solution: 17.0 g sucrose, 7 g peptone, 133 mg $MgSO_4.7H_2O$, 11.1 mg KH_2PO_4, 16.1 mg K_2HPO_4

High P solution: 17.0 g sucrose, 7 g peptone, 133 mg $MgSO_4.7H_2O$, 33.1 mg KH_2PO_4, 47.9 mg K_2HPO_4

The P:C ratios of the nutrient solutions are shown in Table 7:

Table 7. Phosphorus and carbon analyses of fungal nutrient solutions.

Solution	P:C ratio (mg P g^{-1} C)	P concentration (mM total P)
low P	1.1	0.40
intermediate P	2	0.74
high P	4	0.94

The experiment was performed in two parts. In Part 1 fungi were grown in shaken culture at room temperature in loosely capped 60 ml polypropylene centrifuge bottles containing 30 ml of solution. Growth curves were determined for each solution and fungal species. Fungi were harvested by filtration (Whatman GF/C filters), washed twice with H_2O, then transferred to intermediate P nutrient solution (shaken). Every three hours, a 2 ml sample of nutrient solution was analyzed for P_i and total P.

Due to variation in hyphal weights within each treatment, growth and hyphal P concentration during uptake from intermediate P solution was determined on a separate series of samples in Part 2. Mycelium for this part were grown under the same conditions as those in Part 1, but were cut in half at harvest. One half was immediately dried, weighed and digested for total P. The other half was placed into solution of intermediate P concentration for 12 hours, then dried, weighed and analyzed for P. Changes in dry matter and hyphal P concentration from hyphae in Part 2 were applied to samples in Part 1 and allowed a P balance to be calculated for the system, including hyphal growth and changes in hyphal P concentration during the experiment. Hyphal variability in Part 2 was less than that in Part 1, as data for dry matter and hyphal P concentration were obtained from portions of the same hyphae.

Hyphae were oven-dried (24 hours at 90°C) after uptake from intermediate P nutrient solution for dry matter determination. Polyphosphate content before uptake from nutrient solution of intermediate P concentration was determined on a separate series of samples grown under the same conditions. Four replicates of all treatments were used.

Analyses

Standard analytical methods were used for polyphosphate analysis of bacteria and fungi as the objective was to test theories using proven methods, rather than to focus on method development. No cross-comparison of the efficiency of the extraction methods was made.

When initially harvested from low or high P nutrient solution, the bacterial culture was sampled for polyphosphate analysis of bacteria (Poindexter and Eley 1983). Duplicate 50 ml samples were centrifuged, washed and dissolved in 12.5% $NaHClO_4$ for 1 h. Samples were centrifuged and suspended in 1 mM EDTA before a final centrifugation. The residue was hydrolysed in 1N HCl at 100°C for 10 minutes and P released by this hydrolysis was taken as polyphosphate.

Qualitative confirmation of this method was given by a simple microscopic staining technique for polyphosphate using methylene blue stain (McEwen 1949). Bacteria containing P labile in 1N HCl were lightly stained overall with small areas of intense staining. Bacteria without labile P were clear and difficult to see. Labile P extracted with $NaHClO_4$ will now be considered synonymous to polyphosphate.

Separate bacterial samples were centrifuged, dried and weighed for dry matter analyses. Dried samples were digested in nitric/perchloric acid for determination of total P.

Polyphosphates were extracted from fungal hyphae with distilled water (30 minutes at 4°C) after a pretreatment of cold ethanol/ether (3:1 solution, 4°C for 10 minutes) as recommended by Kulaev (1979) for extracting unmodified polyphosphate compounds. Replicates were bulked and concentrated (to provide the high P concentrations required), and analyzed by ^{31}P nmr. Recovery of added sodium tripolyphosphate (1.0 mg per sample) showed no significant hydrolysis during the concentration procedure.

Samples of hyphae and nutrient solution were digested in nitric/perchloric acid for total P (Olsen and Sommers 1982). All samples for P determination were analyzed on an autoanalyser using the molybdate-ascorbic acid method of Murphy and Riley (1962).

Each microbial species was analysed separately in all cases. Pairwise statistical comparisons were made between each treatment at each sampling time.

Results

Bacteria

There were no significant dry matter changes (growth) during the experiment as shown in Figure 6 a) and b). Differences in initial dry matter were due to different amounts of inoculum added.

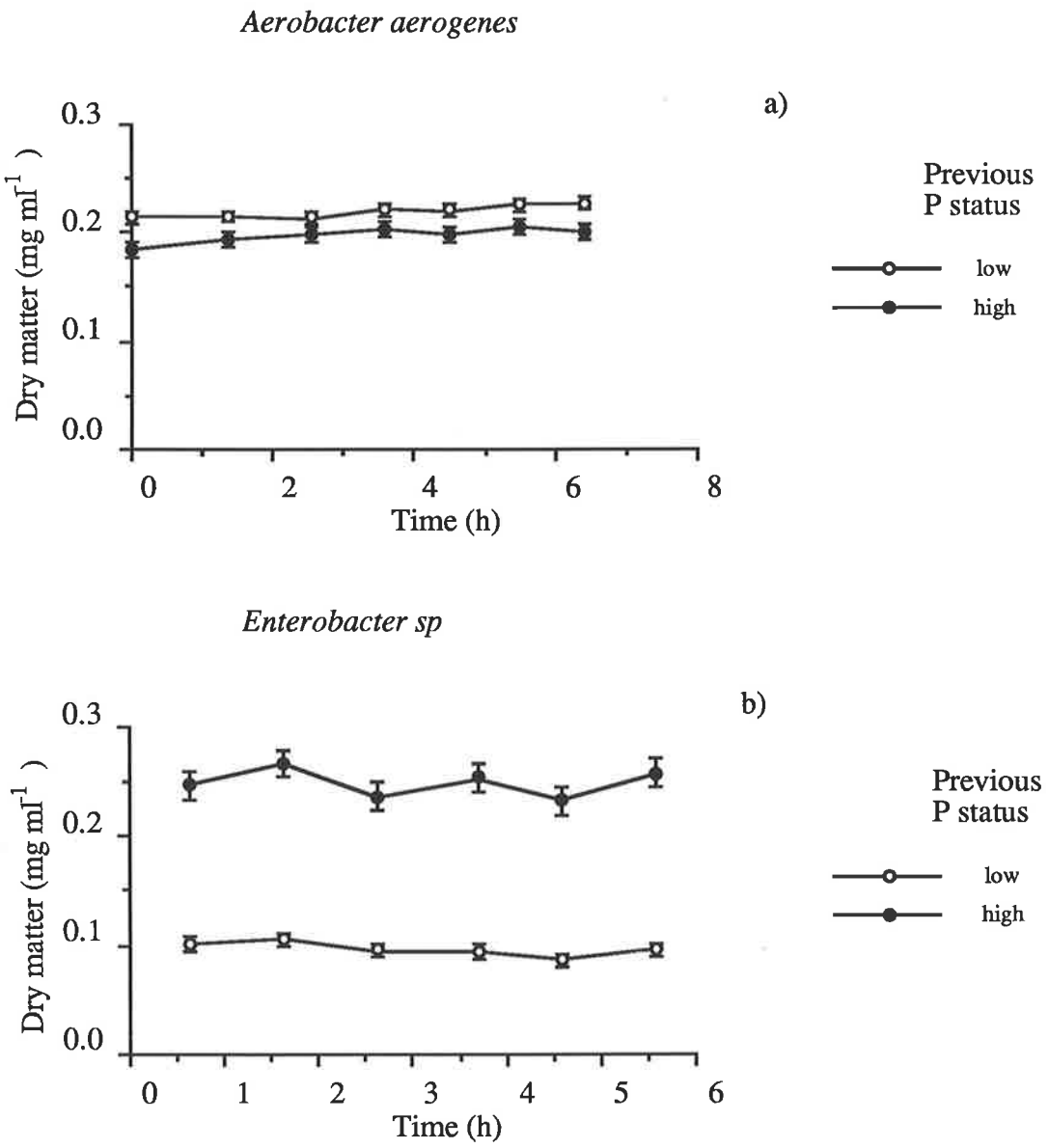


Figure 6. Dry weight of a) *Aerobacter aerogenes* and b) *Enterobacter sp* pretreated in low P or high P nutrient solution. Error bar = SD.

Total P concentration in bacteria was not related to previous P nutrition (Table 8). Total P concentrations are within the range of 10-30 mg P g^{-1} D.M. generally found in bacteria (Alexander 1977).

Table 8. Total P concentrations of *Aerobacter aerogenes* and *Enterobacter sp* grown in nutrient solution of low or high P concentration.

	concentration (mg P g^{-1} D.M.)	standard deviation
Aerobacter aerogenes		
low P	12.1	0.8
high P	16.9	1.5
Enterobacter so		
low P	21.1	2.5
high P	16.9	1.5

Effect of P was not significant at $p < 0.05$

Bacterial P uptake is shown in Figure 7. Bacterial nutrient solutions were not digested to determine total P in solution, as they contained P as orthophosphate only, and the determination of total P in solution was not necessary. "Low P" bacteria took up more P from solution than "high P"bacteria.

Figure 8 shows bacterial polyphosphate concentration. "High P" bacteria contained significantly more polyphosphate per gram dry matter than "low P" bacteria. A larger difference was found in *A. aerogenes*, than *Enterobacter sp*.

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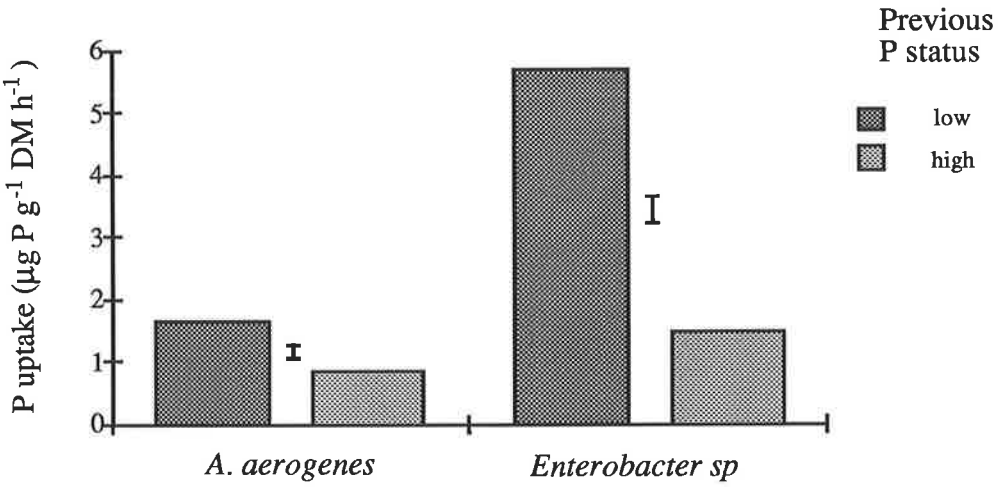

Figure 7. Phosphorus uptake by *Aerobacter aerogenes* and *Enterobacter sp* from intermediate P solution after transfer from low P or high P nutrient solution. Error bar = l.s.d. (p=0.05).

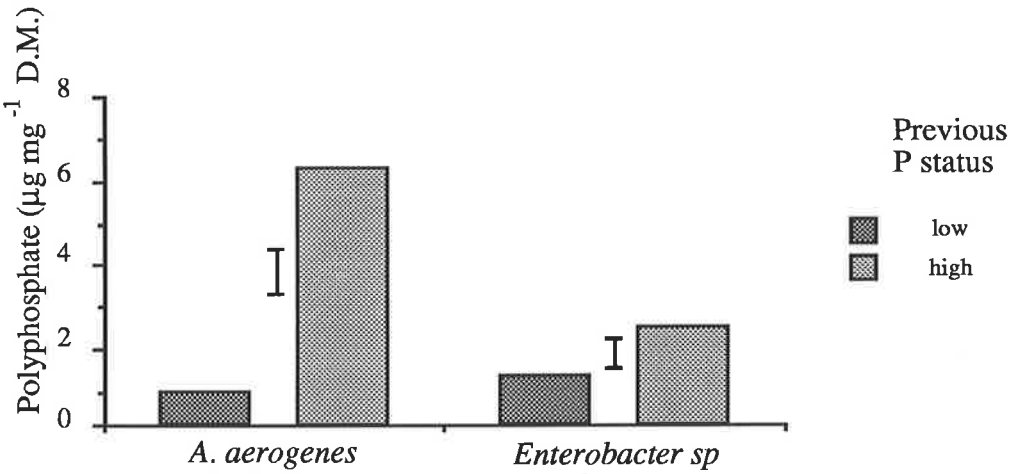

Figure 8. Polyphosphate concentration of *Aerobacter aerogenes* and *Enterobacter sp* grown in low P or high P nutrient solution, sampled before transfer to intermediate P nutrient solution. bar = l.s.d. (p=0.05).

Fungi

"Low P" fungi grew more slowly, attained lower dry matter yields and took up more P from intermediate P solution than "high P"fungi.

Changes in dry matter and hyphal P concentration are shown in Table 9.
There were no significant changes in hyphal weight during uptake from solution of intermediate P concentration. "Low P"*T. cucumeris* hyphae increased hyphal P concentration. *M. racemosus* hyphal P concentration increased in Part 1 but decreased in Part 2.

Figures 9 a) and b) show uptake of inorganic and total P by *T. cucumeris*. "Low P" fungi took significantly more P_i from solution than "high P" fungi. A net uptake of P from solution was shown in both cases. Fungal nutrient solution contained P in both organic and inorganic forms (peptone contains 2.2 mg P g^{-1}), and both orthophosphate and total solution P were determined.

Solution orthophosphate concentration during growth of *M. racemosus* is shown in Figure 10 a). There was a net loss of P from hyphae to solution, "low P" hyphae released significantly less P than "high P" hyphae.

Total P in solution during growth of *M. racemosus* is shown in Figure 10 b). Although there was a net loss of P by hyphae of both treatments, but hyphae grown in low P solution lost less than those grown in high P solution.

Table 9: Dry weight and P concentration of *M. racemosus* and *T. cucumeris* hyphae during P uptake from solution of intermediate P concentration (DBI) after transfer from solution of low and high P concentration.

	Hyphal P concentration (mg P g^{-1} D.M.)			Dry weight (mg $sample^{-1}$)		
Previous P status	before DBI	after DBI	difference	before DBI	after DBI	difference
Thanatophorus cucumeris						
low P	**4.4**	**8.0**	**			
	4.2	7.4	**	43.3	38.6	n.s.
high P	**9.8**	**10.8**	n.s.			
	9.3	10.6	n.s.	47.6	47.0	n.s.
Mucor racemosus						
low P	**16.3**	**18.2**	**			
	19.8	18.9	n.s.	10.5	10.6	n.s.
high P	**25.7**	**28.2**	*			
	27.9	23.9	*	14.7	16.3	n.s.

significance: n.s. $p>0.05$ **bold numerals = Part 1**

* $p\leq0.05$ plain numerals = Part 2

** $p\leq0.01$

Part 1 = hyphae used to determine P uptake from solution.

Part 2 = hyphae used to determine changes in dry matter and P concentration of hyphae during P uptake from solution.

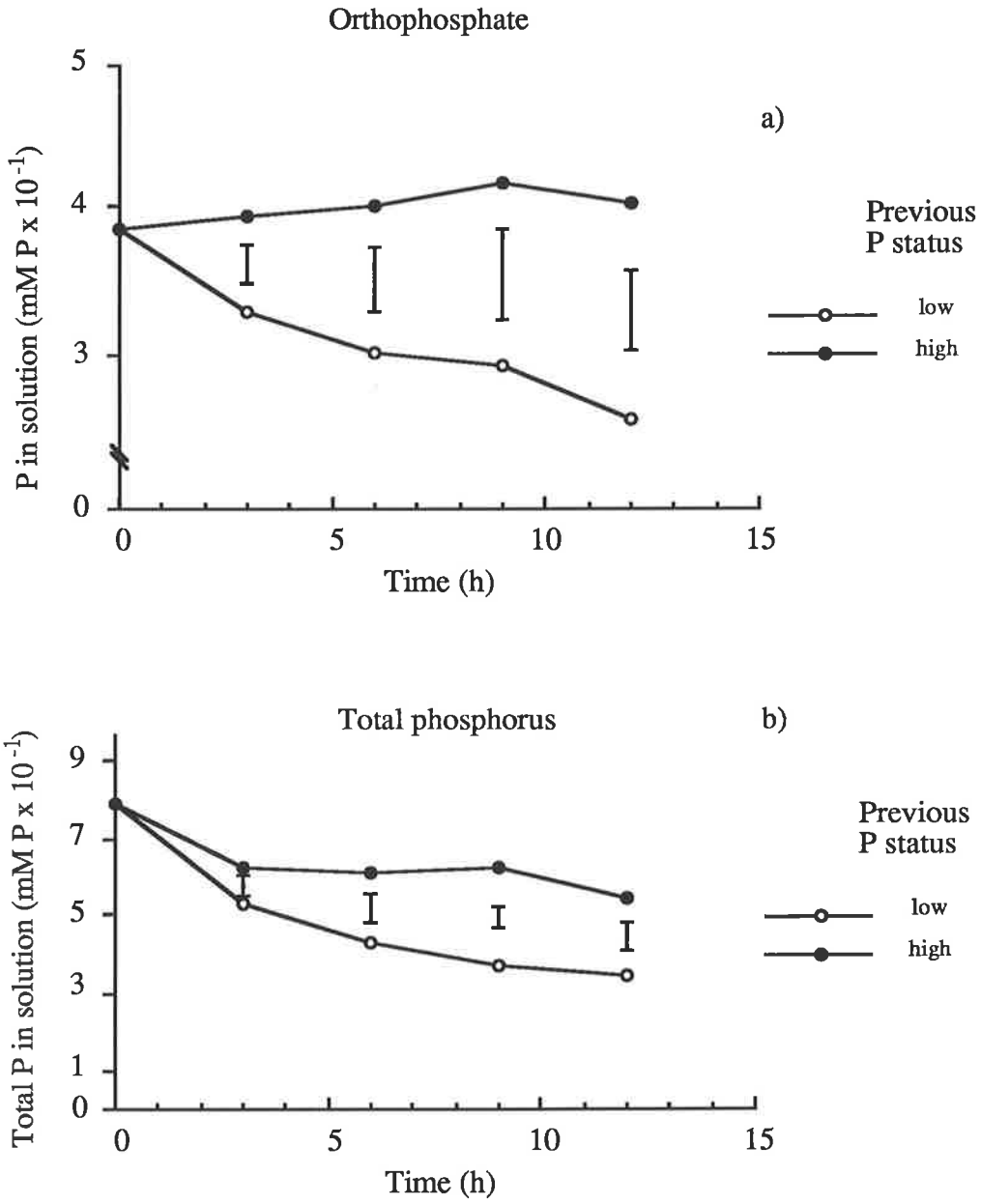

Figure 9. Change in orthophosphate a) and total phosphorus b) concentration of intermediate P nutrient solution due to P uptake by *T. cucumeris* after transfer from low P or high P nutrient solution. The difference between total P and orthophosphate is taken to be organic P. Error bar = l.s.d. (p=0.05).

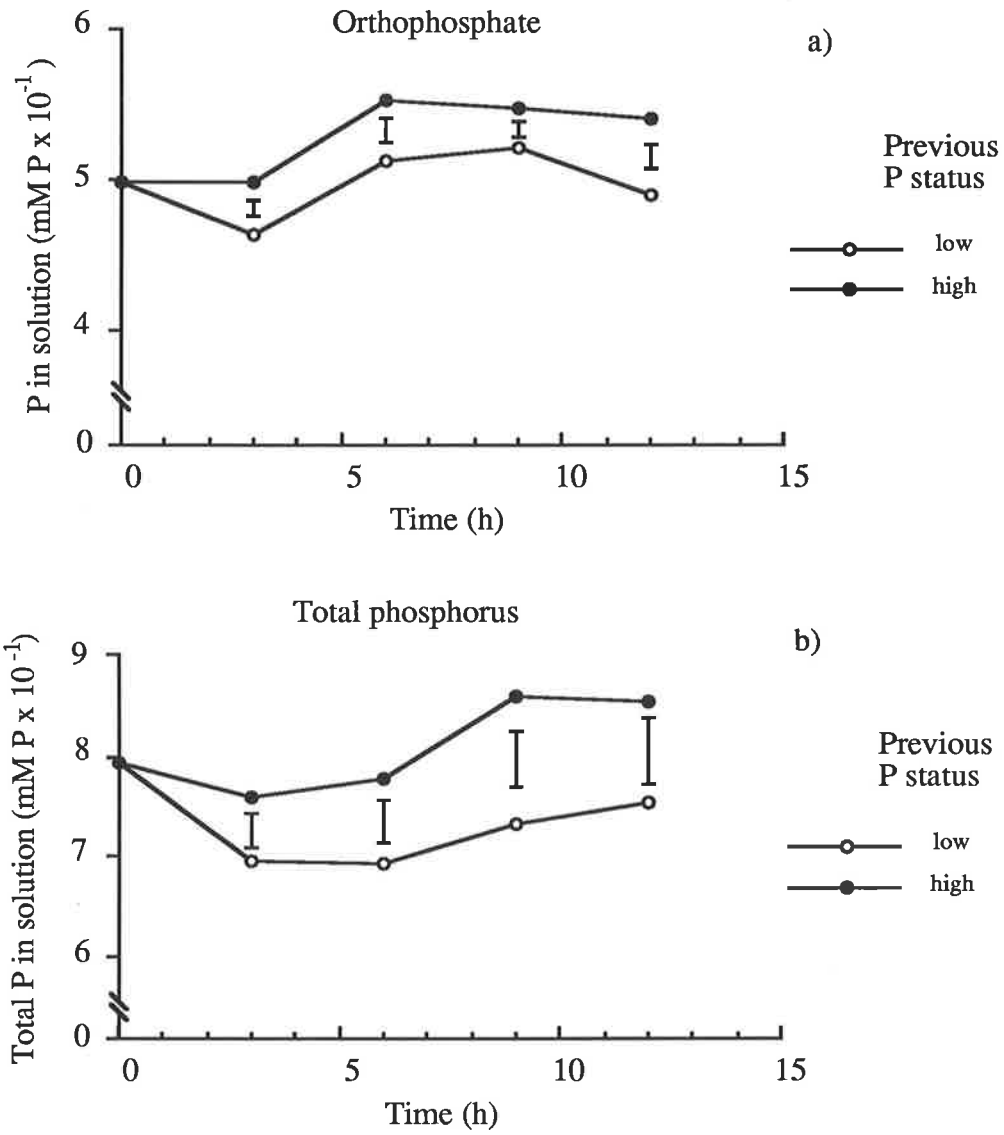

Figure 10. Change in orthophosphate a) and total phosphorus b) concentration of intermediate P nutrient solution due to P uptake by *M. racemosus* after transfer from low P or high P nutrient solution. Error bar = l.s.d. (p=0.05).

^{31}P nmr analysis of water extracts of hyphae showed no polyphosphates in *M. racemosus* or *T. cucumeris* .

Discussion

Bacteria

Both species, when grown on "low P" medium took up more P from solution than those grown on "high P" medium. "Low P"*Enterobacter sp* , took up significantly more ($p<0.01$) P from solution than "high P" *Enterobacter sp* per unit weight, despite having a lower density of bacterial cells per ml of solution. The disparity in dry weight of *Enterobacter sp* per ml of solution between low P (0.1 mg ml^{-1}) and high P (0.25 mg ml^{-1}) was not desirable for comparisons of bacterial P uptake on a unit-weight basis. Ideally, both solutions should have had the same density of bacterial cells. However, the findings were not changed by corrections for bacterial density of suspensions during the experiment, so I consider that they are valid.

There was no bacterial growth during the experiment. The aim of following dry matter changes was to see if higher P uptake in the P-deficient bacteria was due to a physiological difference between bacteria subjected to different treatments, or whether it was an example of normal, but accelerated growth by P-starved micro-organisms due to the sudden availability of P. The lack of bacterial growth during the experiment established that differences in bacterial P uptake caused by pretreatment were due to physiological differences between bacteria in each treatment and not just a spurt of compensatory growth.

Both bacterial species contained significantly more polyphosphates when grown in high P solution than in low P solution. Bacteria grown in high P solution took up P in sufficient excess to store it as polyphosphate. Bacteria containing polyphosphates took up less P from intermediate P nutrient solution than bacteria not containing polyphosphates.

This raises the possibility of using the presence of polyphosphates as an indicator of potential microbial P uptake in other situations, such as in field soil. The author is not aware of previous work relating microbial polyphosphate content to phosphorus uptake from solution. Most research on polyphosphates has investigated conditions conducive to polyphosphate synthesis and breakdown, identification of micro-organisms exhibiting polyphosphate metabolism, and cytological location of polyphosphates in cells (see Chapter 2).

Fungi

The two species of fungus reacted somewhat differently in growth and P uptake when the P concentration of their nutrient solutions was changed. However, "low P" hyphae of both species took up more P from intermediate P solution than "high P" hyphae.

There were no significant changes in hyphal weight during uptake from solution of intermediate P concentration.

P concentration in *M. racemosus* ranged from 16 mg P g^{-1} in "low P" hyphae grown in nutrient solution of 0.40 mM P to 28 mg P g^{-1} in "high P" hyphae grown in 0.90 mM P. These hyphal P concentrations are within the range found by James and Casida (1964), who grew *M. racemosus* hyphae with P concentrations from 3 mg P g^{-1} in low P nutrient solution (0.15 mM P) to 60 mg P g^{-1} in solution containing 14.5 mM P. P concentration in hyphae of *T. cucumeris* ranged from 4. 2 to 10.8 mg P g^{-1}, which is close to the general range of 5 - 10 mg P g^{-1} for fungal hyphae (Alexander 1977).

The contrasting changes of P concentration in *M. racemosus* hyphae during Part 1 of the experiment and Part 2 were not expected. The P concentration of *M. racemosus* hyphae increased during Part 1 but decreased during Part 2. These changes were, however, relatively small compared to those of *T. cucumeris* hyphae grown in solution of low P concentration. An increase in P concentration of low P hyphae was expected, with little or no change in high P hyphae (as found in the Part 1). In Part 2, however, hyphal pads were cut in half before being placed in intermediate P solution. This may have allowed some soluble P to leak from the cut hyphae. *T. cucumeris* hyphae do not seem to have been affected by this, perhaps due to their lower P concentration.

The extent of the loss of P from whole *M. racemosus* hyphae to solution in Part 1 was unexpected. However, James and Casida (1964) reported release of P from *M. racemosus* grown in media of different P contents. They discovered that polyphosphates leaked into solution during growth. From microscopic examination they concluded that polyphosphates had leaked from necrotic cells. The breakdown of polyphosphates into orthophosphate in solution effectively buffered the solution concentration of orthophosphate. This may explain the net release of total P to solution over time by the high P cultures. Some P loss may also be due to hyphal damage by the filtration process. As James and Casida found that the relatively gentle action of a reciprocating shaker was enough to damage growing *M. racemosus* hyphae, the hyphae must be considered fragile, whereas *T. cucumeris* hyphae may be relatively robust.

Fungi took up both orthophosphate and organic P from solution, as shown by the differences between graphs of orthophosphate and total P in solution (Figures 9 and 10). For example, *T. cucumeris* hyphae grown in solution of high P concentration did not affect solution orthophosphate concentration during uptake from intermediate P solution but a net uptake of total P (Figure 9 a,b). Presumably organic P was broken down into orthophosphate prior to uptake.

General discussion and conclusions

Polyphosphate is ephemeral in bacteria and fungi and may be broken down within a few hours by the activity of intracellular enzymes synthesized in response to a demand for P or energy (Harold 1963; Kulaev 1979). Polyphosphate is also short-lived in soil, as found by Pepper et al (1976) when the difference between 2 or 9 days after addition of orthophosphate meant finding a large amount (33 μg P g^{-1} soil) or no polyphosphate in a soil sample. This ephemeral quality may provide the basis for useful information if the presence of polyphosphates is to be used as an indicator of P uptake by the biomass. Polyphosphate would only be present in soil when soil micro-organisms are able to take up luxury amounts of P. From the results of this experiment, micro-organisms containing polyphosphates are likely to take up less P from solution than those not containing polyphosphates. A standard method for measurement of polyphosphate as well as orthophosphate could increase the accuracy of tests for extractable soil P.

Although no polyphosphate was found in fungal hyphae in this experiment, pretreatment with high P was shown to reduce potential P uptake by bacteria and fungi. It is possible that differences in P concentration between solutions of low and high P concentration may not have been enough to induce polyphosphate accumulation; or that higher P concentrations may have been necessary.

Lapeyrie et al (1984) grew the ectomycorrhizal fungus *Paxillus involutus* in nutrient solutions of 0, 1, 5 and 25 mM P. They found formation of metachromatic ("polyphosphate") granules in all hyphae grown in P-containing media. Although 1 mM P is close to the highest P concentration of fungal nutrient solution used in current experiments (0.94 mM P), differences between fungal species may have accounted for the lack of polyphosphate accumulation in *M. racemosis* and *T. cucumeris*. Furthermore, the ^{31}P nmr method used for the identification of polyphosphates in this experiment may not be as sensitive as microscopic identification of metachromatic granules.

It was considered that a comparison between micro-organisms pretreated with three- to five-fold differences in P concentration would be more realistic of field conditions than comparing micro-organisms grown in P-free media to those grown with abundant P. Dramatic changes in the P status of micro-organisms are known to affect their subsequent P uptake. Lapeyrie et al (1984) found that *Paxillus involutus* hyphae completely starved of P (grown in P-free media) could synthesise polyphosphates at solution P concentrations as low as 0.05 mM P.

The P concentrations used for growth media of bacteria (0.15 - 0.70 mM P) and fungi (0.4 - 0.94 mM P) in this project may also be considered a reasonable parallel to those found in some soils, especially close to fertilizer granules. As polyphosphates are found in field soil, suitable conditions for their accumulation must occur in the field. Investigation of the occurrence of polyphosphates in field soil and their relationship to microbial P uptake is required.

The first aim of this experiment was to determine whether polyphosphate synthesis could be initiated by the change from nutrient solution slightly deficient in P to nutrient solution supplying adequate P. This was confirmed by the presence of polyphosphate in "high P" bacteria, but was not confirmed in fungi.

The second aim was to determine if the presence of polyphosphates could be used to indicate potential P uptake from solution. The previous P nutrition of bacteria and fungi significantly affected their P uptake from solution of intermediate P concentration. Although polyphosphates were not found in fungal hyphae, changes in potential P uptake were found to precede accumulation of polyphosphates. Thus, where polyphosphates are detected in these micro-organisms, their potential P uptake has already been reduced.

Differences in P uptake by bacteria were accompanied by significant differences in polyphosphate content. This raises the possibility of polyphosphate being used as a indicator of microbial P uptake over a larger range of micro-organisms and in the soil environment.

CHAPTER FIVE: Polyphosphate content of soil with different plant types and extractable P concentrations

Introduction

Polyphosphates are present in varying amounts in most living organisms (Kulaev 1979). They are associated with internal energy and phosphorus (P) storage systems. They may accumulate when growth is limited by factors other than P deficiency, or when P deficiency is released by a large addition of orthophosphate.

The presence of polyphosphates in micro-organisms has been linked to reduced uptake of phosphorus from solution (Chapter 4). High intracellular orthophosphate concentrations may also reduce P uptake (Rolin et al 1984) but are not as readily identifiable in soil as the presence or absence of polyphosphates. It remains to be seen if polyphosphate can be a measure of potential microbial P uptake in soil. A reliable indicator of the potential P uptake of soil micro-organisms may enhance the efficiency of fertilizer use by predicting how strongly the biomass may compete with plants for P fertilizer added to soil.

Only trace amounts of polyphosphates occur in plants (Kulaev 1979). Polyphosphates present in soil are of microbial origin. Polyphosphates have been found in soil, both induced by P amendment (Ghonsikar and Miller 1973, Pepper and Miller 1974, Pepper et al 1976) and identified in ^{31}P nmr analyses of undisturbed soil (Adams and Byrne 1989, Tate and Newman 1982). Ghonsikar and Miller (1973) found naturally-occurring polyphosphates in four of eight soils tested. The authors were unable to say if these polyphosphates were present in micro-organisms, microbial debris or associated soil components.

This experiment had two aims: Firstly, to investigate the occurrence of polyphosphates in samples of the same soil taken from beneath two plant species (medic and wheat) over a range of extractable soil P concentrations. Secondly, to determine the origin of these polyphosphates in soil, as determined by a sequential chemical extraction. Sequential extraction may determine whether polyphosphates are present in the microbial biomass, relatively stable forms of soil organic matter, or as chemically stabilized complexes in soil.

Methods and Materials

Soil samples were extracted with perchloric acid, evaporated to increase P concentration, then extracted with activated charcoal to remove organic compounds. P in the charcoal-treated perchloric acid extract which was hydrolyzed in hot 1N HCl was taken as a measure of polyphosphate (Pepper et al 1976).

Soil samples were collected (0-100 mm) from a wheat/medic rotation experiment at Mallala, South Australia (19 September 1989). The soil and site have been described previously (McLaughlin and Alston 1986). Soils were sampled under three fertilizer levels (low, intermediate and high) and two crops (wheat and medic). For details of soils and treatments, see Table 10.

Table 10. Description of soil*

Soil treatment		low P	intermediate P	high P
Total P fertilizer† (kg P ha^{-1})		0	40	100
Olsen P	medic	2.79	5.21	12.32
(μg P g^{-1})	wheat	2.74	6.05	15.62

* see Chapter 3 for further description.

† since the experiment was established in 1985.

Samples (100 g air-dry soil) were sieved (2 mm) and extracted on an end-over-end shaker for 4 hours with 0.5 M perchloric acid (PCA) at 4°C (soil:extractant ratio = 1:5). Samples were centrifuged, the supernatant filtered (Whatman no. 42), partially neutralized with 1 M KOH (to prevent hydrolysis of polyphosphates by strong PCA), and concentrated *in vacuo* at 38°C to 100 ml. The concentrated samples were extracted with Darco G-60 activated charcoal (60 mg g^{-1} soil) for 30 minutes to remove organic compounds and filtered (Whatman no. 42). Orthophosphate was measured before and after hydrolysis with 1 N HCl at 100°C for 20

minutes. P labile in HCl was taken as a measure of polyphosphate. To confirm the presence of polyphosphates indicated by this method, a sample containing P labile in HCl (7.4 $\mu g\ g^{-1}$ soil) was purified on a gel column (Sephadex G-25 fine, 2.5 x 45 cm, eluent 0.1 M KCl, flow rate 0.5 ml min^{-1}) and analyzed by ^{31}P nmr, which showed the presence of both orthophosphate and polyphosphate.

Two soils, one containing polyphosphates (medic, high P) and one not containing polyphosphates (wheat, low P) were fumigated and sequentially extracted by the method of Hedley and Stewart (1982) with modifications for the measurement of microbial P (McLaughlin and Alston 1986) and removal of organic compounds from soil extracts.

Fumigation lyses microbial cells and releases a proportion of their contents. The P released from micro-organisms can be estimated by extracting soil with a relatively mild chemical which will not rupture microbial cells (0.5 M $NaHCO_3$) and comparing the P content of this to extracts of unfumigated control samples. Subsequent extractions with more reactive chemicals provide estimates of increasingly recalcitrant fractions of soil P. NaOH removes chemically sorbed P bound to Fe, Al and organic matter. Sonication disrupts aggregates and allows access to compounds which previously were physically protected from the extracting solution. HCl removes stable inorganic P, calcium minerals and some occluded P. Residual P (chemically stable P_o and relatively insoluble P_i) was not determined as the required digestion would have hydrolysed polyphosphates had they been present. Also, it is unlikely that residual P would be extracted by the 0.5 M perchloric acid used to extract soil polyphosphates. Activated charcoal removes organic compounds, including enzymes, but leaves inorganic compounds such as orthophosphate and polyphosphates in solution. Test extractions of orthophosphate and polyphosphate standard solutions with activated charcoal showed that charcoal did not remove orthophosphate or polyphosphates from solution (data not shown).

Samples (2.5 g, four replicates) were extracted with 0.5 M $NaHCO_3$ for 30 minutes or fumigated with hexanol for 24 h and then extracted with $NaHCO_3$. Fumigated samples were extracted with 0.1 M NaOH (16 h), sonicated for three minutes (Branson 1200 w, full power, sample in ice bath), then extracted for 16 h with firstly 0.1 M NaOH, then 1N HCl. Samples were centrifuged between each extraction, and the supernatant immediately shaken with Darco G-60 activated charcoal (30 minutes) and filtered (Whatman no. 42). Activated carbon was used at a rate of 300 mg g^{-1} soil for $NaHCO_3$ and NaOH extracts (high organic content) and 100 mg g^{-1} soil for sonicated NaOH and HCl samples (low organic content). All extractions were carried out at 4°C to minimize enzymatic hydrolysis of polyphosphates. P labile in 1N HCl (100°C, 20 minutes) was taken as a measure of polyphosphate.

Results

Polyphosphate in perchloric acid extracts increased with soil P concentration (Figure 11).

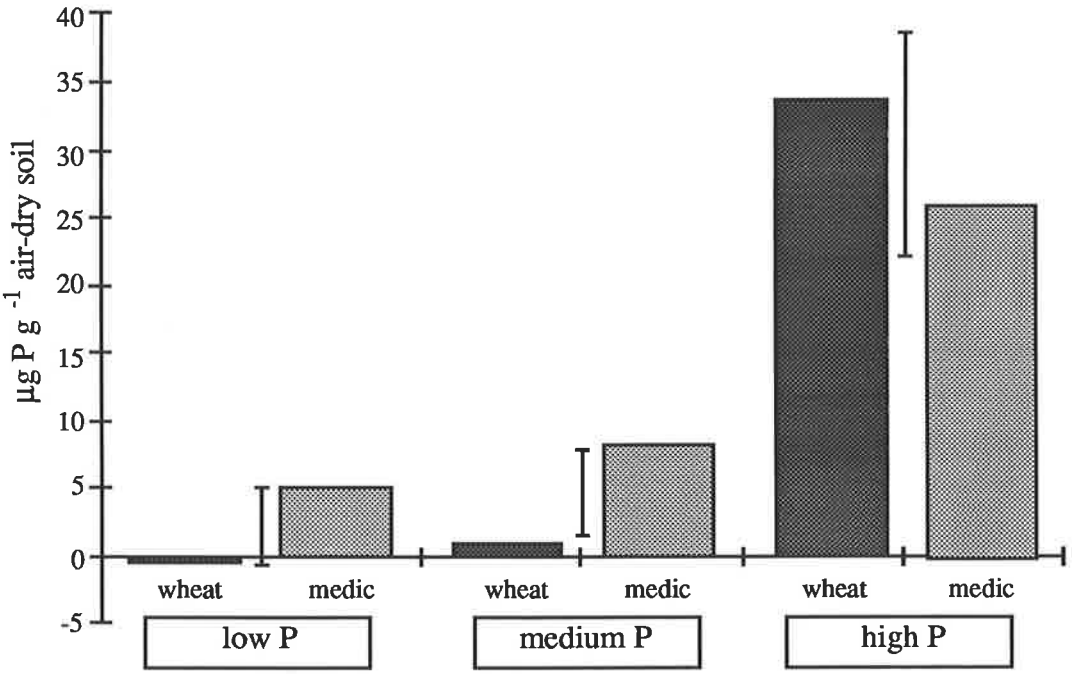

Figure 11. Polyphosphate in 0.5 M perchloric acid extracts of field soil of low, medium or high P status under wheat or medic plants. Error bar = l.s.d. ($p = 0.05$).

Simple linear regression of polyphosphate concentration on soil P concentration (low, intermediate, high) was significant at $p < 0.001$. Medic low P and intermediate P soil contained more polyphosphate than wheat low P and intermediate P, respectively. Medic high P and wheat high P extracts contained significantly more polyphosphate than all other extracts.

P released in soil by fumigation and extracted with sodium bicarbonate is considered to come from the microbial biomass. Sodium bicarbonate extracts from fumigated samples of medic high P soil contained significantly more polyphosphate than all other sodium bicarbonate extracts (Figure 12). The amount of polyphosphate shown in extracts from fumigated samples of wheat low P soil is significantly greater than zero at $p < 0.10$.

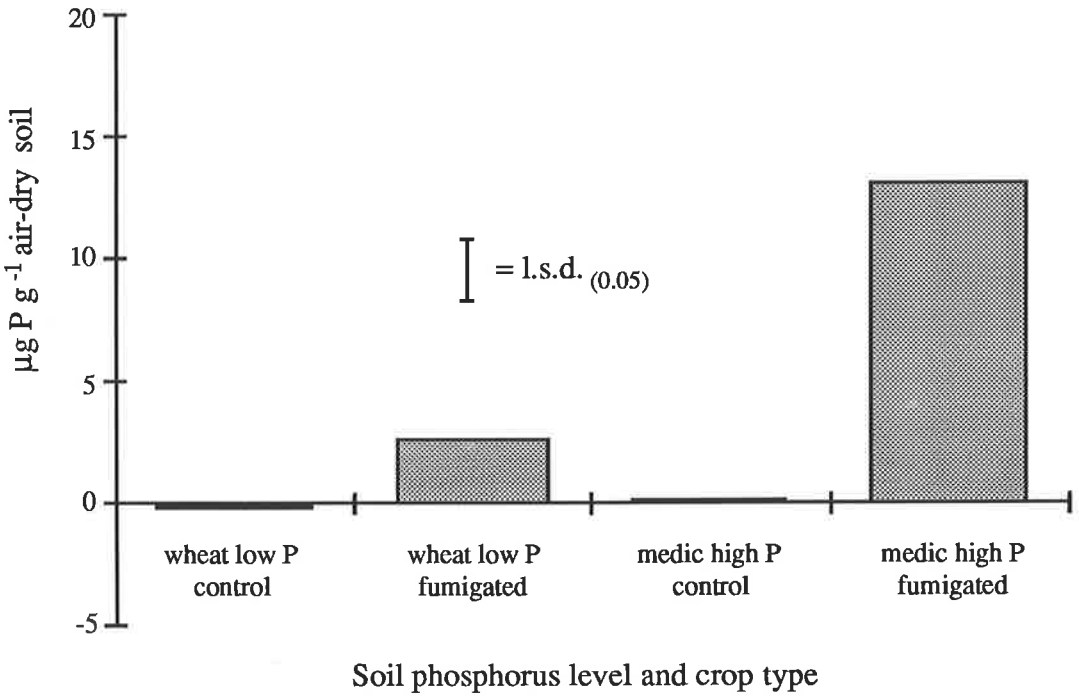

Figure 12. Polyphosphate in 0.5 M sodium bicarbonate extracts of medic high P soil and wheat low P soil. Error bar = l.s.d. ($p = 0.05$).

There were no significant amounts of polyphosphate in other soil fractions (Figure 13).

The polyphosphate concentration of wheat and medic high P soil during storage at 4° C is shown in Figure 14. The polyphosphate concentration of wheat soil declined more rapidly than that of medic soil.

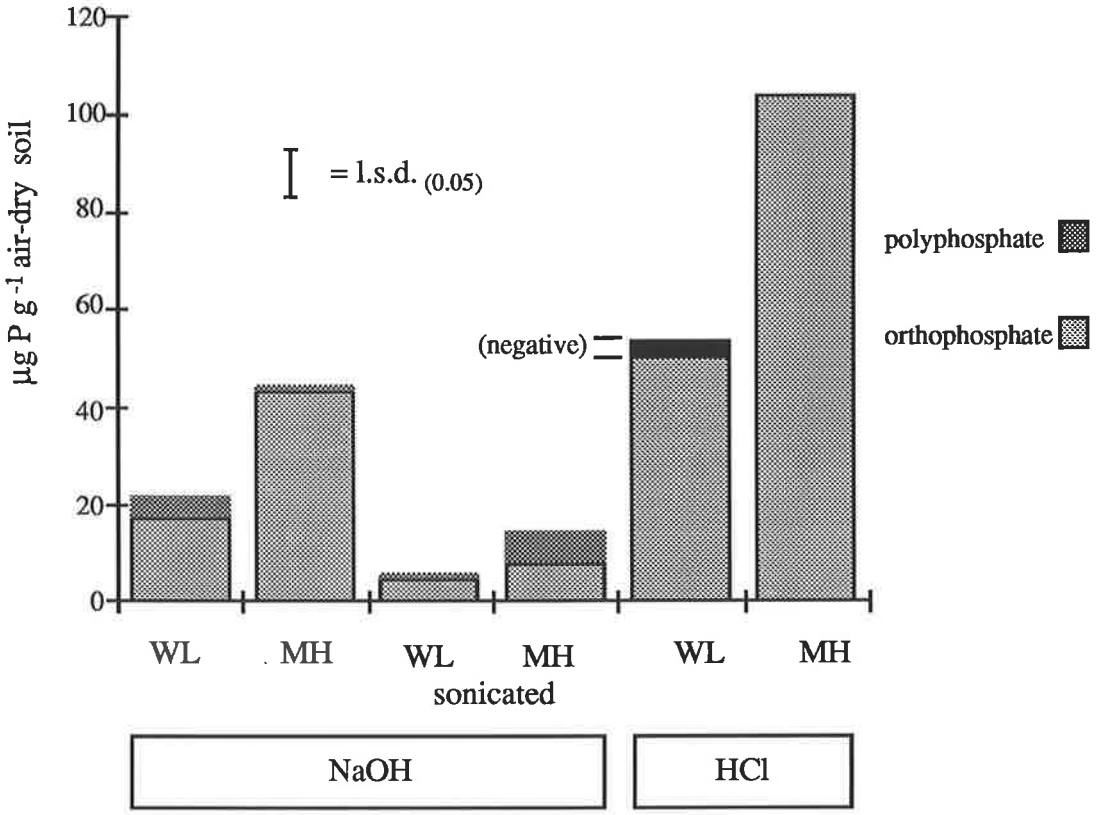

Figure 13. Polyphosphate and orthophosphate in sodium hydroxide, sonicated sodium hydroxide and hydrochloric acid extracts of wheat low P (WL) and medic high P (MH) field soil. Error bar = l.s.d. (p = 0.05). Black shading shows a negative value (value of control sample greater than that of hydrolysed sample).

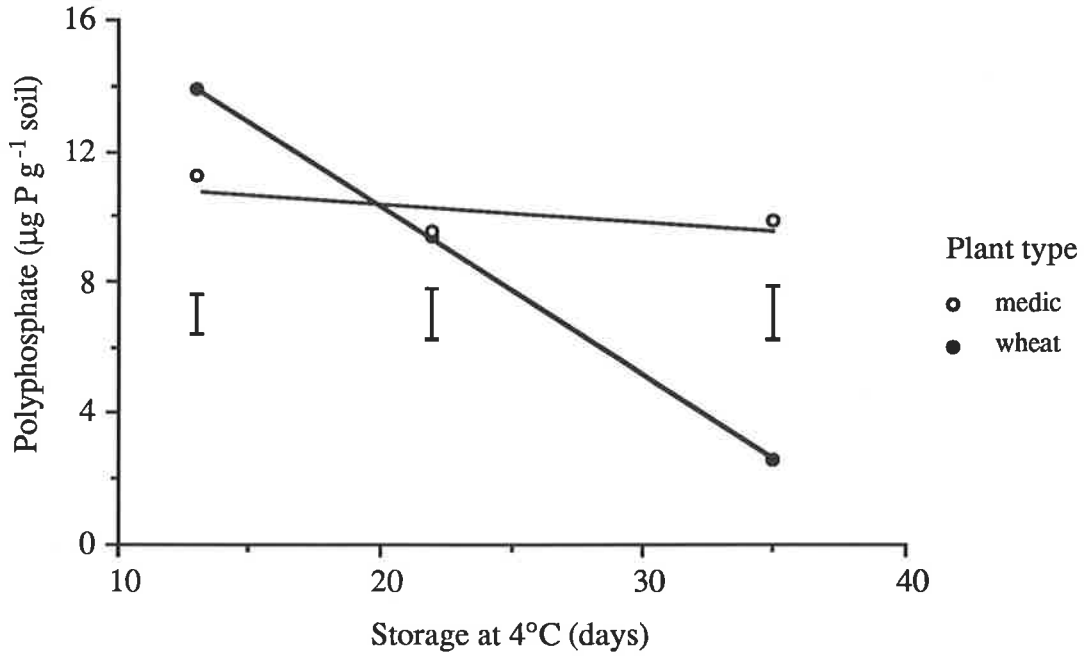

Figure 14. Effect of storage at 4°C on polyphosphate concentration of air-dry soil of high P status from under wheat or medic plants. Error bars = S.E.D.

Discussion

Soil polyphosphate concentration increased with rate of P fertilizer which had been added to the soil over the previous four years. This agrees with the theory that accumulation of polyphosphate increases as the P status of micro-organisms increases.

Soil samples from beneath medic plants contained more polyphosphate at low or intermediate P levels than soil from under wheat plants. Although it is not proven that microbial populations selectively adapt towards certain plants, it is likely that different bacteria and fungi will respond in different ways to specific plant residues (Anderson and Domsch 1990). The differences in soil polyphosphate concentration between samples under different plant species may be due to many things, including soil water regimes determined by plant water uptake, the effect of plants on nutrient status of soil and the composition of root exudates (Rovira 1956a).

It is likely that differences in root material and exudates between wheat and medic plants may have affected the composition of the rhizosphere microbial population. This was implied by the results of experiments described in Chapter 3, and may have affected the concentration and longevity of polyphosphates in soil. Soil water may have caused differences in soil

polyphosphate concentration. Although samples were taken from the same site at the same time, wheat plants were much larger than medic plants (about three times the size). It is possible that rhizosphere soil under wheat plants was drier than corresponding soil under medic plants due to water uptake by plants. Although the measured water content of bulk soil under wheat and medic plants was the same, this may not reflect rhizosphere soil conditions. However, soil water effects would not explain the differences found in storage of polyphosphates at 4°C.

The polyphosphate content of soil under wheat was more affected by soil P concentration than soil under medic as there were no polyphosphates in low P soil under wheat, whereas medic soil contained polyphosphates at all P concentrations. Either medic provided better conditions for synthesis of polyphosphates, or the soil microbial population under medic was better able to make use of similar concentrations of available P. This may have been due to differences in the composition of the microbial populations under wheat and medic plants, with some micro-organisms able to accumulate polyphosphates and other not able to do so. Other differences were found in polyphosphate turnover between wheat and medic soil. After 35 days storage at 4°C, wheat high P soil contained only 19% of its original quantity of polyphosphate, while medic soil contained 88% (Figure 14).

This implies a difference in synthesis and breakdown of polyphosphates between micro-organisms in soil under these two plant species. The compounds were presumably degraded within micro-organisms during that time, as storage at 4°C is not likely to lyse microbial cells. Polyphosphates are known to be labile in soil during incubation at room temperature (Ghonsikar and Miller 1973) but degradation during storage does not appear to have been investigated at lower temperatures. The labile quality of polyphosphates is similar to that of ATP, another cell metabolite, which may also be broken down internally by micro-organisms at 4°C (Ross et al 1980).

Differences in polyphosphate turnover between soil samples under medic and wheat plants may be due to the stimulation of different microbial species by each plant. Micro-organisms stimulated by medic plants may have a different "threshold" of polyphosphate accumulation from those stimulated by wheat, or perhaps different environmental conditions for the micro-organisms (e.g. water, nutrient, structure) are created by each plant species. Differences in chemical composition between soil micro-organisms associated with medic and wheat plants were found in the experiments described in Chapter 3. Soil was incubated with young, chopped roots of either medic or wheat plants. From the microbial C and P flushes produced, it was found that micro-organisms grown in soil amended with medic roots had a lower C:P ratio than those grown in soil amended with wheat roots. This difference was much greater

than that between the relative amounts of P added in medic and wheat roots, and implies that these micro-organisms enjoyed a more favorable P regime than those grown on wheat roots.

As expected, all polyphosphate in the soil was found in the $NaHCO_3$ extracts. For polyphosphate concentrations to change as rapidly as they do in soil, polyphosphates must be part of a very labile component of the soil. Micro-organisms are one such component, and the flush of polyphosphate released by fumigation confirms the presence of polyphosphates within microbial cells. There were no significant amounts of polyphosphate in any other soil fraction. This confirms the findings obtained by use of the perchloric acid extraction method, that large amounts of polyphosphate were present in medic high P soil with little or no polyphosphate found in wheat low P soil. Soil fumigation and fractionation also showed the microbial biomass to be the origin of soil polyphosphates.

Pepper et al (1976) investigated polyphosphate synthesis in soil by adding various amounts of glucose and orthophosphate to soil (see Chapter 2). Polyphosphates accumulated after this treatment had different chain lengths from those found before soil treatment (Table 11). This suggests that the polyphosphates were formed, if not by different processes or micro-organisms, at least under conditions different from those which prevailed during formation of the polyphosphates initially present. The polyphosphate found in Mallala soil ($n\approx4$) is within the range shown in Table 11.

Table 11. Chain length of soil polyphosphates found by Pepper et al (1976) before and after treatment of soil with glucose and orthophosphate.

Soil	Before	After
Brookston	n=2†	n=10
Hoytville	n=20	n=5
Warsaw	n=3,5	n=60

† n = number of orthophosphate residues in polyphosphate chain.

Polyphosphates found in soil originate as orthophosphate molecules taken up by micro-organisms . These are built firstly into short-chain-, then long-chain-polyphosphates (Beever

and Burns 1980). The polyphosphate compounds may be broken down again as necessary by the reverse process. It is possible that the polyphosphates measured by Pepper et al (1974) before and after soil treatment with glucose were merely at different stages of this cycle of polyphosphate synthesis and decomposition. Although the timed addition of glucose and orthophosphate to soil may synchronize the microbial P cycles between soils to a certain extent, this cannot be certain, because textural differences between soils may cause glucose and phosphate to be utilized at different rates. In a clay soil, glucose may be able to diffuse into small pores too small for micro-organisms to enter (Foster 1985). This physical protection is less likely to be available to phosphorus, which has more restricted diffusion in soil than glucose.

Harold (1966) defined two patterns of polyphosphate accumulation in micro-organisms. The first was a gradual accumulation when growth was limited by some factor (e.g. S deficiency). The second occurred when P starvation was suddenly released by addition of large amounts of orthophosphate (polyphosphate overplus). Pepper et al (1976) endeavored to induce synthesis of polyphosphates with the intention of investigating a soil reserve of labile P, which it was hoped would be available for uptake by plants. The work presented here is concerned with the gradual accumulation of polyphosphates through restricted microbial growth, such as that found by Pepper et al (1976) before treatment of the soil. Although it is not known what factors affect the chain length of polyphosphates accumulated in soil, the differing chain lengths of compounds found by Pepper et al (1976) after soil amendment (Table 11) certainly implies that they may not be the same as those present before soil amendment.

The results from this experiment show that soil polyphosphate content varies with both extractable P concentration and plant type. Polyphosphates are found in the soil microbial biomass and not free in soil. This raises the possibility of the presence of soil polyphosphates being used as a predictor of microbial P uptake, with implications for tailoring fertilizer rates to the needs of both crop plants and soil micro-organisms (see Chapter 6).

Conclusions

In relation to the aims of the experiment it has been clearly demonstrated that:

1) The occurrence and amount of polyphosphates in soil varies with both plant species and soil P concentration and increases with soil P concentration. Soil under medic

plants contained more polyphosphate at low and intermediate P concentrations than soil under wheat plants at similar P concentrations. Differences in the polyphosphate content of soil under each plant species may be due to changes in either microbial populations or soil environmental conditions imposed by plant growth.

2) Soil polyphosphates are found within microbial cells, as shown by fumigation and sequential fractionation of the soil.

CHAPTER SIX: GENERAL DISCUSSION

This project set out:

1) to determine whether P uptake by selected micro-organisms could be related to their polyphosphate concentrations; and

2) to examine differences in the P status of the microbial biomass under wheat and medic plants.

Investigations relating microbial P uptake to polyphosphate concentration proceeded in three steps. Firstly, ascertaining whether high P nutrition of micro-organisms enabled them to accumulate polyphosphates. Secondly, determining whether micro-organisms pretreated with low P took up more P from solution than micro-organisms pretreated with low P. Thirdly, linking these two steps to see if the polyphosphate concentration of micro-organisms could be used as a guide to their P uptake.

Relation of previous P nutrition to polyphosphate concentration

Polyphosphates were found in bacteria under high P nutrition, but not in fungi. The previous P nutrition of micro-organisms has been related to polyphosphate content by many workers studying polyphosphate synthesis in bacteria and fungi (see Harold 1966, Kulaev 1979, Harley and Smith 1983 for reviews). However, most investigations into polyphosphate metabolism have been carried out using micro-organisms either starved of P (grown in P-free media) and then given large amounts of orthophosphate, or micro-organisms initially grown in media of relatively high P concentration (generally between 3.2 and 32 mM P). The experiments described in this project were designed to investigate changes in polyphosphate concentrations of micro-organisms growing under conditions in which P was just-deficient or just-adequate for microbial growth.

It was thought that if differences in polyphosphate synthesis and P uptake were determined close to the point of P deficiency/sufficiency, differences found between P-limited and non-P-limited micro-organisms would apply to a wider range of conditions either side of the point of adequate P supply. P-free media could have been used to grow P-limited micro-organisms, and very high-P media to grow non-P-limited micro-organisms. The latter would have assured the accumulation of polyphosphates in *M. racemosus* at the least. However, results gained from these "extreme" conditions may not be applicable to most field situations where P supply is likely to be somewhere between these extremes. Thus it was considered realistic

to use media with relatively small (four-fold) differences in P concentration and to determine the effect of changes in P nutrition over this small range.

The author is not aware of any work linking previous P nutrition of micro-organisms with both the occurrence of polyphosphates and subsequent P uptake. Moreover, little work has been published regarding the critical P concentrations of growth media which allow accumulation of polyphosphates in micro-organisms, and the results that are available appear to be inconsistent. For example, Rolin et al (1984) found that the polyphosphate concentration of ectomycorrhizal fungi was not related to the P concentration of solution ranging from 5 μM P to 2.5 mM P. However, they did find that the orthophosphate concentration of these fungi increased with solution P concentration.

Lapeyrie et al (1984) grew *Paxillus involutus* in nutrient solutions containing 0, 1, 5 and 25 mM P. Metachromatic granules (polyphosphates) were present in all hyphae grown in solutions containing P. Hyphae did not accumulate polyphosphates in experiments reported in Chapter 4, although the P concentration of high-P nutrient solution (0.94 mM P) was close to the lowest concentration (1 mM P) used by Lapeyrie et al (1984). Differences between fungal species or in the sensitivities of the analytical techniques used may have caused this disagreement. Optical microscopy (Lapeyrie et al 1984) is likely to be a more sensitive test for the presence of polyphosphates than the chemical extraction technique used in experiments reported in Chapter 4. Although it was reported in Chapter 4 that hyphae did not accumulate polyphosphates, fungal growth was limited by P deficiency in low P nutrient solution and P uptake by P-deficient hyphae was greater than that by P-sufficient hyphae. Polyphosphate was accumulated by bacteria under high P nutrition. Likewise, growth of bacteria was limited by low P nutrition, but under high P nutrition, not only was uptake greater but polyphosphates were accumulated.

Relation of previous P nutrition to P uptake

Pretreatment of bacteria and fungi with high P decreased subsequent P uptake., while pretreatment of bacteria and fungi with low P increased subsequent P uptake. As discussed earlier, there was only a four-fold difference in P concentration between "low P" media and "high P" media. The effects of pretreatment on subsequent P uptake are qualitatively similar to those found by many other workers who used much greater changes in P concentration in studying the effects of pretreatment at low or high P concentrations on hyphal P uptake. For example, Clipson et al (1987) found that basidiomycete hyphae pretreated with 10 μM P took up more P from solution than hyphae pretreated with 10 mM P (1000-fold difference in P

concentration). Thomson et al (1990) found that hyphae grown in P-free media took up more P from solution than those grown in media containing 1 mM P.

Relation of P nutrition to subsequent P uptake and polyphosphate concentration

The polyphosphate concentration of selected micro-organisms was clearly related to both previous P nutrition and subsequent P uptake. The presence of polyphosphates in micro-organisms could be used to indicate lower subsequent P uptake than that of micro-organisms not containing polyphosphates.

The author is not aware of the presence of polyphosphates in micro-organisms having previously been used as an indicator of subsequent P uptake. Published work on polyphosphates in non-mycorrhizal micro-organisms has focused on the fate of the polyphosphates themselves, not the subsequent uptake of P by micro-organisms.

The presence of polyphosphates in bacteria was accompanied by reduced subsequent P uptake (Chapter 4). Although polyphosphates were not found in fungal hyphae, differences in P uptake due to previous P nutrition were detected . A reduction in subsequent P uptake relative to that of P-starved micro-organisms appeared to require smaller increases in P supply than those required for accumulation of polyphosphates. This implies that when polyphosphates do accumulate in hyphae, potential P uptake will already have been reduced.

It has been shown that changes in P nutrition affect subsequent P uptake. Nutrition with high P decreased subsequent P uptake, while nutrition with low P increased subsequent P uptake. Pretreatment at greater P concentrations than those required to decrease subsequent uptake are needed to cause the accumulation of polyphosphates. Therefore, if polyphosphates are found in micro-organisms, subsequent P uptake will already have been affected.

Previous P nutrition of micro-organisms is known to affect the presence of polyphosphates (Harold 1966; Chilvers et al 1985), and to affect the subsequent uptake of P from solution (Beever and Burns 1980). It has been shown that changes in P nutrition sufficient to affect the growth of selected bacteria and fungi, and to induce polyphosphate accumulation in bacteria, significantly affect subsequent P uptake (Chapter 4).

The results obtained from two species of bacteria and two species of fungi cannot be directly extrapolated to all micro-organisms. There is, however, some justification for inferring that the changes in P uptake of micro-organisms reported in Chapter 4 may apply to many other micro-organisms. Most bacteria (Kulaev 1979) and fungi (Chilvers et al 1985) can

accumulate polyphosphates under conditions of abundant P supply. Lapeyrie et al (1984) considered that their findings using a cultured mycorrhiza were consistent with earlier studies on symbiotic mycorrhizal tissues, and with conclusions made by Beever and Burns (1980) from work on cultures of non-mycorrhizal fungi. Kulaev (1979) considered that the metabolism of polyphosphates is very similar in all of the fungi which have been examined. It is likely that the P uptake of most micro-organisms will be affected by previous P nutrition similarly to that of the standard micro-organisms studied. Complementary to solution culture studies, soil from under wheat and medic plants was examined to determine differences in P status of the biomass. This was done in three steps: Firstly, soil was amended with wheat or medic roots to determine the effects of amendment on microbial P and C. Secondly, soil from under wheat or medic plants, over a range of extractable P concentrations, was examined for the presence of polyphosphates. Thirdly, a soil containing polyphosphates was sequentially fractionated to determine the origin of these polyphosphates.

Amendment of soil with wheat and medic roots

The addition of wheat and medic roots to soil affected soil microbial P and C differently. Biomass stimulated by medic roots had a higher P concentration than that stimulated by wheat roots. It was established in Chapter 3 that plant type may affect both the size and the chemical composition of the microbial biomass. Plants are the driving force in soil biological reactions (Newman and Watson 1977), and it is generally accepted that plant species can affect the type of micro-organisms growing in soil. The fungal:bacterial ratio of soil biomass is generally higher under pasture than under arable crops (Clark and Paul 1970) and root exudates of different plants stimulate different groups of soil micro-organisms (Rovira 1956b).

Examination of wheat and medic soil for polyphosphates over a range of extractable P concentrations

Perhaps partly as a result of changes in root exudates, microbial populations, etc caused by wheat and medic plants, there were variations in soil polyphosphates under different plant species. The occurrence and amount of polyphosphates in soil varied with plant type and soil P concentration. Soil under medic plants contained polyphosphates at all levels of extractable P, whereas low-P soil under wheat contained no polyphosphates. Soil polyphosphate concentration increased with extractable P concentration, which agrees with the theory that accumulation of polyphosphates will increase as the P status of micro-organisms increases.

Differences in polyphosphate metabolism in micro-organisms under each type of plant are likely to to be one of the factors affecting the validity of using a constant value for the k_p factor used in the fumigation technique for measurement of microbial P in soil. This is because differences in polyphosphate metabolism may indicate differences in microbial populations. k_p is known to vary between bacteria and fungi, and differences within each group are likely (Hedley and Stewart 1982).

The longevity of soil polyphosphates differed between soil from under wheat and medic plants. The polyphosphate concentration of wheat soil declined more rapidly during storage than that of medic soil.

From differences found in polyphosphate accumulation and decline it can be concluded that there are significant differences in polyphosphate metabolism between soil from under wheat and from under medic plants.

Sequential fractionation of soil containing polyphosphates

Fractionation of soil containing polyphosphates showed them to be present in the microbial biomass. While this was expected, it had not previously been shown. Plants contain only trace amounts of polyphosphates (Kulaev 1979) so micro-organisms are the only major source of polyphosphates in soil. The absence of polyphosphates in soil organic matter (other than microbial) and in chemisorbed soil P fractions shows that polyphosphates do not exist free in soil long enough to be incorporated into stable organic matter or occluded fractions. This is in accordance with the labile nature of soil polyphosphates, which can disappear entirely over a few days (Pepper et al 1976).

There is no published information on the quantities of polyphosphates in soil or their spatial occurrence. The latter, however, can be assumed to be similar to the spatial occurrence of micro-organisms in soil (see Chapter 2). Polyphosphates are known to be found in mycorrhizal fungi (Harley and Smith 1983) and are postulated to be the major form of P transfer within mycorrhizal fungi (Cox et al 1980). This implies that polyphosphates may be found in soil wherever mycorrhizal fungi are present. Mycorrhizal fungi are common in most soils, as they infect most cultivated crops and most plant species growing in natural ecosystems (Hayman 1978). The quantities of mycorrhizal polyphosphates in soil, however, have not been reliably determined.

In this project, up to 35 $\mu g\ P\ g^{-1}$ as polyphosphates were found in soil. It is possible that some of the polyphosphates measured in soil in experiments described in Chapter 5 were

extracted from mycorrhizal fungi. However, it is unlikely that mycorrhizal polyphosphates would be present at high levels of extractable soil P and not at lower levels, as found in the wheat soil. This argument does not hold for the medic samples, though, as polyphosphates were present in all samples. Some polyphosphates in medic soil may have been of mycorrhizal origin. Increasing extractable P in soil reduces the extent of vesicular-arbuscular mycorrhizal infection in soil (Barea 1991). Soil polyphosphate content increased with extractable P. One may speculate that either the polyphosphates measured were not of mycorrhizal origin, or they were from a small number of mycorrhiza which were not inhibited by high extractable soil P. It is possible that polyphosphates present in medic soil samples were derived from bacteria found in root nodules. Although polyphosphates have been found in cultured rhizobial bacteria (Gourret 1978, Cassman et al 1981, Smart et al 1984) this has not been investigated in soil. This is a potential source of polyphosphate which, however, is not distinguishable from other sources of microbial P.

Polyphosphates as an indicator of potential P uptake by the biomass

For soil polyphosphates to be an indicator of P uptake by the biomass, the uptake characteristics shown in solution culture must be extrapolated to soil conditions. Although this cannot be done directly, the behaviour of micro-organisms in soil shows similarities to that of micro-organisms in solution. Polyphosphate accumulation in cultured micro-organisms may occur in two patterns. The first is the polyphosphate overplus mechanism (Harold 1966) which has been demonstrated many times in solution culture (Harold 1966, Kulaev 1979). Ghonsikar and Miller (1973) found micro-organisms in soil behaved in this way when orthophosphate was added to soil samples following an induced P deficiency caused by addition of glucose.

However, the absence of plants from soil amended by Ghonsikar and Miller (1973) may mean that their findings cannot be applied to field soil except under fallow conditions. The changes in soil polyphosphate content measured by these workers may have been different if plants had been present. Plants provide the main source of substrate for soil micro-organisms (Lynch and Panting 1980b) and determine the food supply and thus the vigour of the soil microbial biomass.

The second pattern of polyphosphate accumulation in micro-organisms grown in solution culture is a gradual accumulation which may occur through nutrient imbalance. Polyphosphates may accumulate in soil without recent amendment of orthophosphate and glucose (Chapter 5). Presumably this accumulation is more gradual than that induced by orthophosphate addition.

Polyphosphates successfully indicated biomass P status, as inferred by bicarbonate-extractable soil P. Soil polyphosphate concentrations could be used to distinguish differences

between soil under wheat and under medic plants at the same concentration of bicarbonate extractable P. It remains to be seen if differences in soil polyphosphate concentration reflect real differences in P uptake by the microbial biomass between these soils at the same concentration of extractable P. One may speculate that soil polyphosphate concentration could "fine tune" estimates of P uptake by the microbial biomass, as inferred from extractable P concentrations.

The use of polyphosphates as an indicator of P uptake by the microbial biomass requires two steps. Firstly, biomass P status must be related to the presence of polyphosphates. Secondly, biomass P status must be related to microbial P uptake in soil. The first step has been accomplished. The second step is a topic for further research.

Further work

Further work would extend investigations into the use of soil polyphosphate concentration as an indicator of P uptake by the microbial biomass. This could be done by adding ^{32}P-labelled plant material to soil which did or did not contain polyphosphates. The uptake of labelled P by micro-organisms in each soil could be related to bicarbonate-extractable soil P and competition with plants for available P. This would determine if soil polyphosphate concentration could predict P uptake by the microbial biomass.

Soil polyphosphate concentration could be investigated as an indicator of the degree of mycorrhizal infection of plant roots. A simple test for polyphosphates may be useful as a chemical assay for the presence of mycorrhizal roots in soil.

Summary

The objectives of this project were to determine whether microbial P uptake could be related to polyphosphate in micro-organisms and to examine differences in biomass P status under wheat and medic plants. Understanding the reasons for differences in the P status of the biomass under wheat and medic plants may allow more efficient fertilizer management in wheat/medic rotations, which provide a basis for one of the major farming systems in southern Australia.

Pretreatment of micro-organisms with low or high P changed both their polyphosphate content and their subsequent P uptake. The two factors were closely related. Pretreatment with low P increased subsequent P uptake, while pretreatment with high P decreased subsequent P uptake. Polyphosphates were detected in bacteria pretreated with high P, but

not in fungi. Reduction of subsequent P uptake required smaller increases in P nutrition than accumulation of polyphosphates.

The polyphosphate concentration of wheat soil was greater than that of medic soil at high P and lower than that of medic soil at low P. Polyphosphates in medic soil were more stable during storage at 4°C than those in wheat soil. Possibly this was due to different microbial populations in wheat and medic soil, as inferred by differences in microbial P and C caused by amendment of soil with wheat or medic roots. Soil polyphosphates were shown to be resident in the microbial biomass.

General trends in soil fertility appear to be reflected in the P status of the microbial biomass and raise the possibility of correlating microbial P uptake with soil fertility. Soil polyphosphate concentration has potential to be investigated as an indicator of likely microbial P uptake in field soil. Further investigation is needed to relate P uptake by the microbial biomass to microbial P status. This would increase our knowledge of P cycling through the biomass and possibly enhance the efficiency of fertilizer use under crop rotations.

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