PHOSPHORUS CYCLING IN SOIL

UNDER WHEAT-PASTURE ROTATIONS

A thesis presented in fulfilment of the requirements

for the Degree of Doctor of Philosophy, Faculty of

Agricultural Science, The University of Adelaide.

by

MICHAEL JOHN McLAUGHLIN

Department of Soil Science

Waite Agricultural Research Institute

The University of Adelaide

NOVEMBER, 1986

TABLE OF CONTENTS

TABLE OF CONTENTS (cont.)

IV

TABLE OF CONTENTS (cont.)

Page No.

CHAPTER 6. TRANSFORMATIONS OF P IN CROPPED SOIL —

           FIELD EXPERIMENT 129

  6.1 Introduction 129

  6.2 Materials and methods 129

    6.2.1 Preparation of ^{33}P-labelled medic

          residues 130

    6.2.2 Wheat growth 131

    6.2.3 Sampling and analyses 135

  6.3 Results and discussion 136

    6.3.1 Plant growth and P uptake 136

    6.3.2 Changes in ^{31}P, ^{32}P and ^{33}P in soil

          fractions 143

  6.4 General discussion 158

  6.5 Summary and conclusions 163

CHAPTER 7. GENERAL DISCUSSION AND CONCLUSIONS 165

  7.1 Measurement of microbial P in soil 165

  7.2 Uptake of fertiliser P by soil

      microorganisms 168

  7.3 P cycling from plant residues 171

  7.4 P nutrition of wheat in wheat-medic

      rotations 177

  7.5 General conclusions 183

BIBLIOGRAPHY 174

APPENDICES 211

LIST OF FIGURES

LIST OF FIGURES (cont.)

LIST OF FIGURES (cont.)

LIST OF TABLES

LIST OF TABLES (cont.)

LIST OF TABLES (cont.)

LIST OF PLATES

LIST OF APPENDICES

SUMMARY

Transformations of phosphorus (P) within the
soil-plant-animal system are complex and are affected by a
combination of physical, chemical and biological reactions. To
date, a large proportion of the research into reactions
involving soil P has focussed on the inorganic components of
the P cycle, despite the knowledge that biological reactions in
the soil can have overriding influences on the transformation
of P from one form to another. There is a need to study the
influence of the microbial biomass on P cycling in soils, and
this has been aided by the development of new methods to
measure P held in soil microorganisms. In Australia this need
is particularly important, as P is the major nutrient applied
to agricultural soils. The objectives of the research reported
in this thesis were therefore
(1) to develop a suitable procedure for measurement of P held
in the microbial biomass in soils under arable rotations,
(2) to assess the importance of the microbial biomass in the
assimilation of fertiliser P,
(3) to determine the role of the microbial biomass in the
turnover and decomposition of cereal root and pasture plant
residues and,
(4) to assess the importance of pasture residues in the P
nutrition of the ensuing cereal crop.

The literature pertaining to the role of microorganisms in P
cycling in soil is reviewed. Factors affecting microbial
activity in soil are considered in relation to the uptake of P

XIV

by microorganisms, and the turnover of P from plant roots and residues is discussed. Methods of studying P cycling are reviewed in relation to the techniques required to study P transformations in field soils.

An improved method for measuring microbial P in field soils was developed. A range of gas, liquid and vapour biocides was tested, in combination with seven extractants, for their ability to release P from soil microorganisms in situ. The biocides tested were chloroform ($CHCl_3$), ethanol (C_2H_5OH), propan-1-ol (C_3H_7OH), hexan-1-ol ($C_6H_{13}OH$), β-propiolactone ($C_2H_4O_3$), formaldehyde (CH_2O), glutaraldehyde ($C_5H_8O_2$), ethylene oxide (C_2H_4O) and methyl bromide (CH_3Br). The extractants tested were $0.5\underline{M}$ $NaHCO_3$(pH8.5), $0.1\underline{M}$ $NaHCO_3$(pH8.5), $0.05\underline{M}$ NaOH, $0.01\underline{M}$ $CaCl_2$, $0.05\underline{M}$ H_2SO_4, $0.03\underline{M}$ NH_4F + $0.1\underline{M}$ HCl and an anion exchange resin in the bicarbonate form. An incubation technique using ^{32}P ensured only microbial P was measured.

Chloroform and hexanol were the most efficient biocides: the latter was preferred because of its less hazardous nature. The best extractant was $0.5\underline{M}$ $NaHCO_3$ (pH 8.5). Mixed populations of soil microorganims were used for calibration purposes, and K_p factors obtained were 0.33, 0.40 and 0.57 for the three soils studied. Since microflora differ from soil to soil, as does the proportion of P released as inorganic P, calibration is necessary for each soil. Incubation is not recommended as a pretreatment for samples used to measure microbial P: soils should be treated with hexanol or extracted immediately after sampling to avoid quantitative or qualitative changes in the biomass. Errors associated with the inclusion of plant root

material in the sample can be minimised by removing the bulk of the roots before fumigating the soil.

The competition between soil microorganisms and plants for fertiliser P is likely to be greatest in the rhizosphere, where root densities are high and substrates for microbial growth are available. This poses problems for the measurement of microbial P in soil adjacent to plant roots. Accordingly, a new technique was adopted to separate the roots from soil with a porous membrane. This technique also allowed an assessment of the loss from the root of P in diffusible exudates. Wheat plants labelled with ^{33}P were grown in thin layers of soil amended with ^{32}P-labelled fertiliser. Over a 22-day growth period, net movement of ^{33}P out of healthy growing roots varied from 0.9 - 4.9% of the total ^{33}P translocated to the root. Over the same period, the plants took up 12.0% and the microbial biomass 14.1% of the fertiliser ^{32}P. On drying and rewetting of the soil after the plants were harvested, a large proportion of root P moved into soil fractions while ^{32}P appeared to accumulate in the biomass and stable forms of P.

The contribution of pasture residues and fertiliser to the P nutrition of wheat was studied in laboratory and field experiments. Wheat plants (Triticum aestivum cv. Warigal) were grown in a Solonised brown soil (Calcixerollic xerochrept) which had been previously cropped with medic (Medicago truncatula cv. Paraggio). In a laboratory experiment, ^{33}P-labelled medic residues and ^{32}P-labelled monocalcium phosphate were added to the soil in factorial combination. Amounts of ^{31}P, ^{32}P, and ^{33}P in the wheat plants and in the

soil microbial biomass were determined. Addition of residues depressed dry weight of wheat, ^{31}P, and ^{32}P uptake, while simultaneously increasing amounts of ^{31}P and ^{32}P incorporated into the microbial biomass. Addition of fertiliser had no effect on the proportion of plant P taken up from the residues, but significantly increased the proportion of microbial P derived from this source. [31]P held in the microbial biomass was significantly increased by addition of both residue and fertiliser P, with the former having the larger effect. Of the total P applied to the soil, medic residues contributed approximately one quarter of that supplied by the fertiliser. Of the total P in the wheat plant, medic residues supplied approximately one fifth of that supplied by the fertiliser.

In a field experiment, ^{33}P-labelled medic residues and ^{32}P-labelled fertiliser were added to open ended pots driven into the soil. Residues were mixed throughout the soil while fertiliser was banded with the seed just below the surface. Dynamics of P uptake by the wheat plants and the microbial biomass in the soil were measured over a 95 day period. [33]P from the residues was rapidly incorporated into the microbial biomass with more than 25% of the applied radioisotope being found in the microbial pool after only 7 days. Little of the ^{32}P from the fertiliser was taken up by the microorganisms presumably because of its location in a band, but the wheat plants were able to assimilate a significant proportion of the fertiliser [32]P. The microbial biomass immobilised a smaller proportion of the P released from the residues under field compared with laboratory conditions, and the residues proved to

be a less effective source of P for the plants in the field.
This fact, coupled with the observation that a large proportion
(50%) of the residue P was quickly converted to organic forms
suggests that the turnover of the microbial population was
faster in the field than in the laboratory studies. The
implications of the results in terms of fertiliser management
and soil P cycling are discussed.

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, except where due reference is made in the text of the thesis.

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

MICHAEL JOHN McLAUGHLIN

November, 1986.

ACKNOWLEDGEMENTS

ABBREVIATIONS

P_i = inorganic P

P_o = organic P

P_t = total P

SE = standard error of mean value $(= \sqrt{S^2/N})$

SD = standard deviation of mean value $(= \sqrt{S^2})$

CHAPTER 1

INTRODUCTION

Phosphorus (P) is unique among elements involved in living organisms in that it acts as an agent of energy transfer through the generation of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) from inorganic orthophosphate (P_i) (Hutchinson, 1970). As pointed out by White (1981) the global P cycle is presently open (Figure 1). World reserves of fertiliser P must be regarded as finite until technology enables us to complete the circle, and economically recover P from natural waters and sediments. However, available evidence suggests that world reserves of phosphate rock are large (Finck, 1982). Agriculturalists should not be complacent however, as the costs of producing P fertiliser from phosphate rock are escalating, as is concern over environmental pollution such as eutrophication of surface waters. In Australia, palaeoenvironmental factors have produced many soils low in phosphorus, compared with those in USA or Europe (Wild, 1958). As a result, it is the amount of P, more than the amount of any other nutrient, which governs the fertility status of Australian soils (Norrish and Rosser, 1983). Accordingly, the main artificial fertiliser used in Australia is superphosphate, although high analysis P fertiliser is gaining acceptance as an alternative source of P. In 1981-82 the total area fertilised was 26.7 million hectares (ha). The most recent data available suggest that the upward trend in total amounts of superphosphate used in Australia is continuing with 2.87 million tonnes being used in 1981-82 (Australian Year Book,
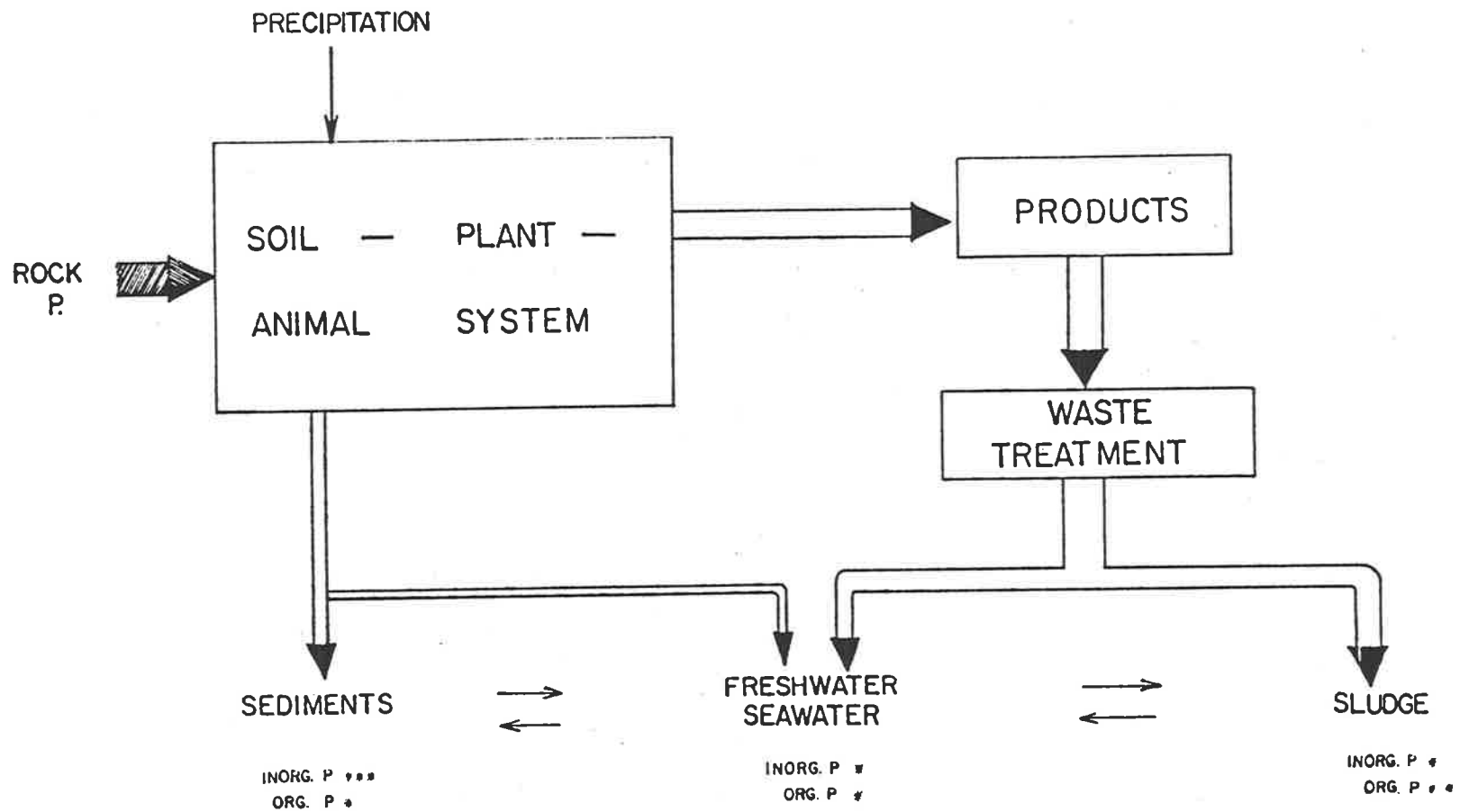
Figure 1. The global P cycle (from White, 1980). * indicates relative abundance of P forms

1984). As Australia has few large deposits of phosphate rock
from which superphosphate fertiliser can be manufactured,
$109.5 million was spent on imports of rock phoshate in
1981-82. At the farm level, the cost of superphosphate
fertiliser has almost trebled since 1975, so that farmers are
looking more seriously at the investment they must make in
terms of nutrient inputs to the soil. Any small percentage
decrease in the use of superphosphate will have large economic
impacts at both the farm level, and nationally in terms of
trade balance. To decrease use of superphosphate will require
changes in farm practice and the development of improved
methods of crop and soil husbandry. To meet this objective,
agricultural scientists require a much better understanding of
the soil P cycle than they have at present, so that each
agro-ecosystem can be managed effectively.

The principal agro-ecosystem in much of southern Australia
is based on cereal-livestock farming. This involves rotational
cropping of legume based pastures, such as subterranean clover
(Trifolium subterranean) or medics (Medicago spp), with cereal
crops such as wheat (Triticum aestivum) or barley (Hordeum
vulgare). Much of the P added to the soil in this system of
cropping is applied during the cereal phase of the rotation.
However, topdressing of the annual legume pastures with
superphosphate has been frequently recommended and responses
are well documented (Donald, 1960; McLachlan, 1968; Cameron and
McGowan, 1969; Rudd,1972). The residual effect of
superphosphate applied in the pasture phase of the rotation on
the subsequent wheat yield has been recognised (Ayres et al,

1977; Osborne et al, 1977), although the mechanism of the
residual effect is still not clear. There is little
information on the residual effect of P contained in cereal
roots (remaining in the soil after harvest) on the P nutrition
of the subsequent cereal crops.

In laboratory studies, the reactions of inorganic P are
often considered with little regard for reactions of organic
matter which can affect availability of residual P (e.g.
Barrow, 1973,1974; Olsen et al, 1983). In a review of the
residual effect of P application to soils, Barrow (1980)
considers mainly those reactions involving inorganic
phosphorus, although he does point out that cycling of P
through organic materials is particularly important under
Australian conditions. Virgin soils are P-deficient, have low
levels of biological activity and low amounts of organic
matter. Inputs of nutrients will therefore have a large effect
on the biological activity in such soils. To understand the
system fully, we must consider the whole P cycle where both
organic and inorganic reactions are occurring simultaneously.
Compared to the processes of precipitation-solubilisation and
adsorption-desorption, the rates and pathways of P through soil
organic matter are poorly understood, and involve complex
interrelationships between detrital processing, mineralisation
of organically bound P (P_o) and P uptake mechanisms (Tate,
1985a). Despite the knowledge that activity of microorganisms
has a large influence on the above reactions, studies of the
role of the biota in soil fertility have been hampered by a
lack of suitable experimental techniques. Recent advances in

techniques to measure P held in the biomass have created new opportunities to elucidate the relationship between inorganic and organic P forms in soil. This has implications not only for agricultural systems, but also in ecosystems undisturbed by man, where cycling of nutrients through the biomass is critical for survival of the system.

The objectives of this study therefore were

(1) to develop a suitable procedure for measurement of P held in the microbial biomass in soils under arable rotations,

(2) to assess the importance of the microbial biomass in the assimilation of fertiliser P using the above technique,

(3) to determine the role of the microbial biomass in the turnover and decomposition of cereal root and pasture plant residue, and

(4) to ascertain the importance of pasture residues in the P nutrition of the ensuing cereal crop.

CHAPTER 2

LITERATURE REVIEW

2.1 The soil P cycle

Transformations of P within the soil-plant-animal system are complex (Figure 2) and are effected by a combination of physical, chemical and biological reactions. As depicted in Figure 1, the P cycle in soil can be either open, where P is added in fertiliser and removed by cropping, or virtually closed as in natural ecosystems where only small inputs of P are provided by rainfall and weathering, and P is recycled within the system by microbial breakdown of dead plant material. In all terrestrial ecosystems, by far the largest proportion of P in the system is held in the soil. For example, the amounts of P (kg ha^{-1} to 30 cm depth) in the various components of a grassland ecosystem were soil 2913, roots and rhizomes 10.7, green plant material 2.1, standing dead plant material 2.1, and soil fauna and microorganisms 19.8 (Halm et al, 1972). The amounts of P in litter, herbivores and carnivores, and in water soluble forms in the soil were all less than 1 kg ha^{-1}. While amounts of P held in each of the pools may vary from one ecosystem to another, there is invariably insufficient water soluble P in most soils to support plant growth over a whole growing season. P removed from the soil solution by plants must be replenished from less soluble sources in the soil. P exists in a variety of both organic and inorganic forms in soil. The forms and reactions of inorganic P (P_i) in soil have received considerable research effort and are well documented - for reviews see Larsen (1967),

- 7 -
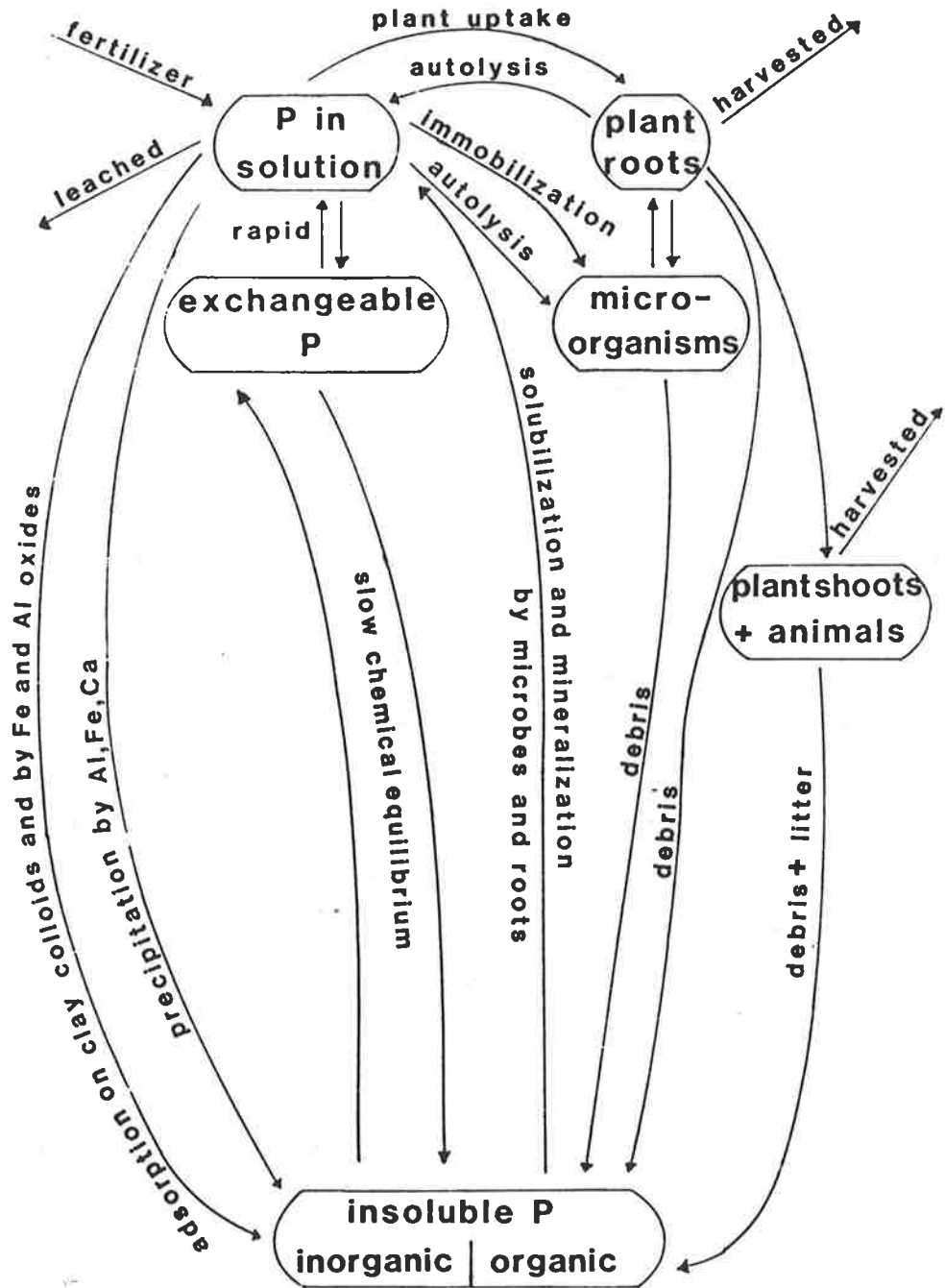
Figure 2. The P cycle in soil (from Hayman, 1975).

Barrow (1978), Parfitt (1978), White (1980) and Sample et al (1980). Considerable research has also been directed toward organic P (P_o) in soil - for reviews see Anderson (1975), Hayman (1975), Halstead and McKercher (1975), Dalal (1977), Anderson (1980) and Tate (1985a,1985b). Agriculturalists however, are more interested in the interactions between P_i and P_o in the soil-plant system. Management of nutrients in any agricultural system has the prime objective of ensuring sufficient amounts of inorganic nutrient (normally in the soil) are converted into organic forms (in plants or animals) capable of being harvested. There are a number of points in the soil P cycle where the fundamental transformation of P between organic and inorganic forms occurs, and all are associated with either growth or death of living organisms. Thus any study of P cycling in soil which has the ultimate aim of improving fertiliser efficiency, must take account of the biologically mediated reactions.

The living component of the soil-plant system can be divided broadly into autotrophs and heterotrophs. Autotrophs include all agricultural crops as well as some free living organisms in the soil - chemoautotrophs such as nitrifying and denitrifying bacteria, and photoautotrophs such as algae. Heterotrophs include all the underground flora and fauna involved in the decomposition of dead plant tissue as well as pathogenic organisms feeding on living plant and animal life. In agricultural research of soil processes, where the emphasis is on cropping, a slightly different classification of organisms has generally been used to facilitate research of P cycling

through the system — higher autotrophs (green crops), and lower
autotrophs plus all heterotrophs (soil biomass).

2.2 The role of plants in P cycling

Plants act as both sources and sinks of P in soil. During
the growth period of a plant its roots act as a sink,
collecting P to be utilised in the synthesis of organic plant
constituents. After the termination of growth and the start of
decay, the flow is reversed and nutrients are released into the
soil from the breakdown of plant debris.

2.2.1 Uptake of P by plants

Uptake of P by plants is the part of the P cycle which has
received most attention by agricultural scientists.   It is
beyond the scope of this review to detail all the factors
involved in P uptake by plants. The movement of P both to and
into the plant root have been extensively studied and several
texts and reviews of the subject are available e.g. Olsen and
Kemper (1968), Bieleski (1973), Bowling (1976), Nye and Tinker
(1977), Loughman (1978), Ozanne (1980) and Mengel and Kirkby
(1982). One feature which should be noted here is that
evidence to date suggest that, with a few exceptions (McKercher
and Tollefson, 1978; Islam et al, 1979), plants absorb P from
the soil solution only as an inorganic ion.

2.2.2 Loss of P by actively growing plants

The main pathway for loss of P by actively growing plants is
through the roots, excepting leaf fall or defoliation by

grazing. Loss of P by plant roots was first recognised by Akhromeiko and Shestakova (1958), who used foliar applications of ^{32}P to demonstrate the loss of P by oak and ash seedlings. Subsequent studies also used ^{32}P (Emmert, 1959; Dove, 1969; Rovira and Bowen, 1970) and confirmed that a range of plants were capable of losing ^{32}P to the surrounding medium which in most studies was a nutrient solution. More recently, Ritz and Newman (1984) demonstrated the transfer of ^{32}P from one plant to another in soil. Transfer can occur between both mycorrhizal and non-mycorrhizal plants (Heap and Newman, 1980). Estimates of the quantitative loss of P by roots through exudation given by Harrison (1978) for forest ecosystems suggest that the loss may be substantial — up to 2 kg P ha^{-1} year^{-1}. To date, no data are available for arable ecosystems.

Other possible losses of P from actively growing plants may be caused by attack of roots by pathogenic organisms, or through natural senescence of parts of the root system during plant growth. While many studies have recognised these processes exist, there are few quantitative data on the losses of P involved.

2.2.3 Loss of P by plants following death

Loss of P by plants following death is considered to be the major pathway by which P in plants returns to the soil, and is intimately associated with the action of microorganisms. Death and the onset of decomposition in plants may be initiated by a number of factors including natural senescence, water or temperature stress, grazing, and disturbance by man (e.g.

addition of herbicide or cultivation). The release of
inorganic P from plant materials following death is often
termed "mineralisation", and a number of studies have examined
the processes involved. Over forty years ago, both Lockett
(1938) and Chang (1939) demonstrated that decomposition of
plant residues involved both the breakdown of P_o in the
residues and the synthesis of P_o by microorganisms. Lockett
(1938) also observed that P could be released into the soil as
orthophosphate on prolonged decomposition of organic materials,
and he presumed this was due to death of the microbial
populations which had developed. These results led to a number
of studies which aimed to determine the factors affecting the
release of P from decomposing plant residues. Kaila (1949)
suggested that net release of P would occur if the P content of
the decomposing organic matter was greater than 0.3%. Using
^{32}P-labelled plant materials, Fuller et al (1956) found that
once the total P content of the materials exceeded 0.2%, net
release of P could be expected. Birch (1961) postulated that
the decomposition of plant P_o was less important than the
utilisation of plant P_i by decomposer populations, and that
release of P_i from the material was due to a rapid decrease in
decomposer populations. This postulate is in agreement with the
observations of Lockett (1938). Birch also noted that a large
proportion of plant P was inorganic, and later work confirms
his observation (Table 1). Martin and Cunningham (1972) found
that P_i could be released from dead plant roots before an
extensive population of microorganisms had developed in
response to the fresh organic matter, and they suggested that

this was due to autolytic activity of plant enzymes.
Air drying of roots was found to increase considerably the
water extractability of root P. A number of studies have
attempted to relate the release of P from decomposing plant
materials to the C:P ratio of the materials being decomposed,
analagous to assessing the critical C:N ratio for N
mineralisation. The results are conflicting, and critical
values reported vary from 55 to over 500 (Table 2). As pointed
out by White (1981) however, the change from net immobilisation
to net mineralisation depends on the C:P ratio of the material
actually being used by the organisms (not the total C:total P
ratio of the material), as well as on the growth yield (CF =
the efficiency with which microbes convert substrate C into
cell C) and the C:P ratio of the microbial cells formed
($C_m:P_m$). White indirectly estimated a critical C:P ratio of 50
- 70 from measured values of C respired (C_r) and net P
mineralised (P_r) according to the equation

$$C:P \text{ substrate} = (1- CF)P_r:C_r + CF / C_m:P_m$$

and the following values from the literature

CF = 0.5 (Jenkinson, 1976)

$C_m:P_m$ = 50 (Alexander, 1977)

$C_r:P_r$ = 0.009 - 0.02 (Thompson et al, 1954; Dalal, 1979).

However, reported CF values range from 0.1 (Alexander, 1977) to
0.6 (Paul and van Veen, 1978), and $C_m:P_m$ values range from 10.6
(Brookes et al, 1984) to 100 (Cole et al, 1977). As
microorganisms will often store P in excess of their immediate
requirements as polyphosphates (Barsdate et al, 1974;

Table 1. Inorganic P as a proportion of total P in plant
          residues.

Material	Reference	%
Tropical grasses, young to mature	Birch (1961)	47-87
Grasses	Floate (1970a)	58-66
Grasses and subterranean clover	Bromfield and Jones (1972)	60-85
Beans	White and Ayoub (1983)	75-82

Table 2. Critical C:P ratios reported in the literature.

Ratio	Reference
55	Barrow (1960)
>77	Dalal (1979)
165-366	Floate (1970a)
133	Kaila (1949)
>183	Singh and Jones (1976)
200	Black and Goring (1953)
200	Alexander (1977)
200	Hannapel et al (1964)
123-251	White and Ayoub (1983)
200-300	Stevenson (1982)
>298	Chauhan et al (1979)
150-300	Blair and Boland (1978)
112-501	Enwezor (1976)

Pepper et al, 1976), variable C_m:P_m ratios are to be expected.
The release of P from decomposing plant materials may be
closely related to the dynamics of the decomposer populations.
For example, Johannes (1965, 1968) found that bacteria in
aquatic ecosystems released little P, but that substantial
quantities of P could be released due to the activity of
bacterial grazers. There is evidence that the cycling of P
from decomposing plant material is not directly linked to the
cycling of C (Barsdate et al, 1974). The usefulness of

considering strict stoichiometric relationships between C and P is therefore questionable (McGill and Christie, 1983).

A criticism of many laboratory studies in which mineralisation has been examined has been the absence of growing plants from the system. Plants compete with microorganisms for available P and act as a sink for P_i. Hence equilibria between solution and solid phase P in the soil are affected. The net mineralisation of P from decomposing plant residues is stimulated by the presence of plants (Blair and Boland, 1978). ^{32}P has often been used in studies of P uptake by plants from decomposing residues, to identify the proportion of P in the residues which enters the plant. For example, Fuller and Rogers (1952) and Fuller et al (1956) found that the plant availability of P in the ^{32}P-labelled plant materials was related to the maturity of the material. P in young materials was more available than P in mature materials. Till and Blair (1978) found a positive relationship between soil temperature and the amounts of ^{32}P and ^{35}S taken up by carpet grass (Axonopus affinis) from clover (Trifolium subterranean) residues (Table 3). Blair and Boland (1978) found that changes in soil water regime had no significant effect on uptake of ^{32}P by oats from labelled clover residues. Both Blair and Boland (1978) and Dalal (1979) found a high proportion of P in the residues was taken up by plants. Dalal (1979) reported that up to 42% of total residue P was recovered in plants after only 10 weeks growth. High recovery of P from residues might be expected where the only source of applied P is the plant residues, and is likely to occur therefore in natural or

Table 3. Recovery of ^{32}P from labelled clover litter (from
Till and Blair, 1978).

Days of growth	Temperature (°C)	Percentage of added ^{32}P recovered			
		Soil P_o	Soil P_i	Plants	Mineralised
14	15/10	3.0	4.8	3.3	8.1
	28/22	5.9	6.1	4.3	10.4
36	15/10	15.5	16.3	13.8	30.1
	28/22	10.7	21.1	14.2	36.3
56	15/10	23.6	19.1	22.2	41.3
	28/22	14.9	21.6	28.3	49.9

agricultural systems where there are no inputs of fertiliser P
to the soil. In fertilised agricultural soils however, the
plant can draw on P from currently applied and residual
fertiliser P sources, in addition to P in native soil reserves
and decomposing residues of the previous crop.

   To summarise, it would appear that while plants can lose P
during active growth the main loss of P occurs following death
and decomposition of the plant material. The release of P from
dead plant material is closely linked to the growth and death
of organisms feeding on the material. Autolytic activity of
plant enzymes following death allows the release of P in an
inorganic form. The importance of organisms appears to be
linked more to their assimilation of P_i released from plant
material rather than the hydrolysis and assimilation of plant
P_o. The usefulness of using plant C:P ratios to predict net
mineralisation or net immobilisation is questionable. There is
a need to include growing plants and/or fertiliser in the

system under study due to the large effects these can have on microbial activity and P equilibria.

2.3 The role of the microbial biomass in P cycling

The microbial biomass, defined as the living part of the soil organic matter excluding plant roots and soil animals larger than $5 * 10^{-15}$ m^3 (Jenkinson and Ladd, 1981), has for many years been recognised as playing a major role in many processes involving P transformations. These can be listed as follows;

1) redox reactions,

2) solubilisation of P_i,

3) alteration of root morphology and/or function,

4) immobilisation of P_i, and

5) mineralisation of P_o.

2.3.1 Redox reactions

There is some evidence that microorganisms can directly change the oxidation state of P (Adams and Conrad, 1953), although these reactions are of limited importance in soils (Hayman, 1975). Of greater significance is the indirect effect of microorganisms in decreasing the redox potential of waterlogged soils, thus affecting the solubility of iron and aluminium phosphates. Only under extremely reducing conditions can insoluble basic calcium phosphates like hydroxyapatite be solubilised in soils through changes in redox potential (Lindsay, 1979). The importance of these reactions is limited in Australian soils. Of more significance are the changes in

pH which accompany changes in redox potential (Ponnamperuma, 1972).

2.3.2 Solubilisation of P.

As long ago as 1908 the potential of certain bacteria to solubilise water-insoluble P compounds was recognised (Sackett et al, 1908). Since then there have been numerous studies in which the ability of a wide range of organisms to solubilise P has been demonstrated. Much of the work in this field was stimulated by the experiments of Gerretsen (1948), who found that the uptake of P by plants from sand supplied with calcium phosphate increased in the presence of microorganisms. The various mechanisms postulated to explain this phenomenon have been reviewed by Nye and Tinker (1977). Organic acids produced by organisms (Rose, 1957; Louw and Webley, 1959) are able to dissolve P by changing pH (Moghimi et al, 1978), and possibly by chelating calcium ions (Swaby and Sperber, 1958). Hayman (1975) suggested that CO_2 and humic substances produced during the breakdown of organic materials may also cause solubilisation of P compounds through alteration of pH and complexation, and there is some evidence to support this hypothesis (Mishustin, 1972). However, Tinker (1984) points out that it is improbable that such effects could be appreciable in soil which is buffered, in which the supply of cations to be chelated is very large, and in which phosphate-competing ions are rapidly sorbed. Nye and Tinker (1977) also point out that the quantities of compounds which microorganisms must generate to produce any significant effects on P compounds make it

unlikely that this process is of any significance.
Solubilisation by materials derived from plant roots directly
is considered much more likely (Hayman, 1975; Tinker, 1984).
In the rhizosphere the amounts of root exudates and microbial
metabolites combined appear to be sufficient to effect
solubilisation of calcium phosphates (Moghimi et al, 1978;
Hedley et al, 1982b).

2.3.3 Alteration of root morphology and/or function

The role of microorganisms in altering root morphology
and/or function is outside the scope of this review. The
alteration of root growth through hormonal activity has been
reviewed by Mosse (1975). There is evidence that
microorganisms can alter the rate of P uptake by roots over
such short periods that no morphological or developmental
effects within the plant are likely (Barber and Rovira, 1975),
and hormonal mechanisms may be involved (Tinker, 1980). Of
greater importance is the effect of mycorrhizas on nutrient
uptake. It is now widely known that plants infected with
mycorrhizas are able to grow and absorb P from P-deficient
soils more effectively than non-mycorrhizal plants (Mosse,
1973; Tinker, 1978). Various mechanisms have been proposed to
account for the mycorrhizal response, and these were summarised
neatly by Bolan et al (1984) as follows.
1) Roots infected with mycorrhizal hyphae can explore a greater
volume of soil than non-mycorrhizal roots (Rhodes and
Gerdemann, 1975).
2) mycorrhizal and non-mycorrhizal plants differ in their

absorption of P from solution. Mycorrhizal plants have faster

uptake rates (Bowen et al, 1975; Cress et al, 1979; Howeler et

al, 1982), and have a lower threshold concentration at which

net absorption of P from solution commences (Mosse et al, 1973;

Howeler et al, 1982).

3) Mycorrhizas may produce exudates which increase the amount

of P available to plants as discussed above (Tinker, 1975).

4) Mycorrhizal roots have a different cation-anion balance to

that of non-mycorrhizal roots and hence may influence

rhizosphere pH (Bulwalda et al, 1983).

5) It has also been suggested that mycorrhizas may mineralise

P_o (Bolan et al, 1984), although there is no evidence to

support this hypothesis.

2.3.4 Mineralisation and immobilisation of P

These processes are the major biological reactions

controlling the availability of P in soils, mineralisation

causing increases and immobilisation decreases in P availabilty

to plants. It is difficult to consider these processes

individually as both occur simultaneously. Indeed, the terms

mineralisation and immobilisation as generally used in the

literature usually refer to net mineralisation and net

immobilisation.   As biological activity proceeds in all but

sterilised soils, immobilisation - mineralisation reactions are

continuous. However, various perturbations which affect

biological activity can significantly affect the rate of, and

balance between, these opposing reactions. These include

changes in available energy(C) or nutrients, trophic

interactions, temperature, available water, oxygen, and soil reaction (Dalal, 1977).

Energy

Biological reactions rarely reach an equilibrium state in most soils due to the inputs of energy (C) by both growing roots (Martin, 1977; Martin and Puckridge, 1982) and dead plant material. It has been known for many years that organic compounds are exuded by healthy undamaged roots (for reviews see Rovira (1962) and Foster (1985)). Recently, techniques using ^{14}C-labelling have shown that a considerable portion of the C fixed by plants and translocated to the roots can be "lost" to the soil. Approximately one third of this C lost to the soil can be attributed to root respiration, with the remainder presumably arising from microbial degradation of root tissue or exudates (Martin, 1977; Sauerbeck and Johnen, 1977). Thus the population of microorganisms present on, and immediately adjacent to, the root surface is much higher than in the surrounding soil. The difference in microbial numbers is commonly expressed by the R:S ratio, R and S being the numbers of microorganisms per gram of soil taken from the rhizosphere and the bulk soil respectively (Katznelson, 1948; Nye and Tinker, 1977). Both direct counts and plate counts have confirmed increased R:S ratios with increasing proximity to the root surface. By direct counting, Foster and Rovira (1976) estimated microbial numbers were $120*10^9$ cm^{-3} within 1 μm of the root surface, and $40*10^9$ cm^{-3} at 10 μm. Papavizas and Davey (1961) showed from results of plate counts that the

Table 4. Populations of microorganisms at different distances
from the roots of lupin seedlings determined from
plate counts (from Papavizas and Davey, 1961).

| Distance from root (mm) | Microorganisms (10^9 g^{-1} soil) | | |
	Bacteria	Strepto- mycetes	Fungi
0[a]	159000	46700	355
0-3	49000	15500	176
3-6	38000	11400	170
9-12	37400	11800	130
15-18	34170	10100	117
80[b]	27300	9100	91

a = rhizoplane, b = bulk soil

"rhizosphere effect" can extend for several mm from the root
surface (Table 4). However, their data lacks statistical
treatment, and Rovira and Davey (1974) later noted that the
numbers of microorganisms recorded by Papavizas and Davey at
distances greater than 3 mm from the root surface were not
significantly different from those in bulk soil. However, the
occurrence of the fungus _Paecilomyces marquandii_ near the root
and its absence in bulk soil shows that there are both
qualitative and quantitative differences between rhizosphere
and bulk soil. Although the plant may provide microorganisms
with C, there is little evidence to suggest that all the other
nutrients required by the organisms are supplied by the plant.
While P appears to move out of the root (see Section 2.2.2) it
is unlikely that P from this source is sufficient to meet
microbial demand, although no data are available to confirm

this hypothesis. Thus microorganisms may compete with plants
for soil P. Estimates of the comparative rates of P uptake by
microorganisms and plants by Beever and Burns (1981) have
indicated that microorganisms have more efficient P uptake
mechanisms than plant roots (Table 5). Bowen and Rovira (1966)
found that uptake of ^{32}P by tomato and subterranean clover from
nutrient solution was greater in non-sterile than in sterile
conditions. Barber (1966) and Barber and Loughman (1967) grew
plants at low concentrations of P in solution culture, and
found that roots contained almost twice as much P, and the
amounts transferred to the shoot were twenty times greater in
the absence of microorganisms than in their presence. Crossett
(1967) and Barber et al (1968) provided visual evidence to
support the results of the solution culture experiments.

Table 5. Potential P_i uptake rate of the soil flora (from
Beever and Burns, 1981).

Organism	Estimated fresh weight biomass in 0-10 cm[a] (g m^{-2})	Calculated P_i uptake rate in 10μm P_i[b] (μmol P min^{-1})	Relative P uptake (plant roots = 1)
Bacteria	103	359	9.0
Fungi	260	71	1.8
Plant roots	5000	40	1.0

a = Biomass per 1 m^2 block of soil, 10 cm deep. Bacterial and
fungal biomass based on Clark and Paul (1970) assuming a dry
weight / fresh weight ratio of 0.2. Root biomass based on
value of 50 g m^{-2} for root length per unit volume given by
Barley (1970) and assuming a conversion factor of 1 mg fresh
weight cm^{-1} root length.
b = calculated from uptake rates of microorganisms (A.
nidulans and E. coli) and millet (Pennisetum glaucum) roots in
solution culture (Beever and Burns, 1981).

Autoradiographs of plant roots grown in non-sterile conditions
showed marked accumulation of isotope at the root surface. The
accumulations were attributed to colonies of bacteria.
Decreased P uptake in the presence of microorganisms in soil
has also been demonstrated. Akhromeiko and Shestakova (1958)
showed that uptake of ^{32}P by oak and ash seedlings growing in
sand was depressed considerably by the addition of an aqueous
suspension of bacteria (Table 6). Also Benians and Barber
(1974) found that P uptake by barley plants from a P-deficient
basaltic loam soil was higher under sterile than non-sterile
conditions.

In many laboratory investigations where microbial activity
has been artificially stimulated by the addition of a C source
to simulate the natural incorporation of organic material,
changes in soil P distribution have been observed. Johnson and
Broadbent (1952) added ground lucerne to ^{32}P-labelled soil and
measured changes in the specific activity of various chemical
fractions of soil P. Accumulation of ^{32}P in organic fractions,

Table 6. Influence of bacteria on the uptake of P by oak and
ash seedlings (from Akhromeiko and Shestakova, 1958).

Species	Treatment	No. organisms present (10^4 g^{-1})	^{32}P content (cpm g^{-1}) Seedling			Sediment[a]
			Leaves	Stems	Roots	
Oak	Control	21	5.1	6.7	23.5	16.8
	+ Bacteria	3200	3.9	3.2	16.7	33.9
Ash	Control	17	24.8	10.6	108.6	14.8
	+ Bacteria	1120	16.8	9.0	82.4	53.8

a = sediment obtained after centrifugation of a water extract
of the sand.

and large and rapid changes in specific activity of other
fractions were attributed to microbial activity. Johnson and
Broadbent (1952) proposed that available P_i in soil be termed
the "metabolic pool". Plants and microorganisms may withdraw P
from the pool (immobilisation) while biological and biochemical
reactions may add to the pool (mineralisation). These workers
pioneered the concept that the microbial biomass in soil was a
labile fraction of organic matter, although many believe this
concept to be a recent one (Jenkinson and Ladd, 1981). In a
series of experiments, Ghoshal (1974, 1975a, 1975b) and Ghoshal
and Jansson (1975) were able to demonstrate indirectly the
immobilisation of fertiliser P by soil microorganisms.
Microbial activity was stimulated by addition of glucose,
cellulose or farmyard manure to the soil, and water soluble P
labelled with ^{32}P was added to the soil. The changes in ^{32}P
activity of an ammonium lactate-acetic acid extract
("available" P) of soil was monitored with time under various
treatments (Figure 3). Chauhan et al (1979) studied the
effect of repeated additions of C, as cellulose (with and
without added P) and as dried grass, on the amounts of P
extracted from soil by various reagents. They found that
approximately 39% and 22% of the P added in grass and with
cellulose, respectively, was found in organic forms of soil P
after 9 months. The addition of cellulose without P caused a
decrease in the amount of P held in plant available form (as
determined by an anion exchange method (Sibbesen, (1977)) due
to immobilisation by microorganisms. Chauhan et al (1979)
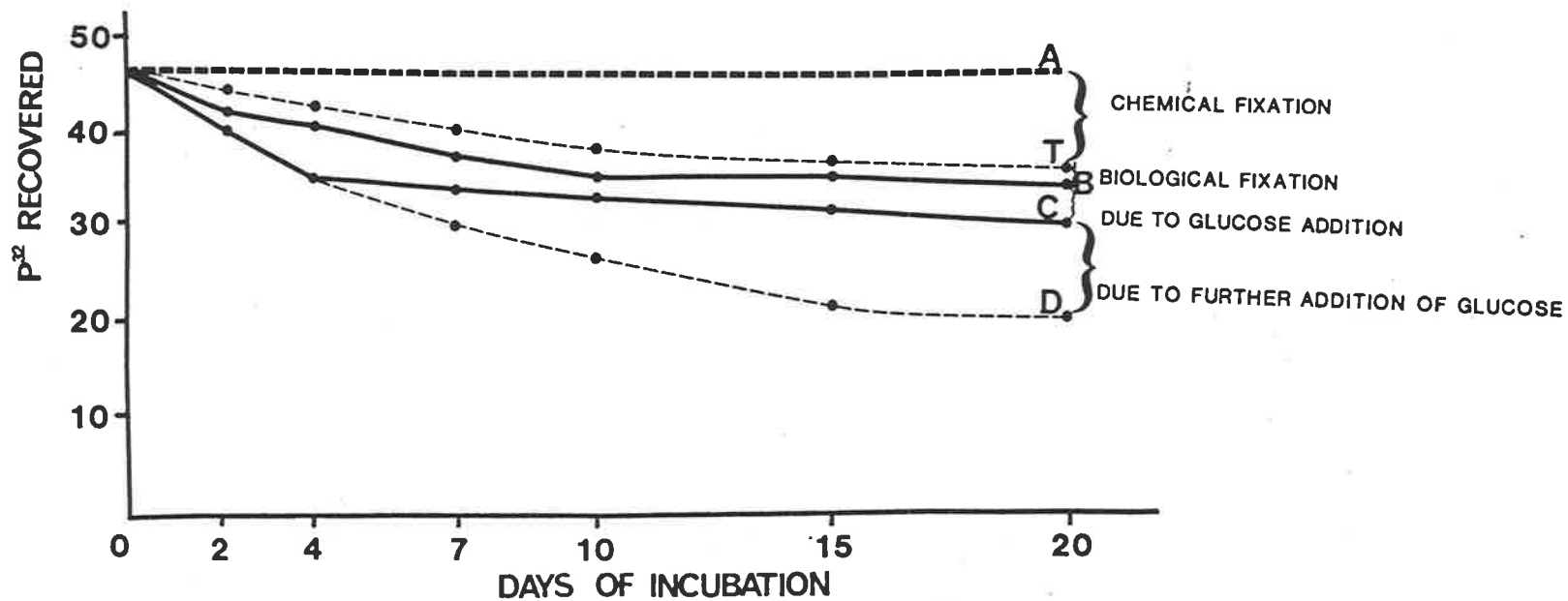
A: Theoretical line for biologically and chemically static condition.

B: 10 mg P^{32}-labelled phosphorus

C: B + 0.2% Glucose-C.

D: Same as 'C' treatment, but further additions of 0.2% Glucose—C on 4th, 8th & 12th day.

T: B + Toluene.

Figure 3. Extractable ^{32}P recovered after various periods of incubation with special reference to glucose addition (from Ghoshal, 1974).

postulated that lack of available P could eventually slow down decomposition of added C, and they subsequently presented data to support this hypothesis (Chauhan et al, 1981). However, to obtain large and measurable changes in ^{31}P extracted by the reagents, Chauhan et al used very large additions of C - equivalent to 4000 kg organic matter ha^{-1} every 30 days. Cole et al (1978) also used high rates of glucose addition in their studies of the incorporation of added P_i into the soil biomass. However, they were the first workers to apply a fumigation technique to estimate P held in the microbial biomass (see Section 2.4.2), although the fumigation technique had been used previously to measure C in the biomass (Jenkinson, 1966). They were able therefore, to measure directly the amounts of P immobilised by microorganisms. Stimulation of microbial activity with glucose decreases the proportion of added fertiliser P entering the plant (Ghoshal, 1975b; Abbott, 1978).

While most of the experimental work detailed above demonstrates that soil microorganisms can effectively immobilise solution P, it should be remembered that net mineralisation of P may also occur. Addition of organic material to soil may provide both a source of energy and of nutrients - nutrients which microorganisms can utilise to build tissue, or nutrients which may be released as the organisms release and assimilate C. This aspect has already been discussed with respect to plant materials in Section 2.2.3. Similar arguments apply to the decomposition of animal manures or other materials such as municipal composts or sludges. (For

a detailed review of the effect of manures and wastes on soil P transformations, see Sommers and Sutton (1982).) Nutrients held within microbial cells however, must be regarded as being only temporarily in this form. Microorganisms synthesise a variety of P compounds including RNA (30 to 50% of total cell P), DNA (5 to 10%), acid-soluble P_i and P_o compounds comprising orthophosphate, metaphosphate and probably polyphosphate, sugar and adenosine phosphates (15 to 20%), and various phosphorylated coenzymes and phospholipids (<10%) (Stewart and McKercher, 1982). Depletion of added C, predation, or environmental stresses (which are discussed below) will eventually lead to a decline in population, and a release of P compounds into the soil. A literature review by Katz and Leith (1974) indicates that all microbial groups undergo dramatic population changes in virtually all ecosystems. Dead organisms act as substrate for new microbial growth (Jenkinson, 1966; Anderson and Domsch, 1978), or autolytic degradation of the microbial tissue may occur irrespective of the number of survivors (Cole et al, 1978). There is evidence that P released from decomposing microorganisms can be utilised by plants. In an experiment similar to earlier studies in which uptake of P from added plant materials was examined (Section 2.2.3), Kapoor and Haider (1982) studied the net mineralisation of (dead) fungal biomass added to soil, and its availability for plant uptake. They concluded that the material was not as effective in supplying P to plants as P_i, and that melanised fungi had a lower availability in this respect than hyaline fungi. Stewart and Hedley (1980) found that a large proportion

of the P held in ^{33}P-labelled bacterial cells was rapidly
mineralised and taken up by other organisms or plants. It
should be remembered, however, that mineralisation of microbial
cells grown <u>in</u> <u>vitro</u> and added to soil may be different from
mineralisation of soil organisms <u>in</u> <u>situ</u>.

Soil disturbance

Disturbance of the soil has the effect of rearranging the
spatial distribution of microorganisms and substrates.
Non-motile microorganisms which deplete the substrate in their
immediate vicinity are brought into contact with fresh sources
of C. This is analagous to an input of C into the system so
that microbial activity is stimulated. The C being
metabolised however is native to the soil, and therefore the
amounts of soil organic C will decrease with repeated
cultivation (Tiessen <u>et</u> <u>al</u>, 1982). It has also been suggested
that cultivation stimulates microbial activity through improved
aeration (Clements and Williams, 1964). Several studies have
shown that P_o decreases in soil under continuous cultivation
(Thompson <u>et</u> <u>al</u>, 1954; Haas <u>et</u> <u>al</u>, 1961; Williams and Lipsett,
1961; Adeptu and Corey, 1977; Hedley <u>et</u> <u>al</u>, 1982a; Tiessen and
Stewart, 1983). For example, Williams and Lipsett (1961) found
that cultivation of soils for wheat in New South Wales caused,
on average, a 30% reduction in organic matter content of the
soil over a 50 - 60 year period. P_o declined by 17% over the
same period, and this was attributed to increased
mineralisation in disturbed soils. Williams and Anderson
(1968) found that inositol phosphates accumulated in soil under

undisturbed leguminous pastures, but that cultivation decreased
organic matter and inositol phosphate contents. More recently,
fractionation techniques (see Section 2.4.2) have been employed
to follow the redistribution of P which occurs with cultivation
(Hedley et al, 1982a; Tiessen et al, 1983). Hedley et al
(1982a) found that cultivation caused a reduction of total P
present in a black chernozem (Udic haploboroll). The soil's
ability to provide P for plant growth deteriorated more than
might be expected from the change in total P content. Not only
was plant available P_i decreased, but so also were the
extractable P_o and residual P fractions. Tiessen et al (1983),
used a combination of physical and chemical fractionation
techniques. They found that cultivation decreased plant
available P to an extent which was closely related to loss of
organic matter. Examination of particle size fractions showed
that P_i contents of coarse fractions increased during the first
few years of cultivation, and that a shift of soil P
composition towards P_i at the expense of P_o occurred in all
size fractions (Tiessen and Stewart, 1983). In most of these
studies, unfertilised cultivated and uncultivated soils were
used for comparative purposes.

The action of invertebrates in disturbing and rearranging
soil particles has a large influence on microbial activity in
soil. Earthworms in particular have a considerable impact on
nutrient cycling in pasture soils (Sharpley and Syers, 1977).
By creating a favourable environment for bacterial activity in
their gut (Edward and Lofty, 1971) and by physical disruption
of soil particles and organic matter, earthworms can increase

the plant availability of P derived from both fertilisers and plant residues (Mansell et al, 1981; Mackay et al, 1982). A concise review of the role of invertebrates in nutrient cycling has been produced by Hutchinson and King (1982).

Inorganic nutrients

There is little information in the literature on the effect of inorganic nutrients on mineralisation-immobilisation reactions. Most of the research undertaken has focused on the effect of P_i on the mineralisation of P_o. Dalal (1977) lists a number of studies where addition of P_i stimulated mineralisation (McCall et al, 1956; Kaila, 1961; Acquaye, 1963; Enwezor, 1966). Other workers, however, have reported no effect of P_i on mineralisation of P_o (Weir and Black, 1968; Ghoshal, 1975a). It is difficult to reconcile these conflicting reports. Dalal (1977) suggests that increased mineralisation of P_o through addition of P_i may be due to displacement of P_o from solid surfaces, thus rendering the compounds liable to chemical or biological hydrolysis.

It has been observed that addition of P_i as fertiliser increases immobilisation as amounts of P_o increase in soils under pastures (Jackman, 1964; Steward and Oades, 1972; Lewis, 1978) and cultivated crops (Owssia, 1966; Van Diest, 1968). Most of the increase in P_o can be attributed to the increase in plant growth stimulated by fertiliser application, and the associated increase in inputs to the soil of non-harvested plant material (Anderson, 1980). Hedley et al (1982a) also suggest that increased amounts of P_i available for microbial

uptake cause greater amounts of P_o to be produced. Supporting
evidence is provided by Chauhan et al (1981), who found a
positive relationship between microbial P uptake and the
concentration of P_i in solution. Hedley and Stewart (1982a)
also showed that the P content of microorganisms varied
according to the P_i supplied.

Addition of P_i may also influence mineralisation through its
repressive effect on the activity of phosphohydrolases (McGill
and Christie, 1983).

Influence of nutrients other than P on mineralisation-
immobilisation reactions of P in soils have received scant
attention. Barrow (1960) studied the effects of varying the N,
S, and P content of pure organic compounds on their
mineralisation. He found that increasing N supply caused
increased immobilisation of P but the effect of S was less
clear. Dalal (1977) states that organic matter contains
definite proportions of C, N, S, and P, and that deficiencies
in one element will reduce organic matter accumulation even if
P is in plentiful supply. However, as pointed out previously
in the discussion of decomposition of plant materials, there
are dangers in adhering to strict stoichiometric relationships
between energy (C) and nutrient elements.

Trophic interactions

The importance of predation in nutrient cycling was first
recognised in aquatic ecosystems (Johannes, 1965, 1968;
Barsdate et al, 1974). Barsdate et al (1974) studied the
decomposition of plant material in microcosms, with and without

bacterial grazers. They found that the turnover of P in the system far exceeded the actual mineralisation of P from the added plant material, and that this cycling, as well as mineralisation, was stimulated by protozoan grazing of the decomposer populations. Cole et al (1978) performed a similar study with soil microcosms and found extractable P (0.5$\underline{M}$ NaHCO$_3$ (pH 8.5)) to increase and microbial P to decrease in systems containing bacterial grazers (Figure 4). In a field experiment, Elliott et al (1984) found large declines in microbial C and P, increases in mineral N and extractable (0.5$\underline{M}$ NaHCO$_3$ (pH 8.5)) P$_i$ and P$_o$ concurrent with increases in protozoan numbers. All these studies emphasise the importance of both the microorganisms, and their population dynamics, in nutrient cycling.

Temperature

Diurnal and seasonal changes in temperature affect biological processes in soils. Large seasonal variation in several labile P fractions has been noted by a number of workers (Blakemore, 1966; Garbouchev, 1966; Nguyen et al, 1969; Halm et al, 1972; Dormaar, 1972; Gupta and Rorison, 1975; Weaver and Forcella, 1979; Haines and Cleveland, 1981; Felleca et al, 1983; Elliott et al, 1984). Saunders and Metson (1971) postulated that the poor response of pastures to applied superphosphate in spring was due to mineralisation of P$_o$. This accords with the observations of Dormaar (1972), who noted increases in soil P$_o$ over winter and decreases in spring in Canadian soils. In laboratory studies, it has been observed
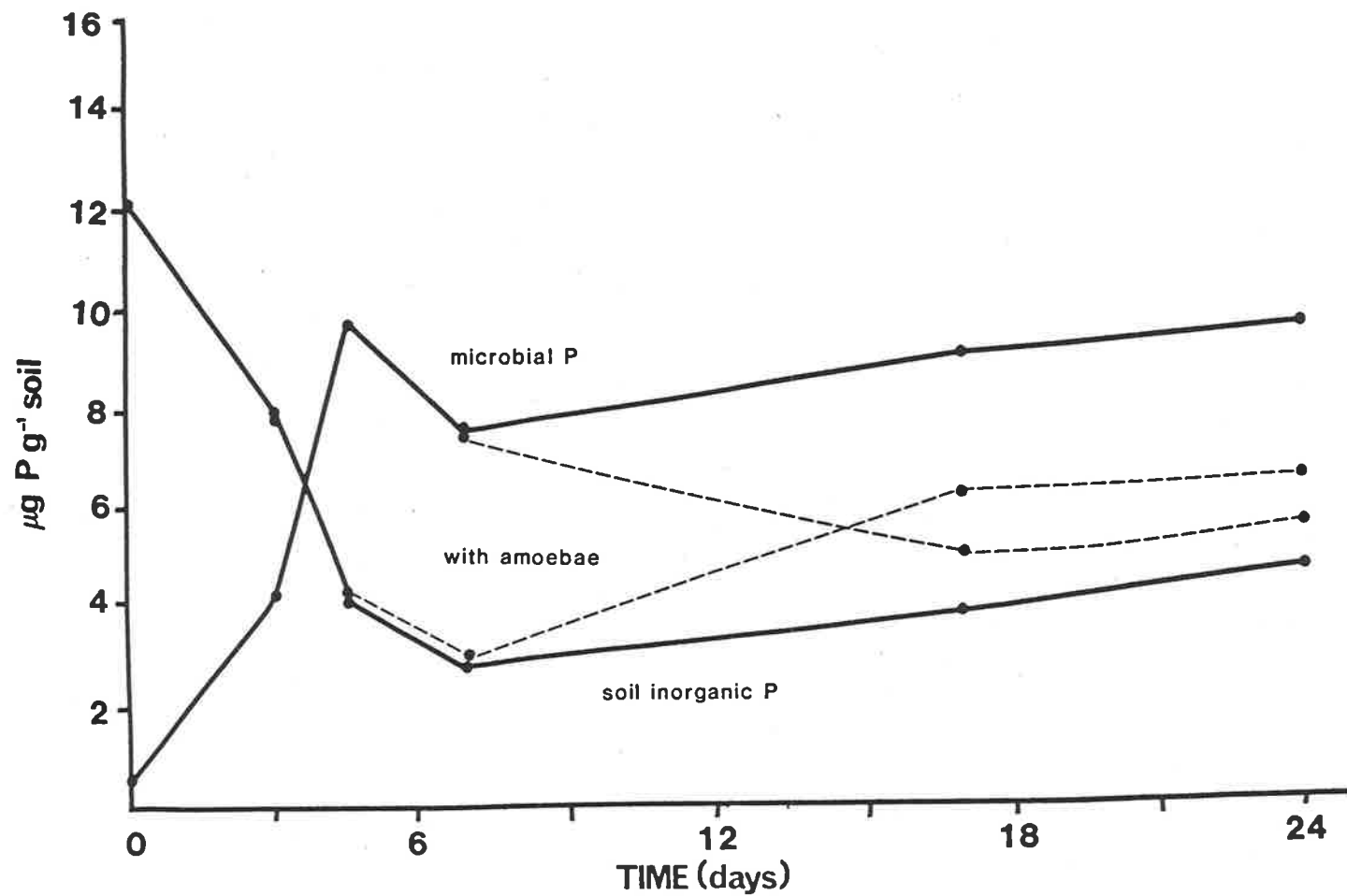
Figure 4. Effects of amoebae on amounts of NaHCO$_3$-P$_i$ and microbial P over
a 24 day incubation in soil microcosms (from Cole et al, 1978).

that temperature can have a large effect on rates of
mineralisation and immobilisation reactions (Floate, 1970b;
Martin and Cunningham, 1972; Till and Blair, 1978). Williams
(1967) reviewed the effects of temperature upon mineralisation
of soil P_o. He concluded that mineralisation was slow at low
temperatures, but that at higher temperatures P_o may be more
sensitive to temperature changes than other components of soil
organic matter. Although low temperatures are usually
associated with the cessation or drastic decreases in
biological activity, there is evidence that organisms naturally
and continuously exposed to such conditions adapt to them.
Organisms in arctic tundra soils can still effectively
decompose organic materials at temperatures as low as -5 °C
(Chapin et al, 1978).

Recently, Harrison (1982a) developed a technique using
^{32}P-labelled RNA to measure rates of labile P_o mineralisation
in forest soils. He found that rates of mineralisation of RNA
were higher in spring than in autumn, and that this was related
to increased phosphatase activity in the soils during spring
(Harrison, 1982b).

Water

All organisms require water if they are to survive. It is
well known that there is a close relationship between microbial
activity and soil water status. Water potential affects the
activity of soil organisms both directly and indirectly. Low
water potential directly influences metabolic activity by
slowing enzyme reaction rates (Acker, 1962; Skujins and

McLaren, 1967). Growth may also be retarded by the need to accumulate solutes to maintain turgor pressure (Adebayo et al, 1971). Indirectly, changes in water potential affect the rate of solute diffusion and degree of microbial motility, and thus respiration rates may decrease at low water potentials due to substrate exhaustion (Wilson and Griffin, 1975). For example, Orchard and Cook (1983) found a log-linear relationship between soil water potential and microbial activity as long as activity was not limited by substrate availability. Thus soil water potential has a large effect on mineralisation-immobilisation reactions in soils. That soil organisms are able to function, albeit at different levels of activity, over a fairly wide range of water potentials (Wilson and Griffin, 1975), may explain some of the conflicting results in the literature relating soil water status to P transformations (Dalal, 1977).

Large and/or rapid fluctuations in water potential have a large influence on P transformations in soil. Lebedjantzev (1924) reported that repeated drying and wetting of a soil brought about a substantial release of P and N from organic sources. Birch and Friend (1961) found that P in an organic soil was completely mineralised after 204 wetting and drying cycles, although temperatures of 100°C were used during the drying cycles. There are many other reports in the literature where a release of plant nutrients has been observed as a result of drying and wetting cycles (e.g. Birch, 1960, 1964; Soulides and Allison, 1961; Harada and Hayashi, 1968; Marumoto and Yamada, 1977). A number of reasons to explain the "flush" of easily extractable P_i or P_o following desiccation have been

postulated. Air drying of plant material causes extensive
degradation and conversion of P_o to P_i, probably through
autolytic activity of plant enzymes (Barr and Ulrich, 1963;
Martin and Cunningham, 1972; Jones and Bromfield, 1982). If
the water potential deficit is sufficiently severe, drying of
soil also results in the death of microorganisms (Lund and
Goksoyr, 1980). That portion of the biomass killed through
desiccation may undergo autolysis and release P_i, thereby
transforming P_o into readily extractable forms (Brookes et al,
1982).

On rewetting of previously dried soil, there is a rapid
burst of microbial activity as the surviving organisms utilise
their dead colleagues as sources of energy and nutrient
(Stevenson, 1956; Lund and Goksoyr, 1980; Orchard and Cook,
1983). Substrates held within aggregates may also be exposed
by drying-rewetting soil due to the disruption of aggregates
(Adu and Oades, 1979). Peaks in bacterial populations in dried
soil occur soon after wetting (Campbell and Biederbeck, 1976).
Fungi respond more slowly (Lund and Goksoyr, 1980).
Respiration and amounts of mineral N in air dried soils
increase dramatically on rewetting. Indeed, the release of
mineral C, N, S, and P is used as the basis for the methods for
measurement of nutrients held in the biomass — death being
caused by fumigation rather than drying (Jenkinson and Powlson,
1976; Ayanaba et al, 1976; Saggar et al, 1981; Brookes et al,
1982). Marumoto et al (1982) observed that the quantities of N
mineralised from dried and rewetted soils was closely related
to the quantities present in the soil biomass before

treatment. The magnitude of any changes in soil nutrient distribution will therefore depend on the quantities of nutrients held in the biomass, and the proportion of the population susceptible to desiccation.

Oxygen

It is difficult to separate the effects of water from those of aeration on P transformations and reactions in soil (Dalal, 1977). Despite the fact that microbial activity is lower in anaerobic than in aerobic conditions, waterlogged soils generally contain less P_o than well drained soils (Campbell and Racz, 1975). It is difficult to attribute lower concentrations of P_o in anaerobic soils to increased rates of P_o mineralisation, as these rates are usually slower in anaerobic conditions (Dick and Tabatabai, 1978). More research is required before the effect of aeration on reactions of P in soil are fully understood.

Soil reaction

Biological activity in soil is depressed at low pH. Thus acidification of soils favours the build up of P_o (Batten and Osborne, 1983) as plant residues are not quickly recycled. Net mineralisation of P_o in acid soils is usually stimulated by liming (Thompson et al, 1954; Halstead et al, 1963; Awan, 1964; Lucas and Blue, 1972; Islam and Ahmed, 1973). The effect is attributed to increased microbial activity (Halstead et al, 1963) and increased solubility of some phosphate esters (Anderson, 1980). Harrison (1982b) recently demonstrated a

strong positive correlation between rates of mineralisation of labile P_o and soil reaction over the range pH 3.1-7.5. Soil reaction, however, also has a large influence on inorganic reactions controlling P availability in soils (Haynes, 1982). Bulk soil pH may not be an accurate reflection of soil reaction in close proximity to root or clay surfaces, as these can alter the pH of their immediate environment (McLaren and Estermann, 1968; Hedley et al, 1982b).

To summarise, it is clear that microorganisms to a large extent determine the reactions which affect P availability in soil. While mycorrhizal fungi have a large effect on plant nutrition, microorganisms in soil not linked to plants through a symbiotic relationship also have a large effect on soil P transformations, and hence the P nutrition of plants. Much of the early work which examined microbially mediated reactions involving soil P was qualitative. The development of methods to measure directly the amounts of P held in soil microorganisms (Section 2.4.2) now allows a quantitative assessment of the role of microorganisms in P cycling.

2.4 Methods of studying P cycling

2.4.1 Modelling

Before reviewing the results of studies utilising modelling techniques, it is pertinent to discuss how these techniques help the scientific study of nutrient cycling.

The construction of simulation models, aided by the advent of powerful computer technology, has stimulated research by complex systems analysis of environmental systems. Simulation modelling aids the interpretation of large amounts of complex data, facilitates the organisation of information, and can direct research into areas of critical interest (Woodmansee, 1978). Another potential use of simulation models is for prediction of situations that are difficult or impossible to observe experimentally e.g.future trends. A distinction should be made between simulation models and nutrient budget models. Simulation modelling involves the integration of existing data into mathematical expressions to provide a subjectively assessed degree of similarity between model predictions and observed events. These relationships can then be extended, with a chosen degree of uncertainty, to new situations for which no data are available. Alternatively, sensitivity analysis of the relationships incorporated into the model can reveal gaps in knowledge where more precise information is required (within the conceptual framework in which the model was developed). Nutrient budget models, on the other hand, involve the collection of large amounts of data from a particular ecosystem (and are therefore site specific and labour intensive), and their organisation into some conceptual framework without the need to validate concepts or predict events. Precise mathematical relationships need not be defined for such models, as their main function is an organisational one. However, nutrient budget models may still demonstrate interesting relationships between components of the system.

Simulation models of P cycling have allowed the integration of the effects of water, temperature, soil properties, plant phenology, microbial decomposition of organic matter, and the effects of management on flows of P in a system (Stewart and McKercher, 1982). One of the first simulation models of P cycling was described by Barsdate et al (1974). The model was designed to simulate P cycling in a system of detrital decomposers and it was calibrated against measured changes in P fractions in microcosms. A more complex model of P cycling in grazed pastures was developed by Blair et al (1976). Various values for reaction rates of P transformations and pool sizes were taken from the literature for incorporation into the model, and the model validated against results from a field grazing experiment. Through sensitivity analysis, the model was able to identify parts of the soil P cycle where information from experimental research was lacking. These included the turnover of organic matter and the subsequent release of P_i and the role of both microconsumers and macroconsumers in mineralisation and recycling processes. Cole et al (1977) developed a simulation model for P cycling in semiarid grasslands. They applied the model to two grassland sites, and compared P flows with field measurement. Topics on which lack of information was identified included mineralisation of P_o, relationship of microbial P turnover to microbial biomass, influence of phosphatases on mineralisation of P_o, and morphology and activity of roots. Rates of uptake of P by populations of decomposers were four to five times greater than plant P uptake. For P_o transformations in a

forest soil ecosystem, Mishra et al (1979) developed a
simulation model from which they predicted that there would be
no direct mineralisation of P_o from leaf litter without passage
of the P through a microbial body. The model was very
sensitive to changes in the efficiency factor used (the
fraction of decomposed C converted into cell C), but there is
some uncertainty about the relationships generated by the model
as the correlation between predicted and observed values was
poor.

In a number of studies, nutrient budgets of specific sites
have been examined and conceptual models of P cycling developed
for the ecosystems at these sites. Halm et al (1972) measured
the P contents of green grass, dead grass, forbs, litter and
roots in a native grassland ecosystem. They also measured P_i
and P_t extracted by 0.5$\underline{M}$ NaHCO$_3$ (pH 8.5), total and organic P
in the soil and P held in soil fauna and microorganisms. Halm
et al found more P was held in the microbial biomass than in
the standing crop. Rates of transfer of P among all the
various components of the system were not assessed however.
Harrison (1978) described the P cycle under two temperate
unfertilised systems (woodland and grassland), and has given
both pool sizes and estimated or measured net annual transfer
rates between some of them. Although most of the P in the
biomass was in the vegetation, the soil fauna and
microorganisms also contained significant quantities. Harrison
emphasised the importance of recycling of P through
decomposition of plant residues, as plant uptake of P depended
largely on this process. Woodmansee and Duncan (1980) also

prepared a nutrient budget model for a grassland ecosystem and were able to estimate annual fluxes between components of the system. They confirmed Harrison's (1978) findings that decomposition of plant residues supplied the plants with most of their P requirements.

To summarise, modelling approaches have provided great insight into the interrelated nature of many of the processes governing reactions of P in soil. The microbial biomass and the organic P in soil are two components which have repeatedly been identified as requiring further experimental research effort.

2.4.2 Chemical methods

The numerous chemical studies involving P_i and P_o in soils may be conveniently classified as either static or dynamic. Static studies include the myriad of investigations into the chemical nature of both P_i and P_o in soil, reviewed by Larsen (1967), Halstead and McKercher (1975), Dalal (1977), Barrow (1978), Anderson (1975, 1980), Sample et al (1980), and White (1980). While a knowledge of the various chemical forms of P in soil provides insight into reactions which could take place, it is the rate of change in chemical form which is most interesting in P cycling studies. Dynamic studies, aim to relate changes in chemical form over time, or with various treatments, to soil processes. Techniques developed to characterise soil P have been utilised in dynamic studies to assess P transformations in soil. Stewart and McKercher (1982) have summarised the analytical techniques used in P cycling

studies, and have recommended a variety of methods to study components of the P cycle (Table 7). Application of fractionation techniques, the direct measurement of microbial P, and the application of radioisotopes are perhaps the most important developments in this field.

Fractionation techniques

Both chemical and physical fractionation techniques have been used in P cycling studies.

Most of the chemical fractionation techniques do not aim to measure specific P compounds, but rather groups of compounds by evaluating their susceptibility to solubilisation by various chemical extractants. Early attempts to fractionate soil P_o used reagents such as NaOH, KOH, NH_4OH and dilute and strong acids to extract various P_o compounds from soil (Anderson, 1961; Martin, 1964). The chemical nature of P_o in the extracts could then be studied using ion exchange chromatography. Bowman and Cole (1978a) developed a fractionation scheme for P_o in grassland soils which was subsequently used by Chauhan et al (1981) in the series of laboratory incubations described in Section 2.3.4. Bowman and Cole (1978b) subsequently used $0.5\underline{M}$ $NaHCO_3$ (pH 8.5) as an extractant to follow transformations of certain P_o substrates in soils.

Table 7. Procedures recommended by Stewart and McKercher (1982) for the study of P cycling in soils.

Method	Reference
P_t and P_o	Saunders and Williams (1955) modified to use 1 $\underline{M}$ H_2SO_4; Anderson (1960); Halstead and McKercher (1975)
P_t plus division into P_i and P_o fractions	Hedley et al (1982a)
P_i fractions only	Peterson and Corey (1966) modified for calcareous soils by Sadler and Stewart (1975); Hedley et al (1982a)
P_o fractions only	Hedley et al (1982a)
Resin-extractable P	Amer et al (1955); Sibbesen (1977)
Inositol P, lipid P and nucleophosphates	Halstead and McKercher (1975)
$NaHCO_3$ extractable P_i P_o	Olsen et al, (1954); Bowman and Cole (1978b)
Microbial P	Hedley and Stewart (1982)
Microbial activity — CO_2 production trapped in NaOH in sealed containers	

Microbial biomass — bacteria
    — fungi
    — total
    — protozoa
    nematodes
	van Veen and Paul (1979)
	van Veen and Paul (1979)
	Anderson and Domsch (1978)
	Anderson et al (1979);
	Oostenbrink (1970);
	Darbyshire et al (1974)

Phosphatases	Gerittse and Van Dijk (1978)
ATP	Paul and Johnson (1977); Jenkinson and Oades (1979)

Chemical fractionation of P_i in soil has also been attempted by many researchers. Perhaps the most widely used procedure is that of Chang and Jackson (1957) or modifications thereof (Fife, 1959, 1962; Peterson and Corey, 1966; Williams et al, 1967, 1980; Hedley et al, 1982a, 1982b). In one of its recent forms (Hedley et al, 1982a), the fractionation scheme makes no attempt to distinguish between aluminium and iron bound P (Figure 5), but includes measurements of P_o fractions. Note that a similar scheme given recently by Tate (1985b) differs from the method of Hedley et al (1982a) in that soil pretreated with resin is used for the subsequent fractionation, and not the chloroform treated soil as recommended by Hedley et al (1982a). Stewart and McKercher (1982) note that a major advantage of this technique is its ability to distinguish forms of P_i commonly used in the description of P compounds in soil, while simultaneously providing information on labile and stable forms of P_o, and amounts of microbial P. Hedley et al (1982a, 1982b) have shown the utility of the technique in observing transformations of P in soils subjected to inputs of organic materials or cultivation, and in studying reactions in the rhizosphere.

Physical fractionation of soils has also been superimposed upon chemical fractionation schemes to assess the effect of cultivation on the redistribution of P in soil (Tiessen and Stewart, 1983; Tiessen et al, 1983). Tiessen et al (1983) fractionated soil into various size categories before chemical analysis. They found that P_i lost from a soil due to continued cultivation was derived mainly from finer (< 2 μm) fractions.

SOIL

Duplicate samples (0.5 g, <150 µm) in 50 ml screw cap
centrifuge tubes. Add 30 ml deionised water plus 0.4 g
resin in nylon bag. Shake 16 h, 24°C. Remove resin bag,
Centrifuge and discard supernatant.

→ RESIN[a]

Allow to sit overnight at 24°C without cap. Add 30 ml $NaHCO_3$.
Shake 16 h, 24°C. Centrifuge and filter supernatant.
Discard soil.

→ EXTRACT[b]

Add 1ml $CHCl_3$, recap tube, leave 1 h with shaking.
Evaporate $CHCl_3$ overnight, Extract with $NaHCO_3$ as for
Extract[b].

→ EXTRACT[c]

Add 30 ml 0.1 M NaOH. Shake 16 h, 24°C. Centrifuge and
filter supernatant.

→ EXTRACT [d]

Add 20 ml 0.1M NaOH to remaining soil. Stand centrifuge
tubes in ice bath or 0°C and sonicate 2 min, 75 watts. Make
to final volume 30 ml 0.1 M NaOH. Shake 16 h, 24°C.
Centrifuge and filter supernatant.

→ EXTRACT[e]

Add 30 ml 1.0 HCl to remaining soil. Shake 16 h.
Centrifuge and filter supernatant.

→ EXTRACT[f]

Digest remaining soil with 5 ml H_2SO_4 and H_2O_2. Filter

→ DIGEST[g]

RESIDUE

Figure 5.   Flow chart of the fractionation of soil P
             into various P_i and P_o fractions (from
             Hedley et al, 1982a);
             (a) Resin-P: most biologically available P.
             (b) $NaHCO_3$-P: labile P_i and P_o.
             (c) $CHCl_3/NaHCO_3$-P: microbial P.
             (d) NaOH-P: moderately labile P_i and P_o.
             (e) Sonicate/NaOH-P: aggregate protected P_i
                 and P_o.
             (f) HCl-P: basic P minerals and occluded
                 P_i.
             (g) Residue-P: stable P_i and P_o.

In both the soils studied, sulphuric acid extractable P
(thought to be mainly apatites) accumulated due to
cultivation, and the increases were recorded mainly in coarse
silt (5-50 μm) fractions.

Physical measurement techniques such as ^{31}P-nuclear magnetic
resonance (^{31}P-NMR), diffuse reflectance infra-red spectrometry
(DRIFTS) and photo-acoustic techniques are promising
developments which could be applied to studies of P cycling in
soils. Most of the studies to date fall under the static
classification i.e. they have been used mainly to characterise
forms of P_o or P_i in soil (Newman and Tate, 1980; Tate and
Newman, 1982; Condron et al, 1984). However, quantitative
application of ^{31}P-NMR recently proved useful to follow changes
in forms of P_o in alkaline extracts of fertilised and
unfertilised grassland soils (Hawkes et al, 1984).

A criticism of ^{31}P-NMR and the chemical analysis of soil
fractions discussed above is that alteration of the forms of P
may occur during extraction from soil. ^{31}P-NMR of soil
extracts requires high concentrations of P in solution to
attain an acceptable signal to noise ratio. This often
requires that soil extracts be concentrated before analysis.
Such treatment may also allow changes to occur in the chemical
form of P. Methods which study chemically untreated soil, such
as solid state cross polarisation ^{31}P-NMR and DRIFTS, offer the
most potential in terms of the characterisation of P compounds
in soil (Williams et al, 1981). If such methods can be
developed to a stage where accurate quantitative analysis of P
compounds in soil can be achieved, significant advances in our

knowledge of P cycling in soil will result.

✗ Microbial P

There have been few studies in which attempts were made to measure indirectly the amount of P held in the soil microbial biomass. Anderson and Domsch (1980) measured the C content of 26 agricultural soils and estimated the microbial P contents of the soils from C:P ratios observed in laboratory grown organisms. They found that the microbial biomass could contain substantial amounts of P: microbial P contents of the soils ranged from 1.7 to 244 kg P ha^{-1} (0-12.5 cm layer). However, due to the uncertainty of microbial C:P ratios determined in the laboratory (van Veen and Paul, 1979), these figures should be treated with caution. With the advent of direct methods to measure microbial P in soils, indirect methods have not been widely used.

The basis for direct measurement of microbial P in soils is the observed increase in extractability of P from soils to which a biocidal treatment has been applied. Birch (1961) was the first to suggest that release of P from a material following treatment with chloroform vapour could be used as an index for the presence of viable organisms. Bowman and Cole (1978b) cite the use of chloroform vapour and extraction with 0.5$\underline{M}$ NaHCO$_3$ (pH 8.5) to indicate changes in microbial P in laboratory incubations, but Chauhan $\underline{et}$ $\underline{al}$ (1979) were the first to document properly a procedure for determination of microbial P in soils using chloroform and 0.5$\underline{M}$ NaHCO$_3$ (pH 8.5). Subsequently two detailed procedures for the determination of

microbial P in soil were reported (Hedley and Stewart, 1982; Brookes et al, 1982). Both these techniques measure the increase in amount of P extracted by $0.5\underline{M}$ $NaHCO_3$ (pH 8.5) following chloroform treatment, and relate this to amounts present in the microbial biomass. The proportion (K_p) of microbial P released by the procedure is determined using laboratory grown (Hedley and Stewart, 1982) or lyophilised organisms (Brookes et al, 1982). The use of lyophilised organisms is questionable as the release of P from unfumigated lyophilised cells is likely to be different than from unfumigated live cells. Organisms cultured in the laboratory are also likely to have different chemical compositions from those occurring in soils. Moreover the microbial flora may vary from soil to soil, so that different K_p factors may be required for different soils. However, until live, undamaged, and uncontaminated organisms can be isolated from soils there appears to be no other suitable calibration technique. There are differences in the actual procedure followed in the two published methods. These are tabulated in Table 8. The methods have confirmed the findings of Anderson and Domsch (1980), that a considerable quantity of P appears to be held within microorganisms in soil.

A major advantage of the chloroform fumigation technique is that the element under consideration is extracted and measured rather than estimated indirectly. This means that the technique can be used in conjunction with tracers to label and identify reactions occurring in the soil in which the microorganisms are involved.

Table 8. Differences in procedure between the methods of
          Hedley and Stewart (1982) and Brookes et al (1982)
          for the determination of microbial P in soil.

	Hedley and Stewart	Brookes et al
Soil	Air-dried, ground, rewetted, aerobic incubation (21 days). Resin extraction pre-treatment	Field-moist, aerobic incubation (10 days)
Sample size	0.5g	10.0g
Chloroform	Liquid, 1h exposure	Vapour, 24h exposure
Extraction Soil:solution ratio	1:20	1:60
Period	16h	30min
Measured parameter	P_t	P_i
K_p value	0.37 but variable depending on sorption	0.34 (P_i) 0.47 (P_t)
Sorption correction	–	Single spike of P_i used

For example, Stewart and Hedley (1980) used the technique in

short term laboratory studies to measure the mineralisation and

plant uptake of ^{33}P from ^{33}P-labelled bacterial cells added to

soils.

Use of radioisotopes

Rather than list all the investigations which have utilised

isotopes in the study of P cycling in soil, most of which have

already been mentioned above, it is perhaps more appropriate to

make a general comment. From the preceding sections it can be

seen that the use of the two radioisotopes of P – ^{32}P and ^{33}P –

has played a major role in furthering our knowledge of P transformations in soil. The use of these isotopes can often provide valuable information unobtainable with investigations using the stable isotope (^{31}P). The new technique to measure mineralisation of RNA in forest soils, using ^{32}P-labelled RNA, is a good example (Harrison, 1982a). Reaction pathways can be identified using radioisotopes, rather than inferred from changes in pool sizes (Stewart and McKercher, 1982). The major limitation of using ^{32}P and ^{33}P as tracers is the short half life of both these isotopes. ^{32}P has a half life of 14.3 days and ^{33}P has a half life of 25.3 days. Experiments are therefore limited to short-term studies. Large quantities of ^{32}P cannot be used to overcome the limitation of short half life, as radiation damage to organisms or plants may occur (Russell et al, 1950; Blume, 1952; Russell, 1953), as well as the increased risk to personal safety. While ^{33}P is safer to use because of its lower mean beta energy, it is approximately ten times more expensive than ^{32}P. ^{32}P has the advantage that activity can be readily measured in solution by Cerenkov counting (Ross 1969), while ^{33}P requires the use of a scintillation cocktail (Robinson, 1969; L'Annunziata, 1979). Because the radioisotopes of P are strongly sorbed to many types of surface, special laboratory precautions are often necessary in the treatment of samples (Hughes and MacPhee, 1983), and in the washing and decontamination of glassware and plastics. Provided the limitations are realised however, ^{32}P and ^{33}P are extremely useful in tracing the flow of ^{31}P in a system.

2.5 Summary of literature

With the continued rise in the cost of P as a fertiliser nutrient, there is an urgent need to identify, and quantify, reactions involving the transformations of P in soil from available to non-available forms and *vice versa*. This information is required so that user-oriented models for calculating fertiliser requirements can be further refined, and extended to more types of farming systems (Bennett and Bowden, 1976; Cornforth and Sinclair, 1982). While much information is available on the reactions of P_i in soil, there is a lack of knowledge of the role of decomposing plant residues and microorganisms in P cycling. Recent developments in computer modelling of agro-ecosystems have identified the shortage of knowledge in this field. New techniques to measure P held in the microbial biomass, and fractionation procedures which allow measurement of a variety of P pools in soil, have opened up new avenues for research into the turnover of P through organic matter.

CHAPTER 3

MEASUREMENT OF MICROBIAL P IN SOIL

3.1 Treatment of soil samples

A disadvantage of current techniques for measurement of microbial P in field soils is that an incubation period is used before fumigation. The method of Hedley and Stewart (1982) uses small portions of dried, ground, rewetted and incubated (21 d) soils, while the method of Brookes et al (1982) uses a 10 d incubation period of sieved field-moist soil. Chemical analysis of soil often requires the soil to be air dried and ground before analysis, while biological analysis often requires refrigeration of the soil between sampling and analysis. An experiment was carried out to examine the effect of soil treatment after sampling on the flush of P caused by fumigation.

3.1.1 Materials and methods

Five soils of varying pH, texture, and water characteristics were chosen (Table 9). After being sampled (0-150 mm layer), the soils were sieved (<2 mm) and subjected to four types of treatment before fumigation.

Treatment 1: the soils were wetted to -66 kPa and incubated for 21 d at 20°C (Hedley and Stewart, 1982).

Treatment 2: the soils were sealed in drums containing beakers of water and soda lime, and incubated at field water content for 10 d at 20°C (Brookes et al, 1982).

Treatment 3: the soils were air dried.

Treatment 4: the soils were stored at -15°C for at least 14 d, and then incubated at 20°C for 24 h.

Table 9. Description of soils

	Roseworthy[a]	Ferris McDonald	Urrbrae	Northfield	Inman Valley
Sand %	88	–	46	35	51
Silt %	7	–	35	11	28
Clay %	5	–	18	42	21
pH_w(1:5)	8.5	7.6	6.0	8.4	6.0
Org.C %	0.73	1.10	2.65	1.29	2.80
Org.N %	0.09	0.08	0.22	0.14	0.25
Gravimetric water at -66 kPa %	7.6	5.7	19.2	33.8	22.4

a = names refer to locations: they are not type or series names.

Soils were fumigated with chloroform and extracted with 0.5$\underline{M}$ $NaHCO_3$ (pH 8.5) according to the method of Brookes et al (1982), except that 5 g (instead of 10 g) samples of soil were used, and the samples were shaken end-over-end instead of orbitally. A soil:solution ratio of 1:20 was used throughout. All treatments were replicated three times. As these studies were comparative in nature, and absolute values of microbial P were not required, no correction for sorption of released P was attempted, and the results were not corrected for incomplete recovery of microbial P.

P_i was determined after filtration of the extracts (Whatman No.42) and acidification to remove CO_2, and P_t was determined after digestion according to the method of Brookes and Powlson (1981). P_i in the extracts and digests was determined by the

ammonium molybdate - ascorbic acid method of Murphy and Riley
(1962) as modified by John (1970).

3.1.2 Results and discussion

The P_i released by chloroform vapour was a variable
proportion (7-99%) of P_t (Figure 6). These results contrast
with those of Brookes et al (1982, 1984), who found that in
most soils the proportion of P_t released by chloroform vapour
as P_i was generally greater than 90%. For all soils, wetting
to -66 kPa and incubation before fumigation produced the
largest flushes of P_t on fumigation. Incubation of the samples
at the water content found at sampling in the field generally
produced lower flushes of P_t on fumigation. However, most of
the soils were sampled after a prolonged dry spell, so that the
water contents at sampling were low (Table 10). Air drying
decreased the P_t flush compared to field moist incubation in
all soils except the Urrbrae loam, with the decrease being
greatest in those soils having higher water contents at
sampling. Freezing as a pretreatment had no consistent effect
on P_t flush, although values were different than those for
field moist samples.

Table 10. Water contents of soils at sampling.

Soil	Gravimetric water content %
Roseworthy	2.1
Ferris MacDonald	1.1
Urrbrae	4.7
Inman Valley	12.0
Northfield	14.1
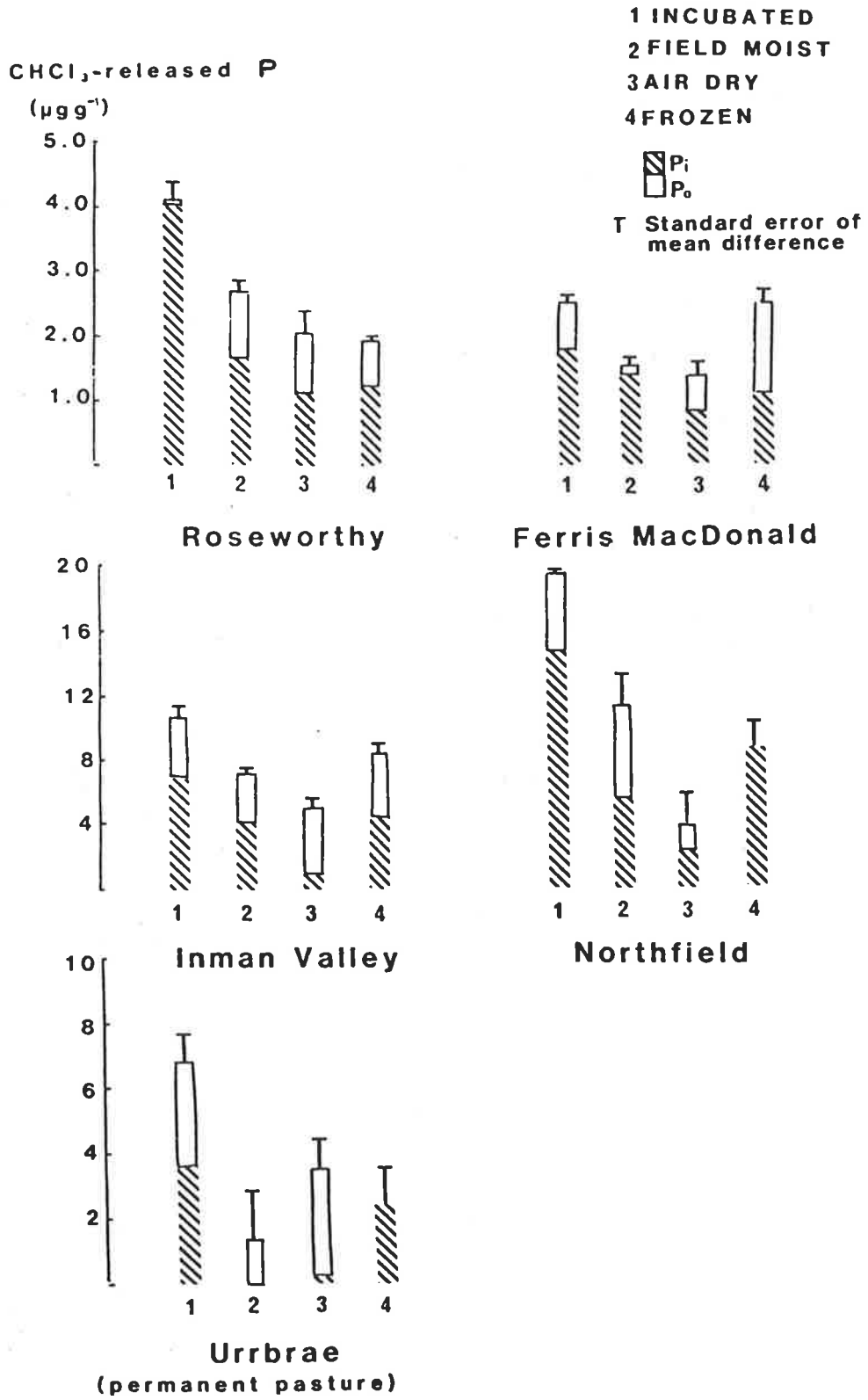
Figure 6. P_i and P_o released from soils by $CHCl_3$ vapour as affected by soil pretreatment.

Hedley and Stewart (1982) recommended that the soil be air dried, ground, and incubated at a water potential favourable for microbial activity before microbial P is measured. However, air drying of soil kills a large proportion of the organisms (Lund and Goksoyr, 1980), and the results presented above show that this will be reflected in values obtained for microbial P. Brookes et al (1982) also demonstrated that air drying of soil can substantially decrease measured values for microbial P. As shown above however, the extent of the reduction will depend on the water content of the soil at sampling. Subsequent incubation of the soil at a water potential favourable for microbial activity will stimulate the organisms to multiply, and this will again be reflected in the values obtained for microbial P. The method of Hedley and Stewart (1982) therefore, is unlikely to provide an accurate assessment of the microbial P content of the soil at the time of sampling.

Methods of chemical and biochemical analyses of soils often require that soils be kept frozen for the period between sampling and analysis. The results obtained here suggest that this is not a suitable storage method before measurement of microbial P in soil. Indeed, alternate freezing and thawing is often used in microbiological procedures to rupture cell membranes, and freezing is likely to have a large effect on organisms in the soil.

It would appear that if the values obtained for microbial P are to reflect those pertaining in the soil at the time of sampling then the method of Brookes et al (1982) should be

used.   This will be discussed further in section Section 3.7.

3.2 Evaluation of biocides

Measurement of any element contained within microbial cells in soil usually involves killing the organisms to release the element under consideration.   Published methods for measurement of microbial P in soil (Brookes et al, 1982; Hedley and Stewart, 1982) utilise chloroform as the biocidal agent - a feature common to many techniques designed to measure nutrients held in the soil biomass (Jenkinson, 1976; Jenkinson and Powlson, 1976).   However, unlike measurements of biomass C and N where the soil must be inoculated with organisms after it has been fumigated, measurements of biomass P and S do not require that all traces of biocide be removed from the soil before extraction.   Indeed, as Brookes et al (1982) point out, any biocide remaining in the soil will help prevent microbial growth during extraction.   Thus a wide range of highly efficient clinical and industrial biocides could be used for measurements of microbial P and S in soils.

3.2.1 Materials and methods

The Roseworthy and Inman Valley soils, having widely differing properties, were selected for screening potential biocides.   The soils were wetted to -66 kPa water potential and incubated at 20°C for 21 d before use, to maintain a high and repeatable level of biological activity between different batches of soil.   A range of vapour, liquid and gas biocides were used - chloroform ($CHCl_3$), ethanol (C_2H_5OH), propan-1-ol

(C_3H_7OH), hexan-1-ol $(C_4H_{13}OH)$, β-propiolactone $(C_2H_4O_3)$, formaldehyde (CH_2O), glutaraldehyde $(C_5H_8O_2)$, ethylene oxide (C_2H_4O) and methyl bromide (CH_3Br). Ethylene oxide and methyl bromide, being gases at room temperature, were introduced into evacuated desiccators containing the soils to be treated (Allison, 1951) (Plate 1a, page 164). Gas pressure was adjusted to 90 kPa. Glutaraldehyde was combined with $NaHCO_3$ extractant as an 0.25% aqueous solution (Borick et al, 1964). The remaining biocides were used both in vapour form according to the method of Brookes et al (1982), or as liquids (2 cm³ 5 g^{-1} oven dry soil equivalent). The water miscible alcohols (ethanol and propan-1-ol) were diluted to give 70% aqueous solutions — this being the most effective concentration against organisms in their vegetative or resting states (Hamilton, 1971). All soils were fumigated for 36 h at 20°C. Vapour and gaseous biocides were removed from soils before extraction by repeated evacuation of the desiccator while soils containing liquid biocides were left for 24 h in a fume hood to allow the biocides to evaporate before extraction. Amounts of biocide and water remaining in the soil before extraction were determined gravimetrically. In the initial experiments, the flush of $^{31}P_i$ and $^{31}P_t$ on treatment of the soil with biocide was measured. Extraction and analysis of released P were done as described previously (Section 3.1.1). As some of the immiscible biocides could not be completely removed from the soil before extraction, any P dissolved in the biocide was assumed to be in equilibrium with P dissolved in the extracting solution. A 16 h extraction period was used throughout.

To ensure that any flushes of P measured by a particular biocide could be attributed to P held only within soil microorganisms, an incubation technique using ^{32}P was adopted. Isotopically labelled tryptic soy broth (TSB) was added to 500 g batches of field moist soils at a rate which supplied (per g oven dry soil) 680 μg C, 225 μg N, 60 μg P, 5 kBq ^{32}P and water sufficient to bring the water potential to -66 kPa. TSB was chosen as this medium is similar to soil extract media in terms of microbial response (Martin, 1975). Soils were fumigated and extracted when microbial activity reached its maximum — any delay could increase amounts of ^{32}P-labelled senescent cells present — so that the flush of ^{32}P could be attributed solely to lysis and extraction of P from living soil microorganisms in situ. Microbial activity was assumed to be related to amounts of CO_2 produced. In a preliminary experiment, amounts of CO_2 produced when TSB solution was added to 50 g batches of soils at the above rate were determined as follows. Immediately after the addition of TSB solution to the soil, the sample was placed in a sealed jar in an incubator at 20°C. Vials containing 1$\underline{M}$ NaOH solution were placed in the jar to trap CO_2 produced. The vials were removed every 12 h and replaced with vials containing fresh NaOH solution. CO_2 in the NaOH solution was determined by titration against HCl. Microbial activity was found to reach a maximum 24-36 h after addition of TSB solution (Figure 7).

Amounts of ^{32}P in the extracts were determined by Cerenkov counting. All counts were corrected for quenching and decay.

All treatments were replicated three times. A completely

randomised design was used.

3.2.2 Results and discussion

Flushes of $^{31}P_i$ and $^{31}P_t$ following treatment of the soils
are shown in Figures 8 and 9. Liquid biocides were generally
more effective than vapour forms in the Roseworthy soil with no
such trend being evident for the Inman Valley soil. The
proportion of $^{31}P_i$ as a percentage of $^{31}P_t$ varied from 2% for
ethanol vapour to 72% for hexanol vapour. Chloroform released
57% of $^{31}P_t$ as $^{31}P_i$ in this soil. This value is lower than the
average value found by Brookes et al (1984).

Glutaraldehyde, formaldehyde and ethanol vapour all produced
small flushes of P. Formaldehyde has previously been found to
be an effective sterilant in soils (Dalton and Hurwitz, 1948)
although Phillips (1977) warns that formaldehyde has poor
penetration into porous surfaces. Formaldehyde gas also
hydrolyses readily in water therefore its penetration into
moist soils could be poor. Glutaraldehyde is twenty times as
effective as formaldehyde as a bactericidal and sporicidal
agent (Hamilton, 1971). However glutaraldehyde may leave cell
membrane and wall structures intact (Labeda et al, 1975)
thus protecting the cell contents against release by the
extractant.   This could explain the very small P flushes
observed in both soils when glutaraldehyde was used as the
biocide.   Methyl bromide is widely used as a sterilant in
horticultural soils, yet this biocide released smaller
quantities of P than chloroform. Both Kolb and Schnecter
(1949) and Munnecke et al (1959) demonstrated that methyl
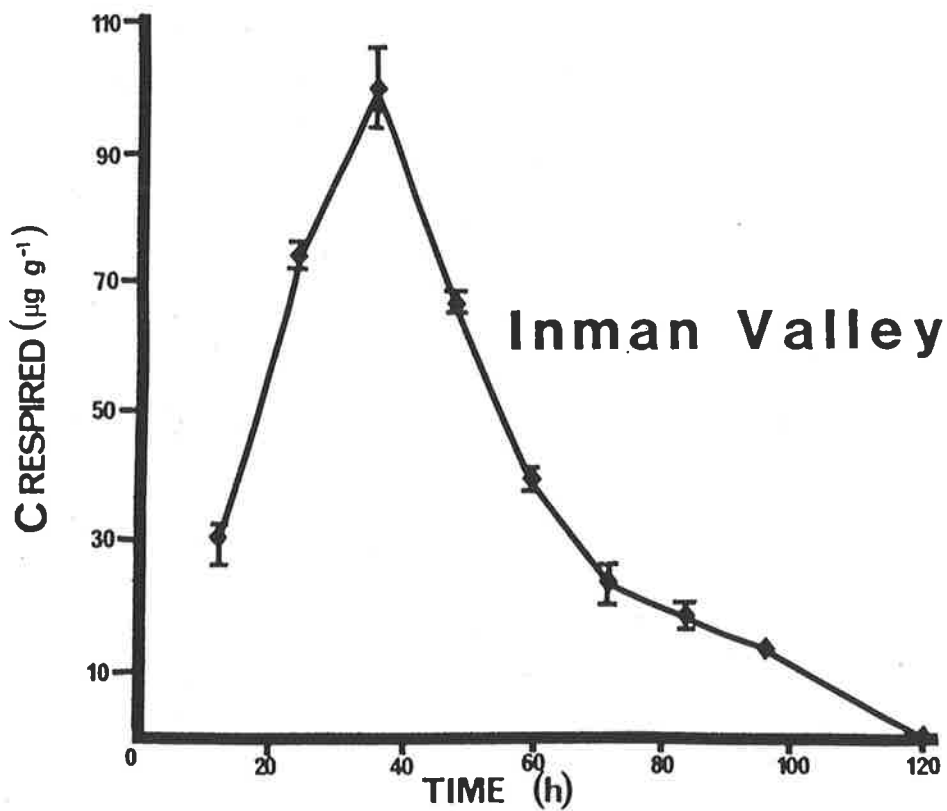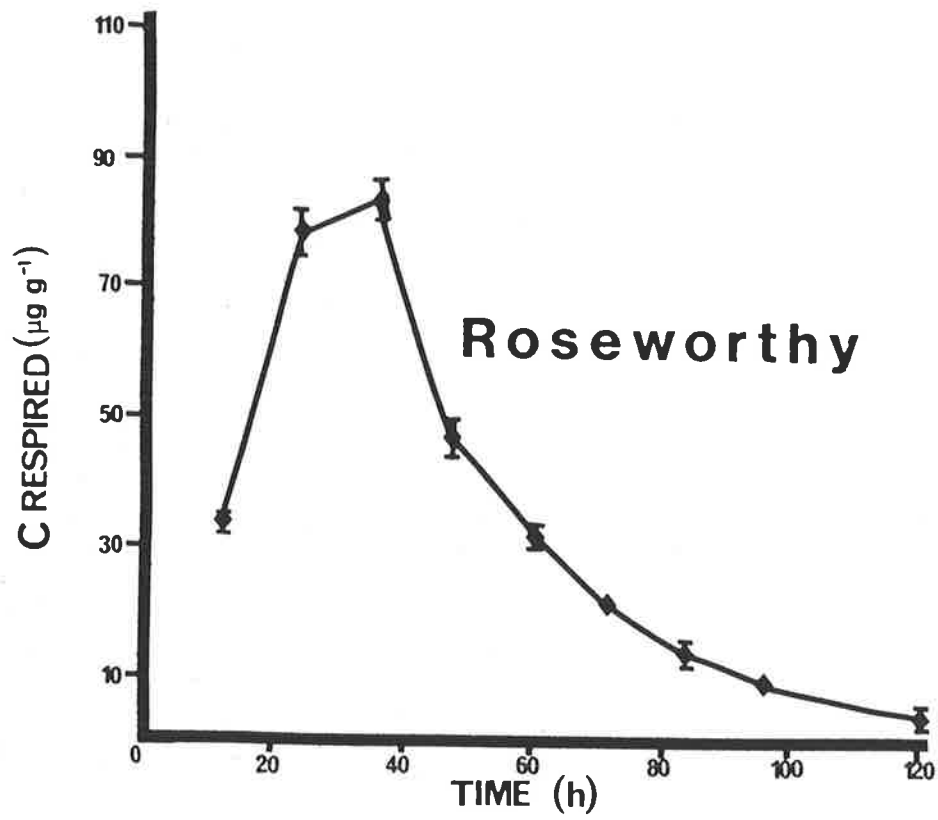
Figure 7. Carbon evolution from soils after addition of tryptic soy broth and incubation at 20°C.
$\mathbf{I}$ = SE of mean values.
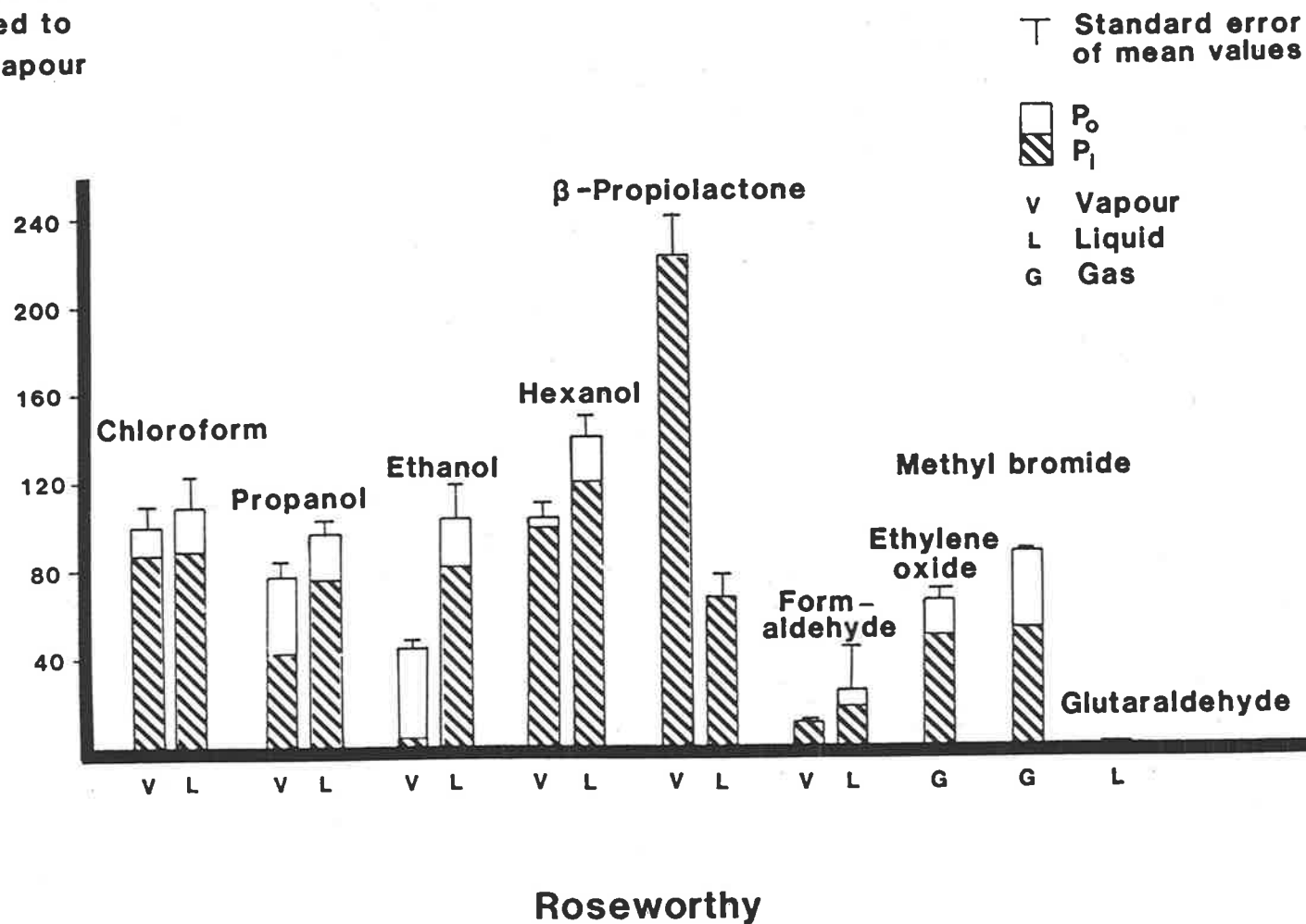
Figure 8. Effect of biocides on the release of $^{31}P_i$ and $^{31}P_t$ from Roseworthy soil compared to CHCl₃ vapour (=100).
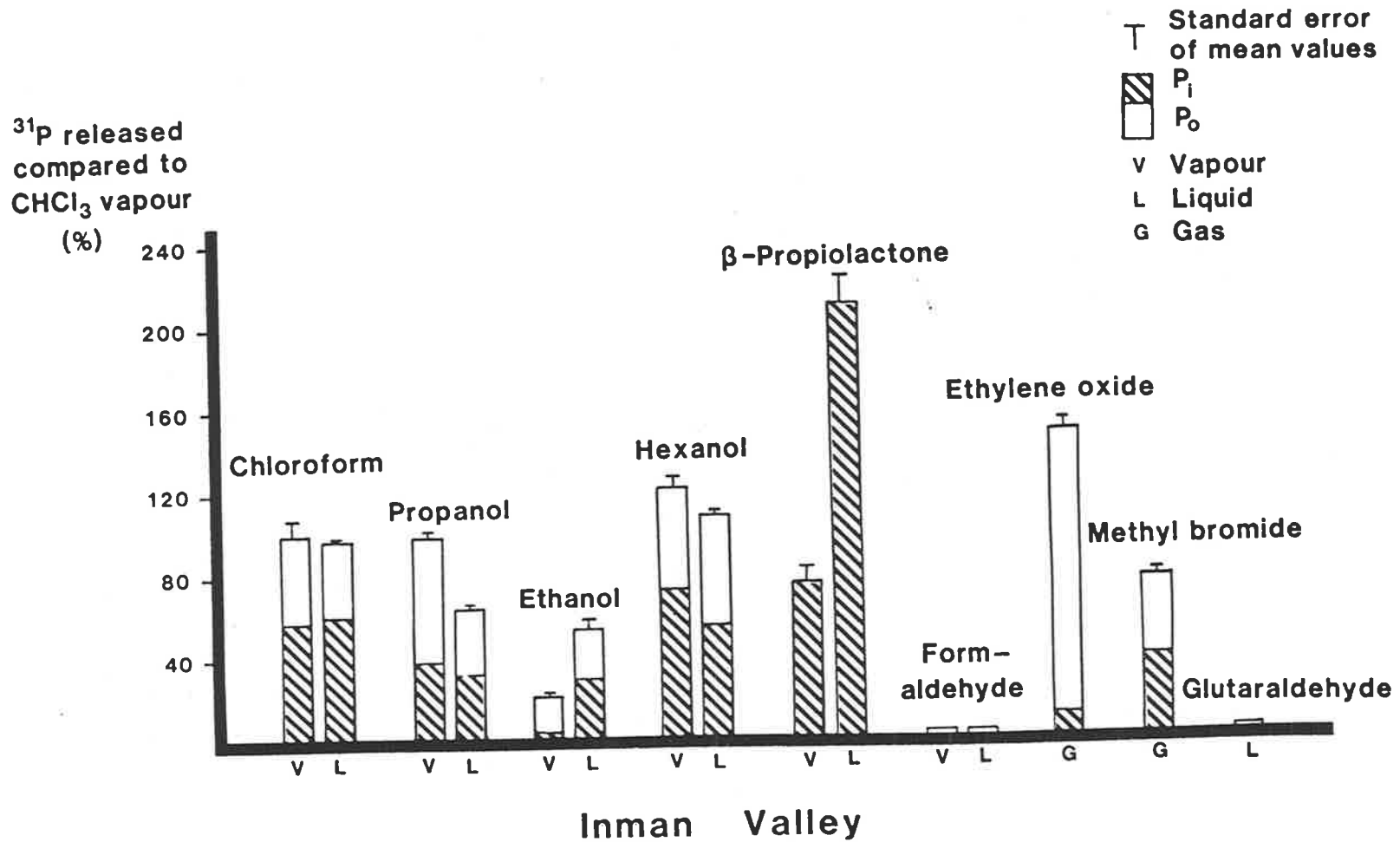
Figure 9.   Effect of biocides on the release of $^{31}P_i$ and $^{31}P_t$ from Inman Valley soil compared to $CHCl_3$ vapour (=100).

bromide is an effective bactericide and fungicide, so that its poor performance in this study could be related to its poor penetration into soil. However, Cuany et al (1985) recently demonstrated that methyl bromide is very effective in penetrating even moist soils. Hence the poor recovery of microbial P when methyl bromide and some of the other biocides were used, may be due to the failure of the biocides to lyse the cell membranes and release the contents for extraction from the dead cells. Phillips (1977) states that ethylene oxide is ten times more effective against organisms than methyl bromide. Although ethylene oxide induced a large P flush from the Inman Valley soil, it was less effective than methyl bromide in releasing P from the Roseworthy soil. A number of workers have found ethylene oxide suitable as a sterilant for soils (Dalton and Hurwitz, 1948; Clark, 1950; Allison, 1951). Hexanol and β-propiolactone produced flushes of P which exceeded that of chloroform in both soils. Hexanol has not been widely used as a bactericidal or fungicidal agent although it is recognised that the effectiveness of aliphatic alcohols increases with chain length and reaches a maximum at 5-8 C atoms (Hamilton, 1971). This trend in effectiveness was evident in the data: observed P flushes decreased in the order - hexanol > propanol > ethanol. Hedley and Stewart (1982) tested the effectiveness of pure ethanol and propanol as fungicides in soil and found them to be less effective than chloroform. β-propiolactone is highly active against vegetative bacteria, bacterial spores, fungi and viruses (Hoffman, 1971; Phillips, 1977).

Because of their general activity, many of the biocides may

also react with complex compounds, so that non-microbial P could also be released. The incubation technique using ^{32}P labelling was adopted to ascertain which of the biocides was most effective in releasing microbial P only.

Recoveries of ^{32}P for the various biocidal treatments in both soils are shown in Figures 10 and 11. Significant effects on recovery of ^{32}P using the different biocides were observed for both soils, with F values being significant at the 0.1% level for the Roseworthy soil, and at the 5% level for the Inman Valley soil. Lower ^{32}P recoveries from the Inman Valley soil than from the Roseworthy soil can be attributed to the Inman Valley soil having a greater ^{31}P content and finer texture giving increased isotopic exchange and sorption respectively. In contrast to the data for ^{31}P, no large ^{32}P flushes occurred when β-propiolactone was used. This suggests that a large proportion of the observed ^{31}P flush came from sources other than the microbial P. As β-propiolactone hydrolyses rapidly in water to form hydracrylic acid, a proportion of the ^{31}P released may have originated in basic phosphates and been released by dissolution. Ethylene oxide and methyl bromide were both less effective than chloroform in rendering microbial P extractable. Hexanol and chloroform gave the largest flushes of ^{32}P, with differences between the two and between vapour and liquid forms being insignificant ($P < 0.05$). Liquid chloroform and hexanol were therefore chosen as the biocidal treatments for evaluation of extracting agents.
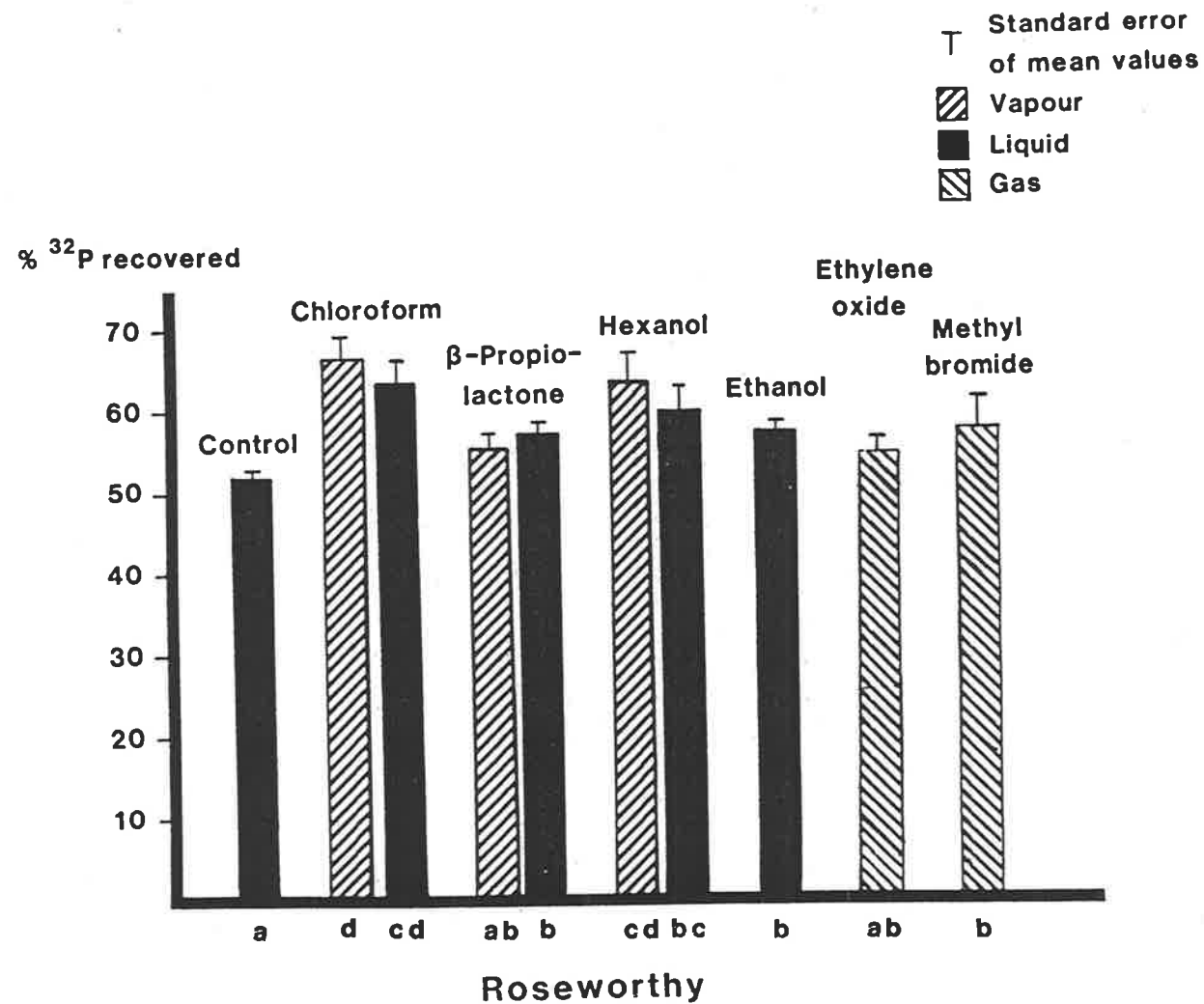
Figure 10. Effect of biocides on the recovery of ^{32}P from Roseworthy soil amended with ^{32}P-labelled tryptic soy broth and incubated until microbial activity reached a maximum. Columns subscripted by different letters are significantly different (P<0.05).
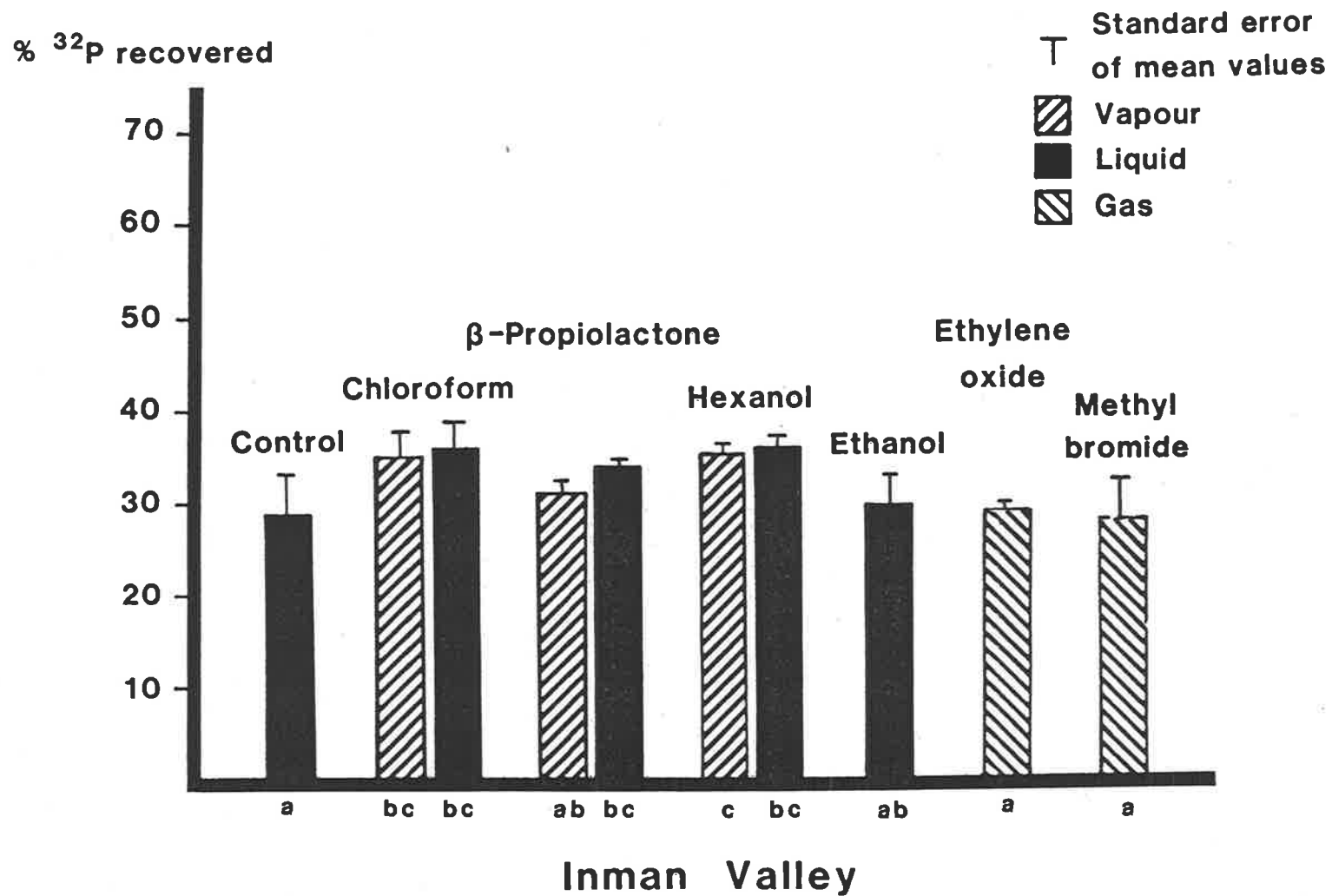
Figure 11. Effect of biocides on the recovery of ^{32}P from Inman Valley soil amended with ^{32}P-labelled tryptic soy broth and incubated until microbial activity reached a maximum. Columns subscripted by different letters are significantly different (P<0.05).

3.3 Evaluation of extractants and extraction period

Once organisms have been killed it is necessary to extract and measure the amounts of P which were held within the cells. However, the extractant must not be so vigorous that it will kill and extract P from organisms in unfumigated soil. This is particularly important when long extraction times are used. Several mild extractants were tested, in conjunction with chloroform and hexanol, for their ability to extract P from killed soil microorganisms. The effect of the biocides and extractants on microorganisms grown from soil isolates in vitro was also investigated.

3.3.1 Materials and methods

Roseworthy and Inman Valley soils were used for this study. To test the ability of various extractants to release P from killed soil organisms in situ, the ^{32}P labelling technique was employed as described previously (Section 3.2.1). A factorial design of two biocides * seven extractants was used. The extractants were 0.5$\underline{M}$ NaHCO$_3$(pH8.5), 0.1$\underline{M}$ NaHCO$_3$(pH8.5), 0.05$\underline{M}$ NaOH, 0.01$\underline{M}$ CaCl$_2$, 0.05$\underline{M}$ H$_2$SO$_4$, 0.03$\underline{M}$ NH$_4$F + 0.1$\underline{M}$ HCl (Bray and Kurtz, 1945), and distilled water with an anion exchange resin in the bicarbonate form (Sibbesen, 1977).

The effect of extractant and shaking period on the release of P from both living and fumigated soil organisms was also investigated. Soil bacterial populations were cultured by adding 1 cm^3 of a 10^4 soil dilution to 750 cm^3 0.3% TSB containing 100 mg cycloheximide. Organisms were cultured in the dark for 5 d at 18°C in unshaken flasks and harvested by

centrifugation (10000g) for 20 min at 4°C. The organisms were repeatedly washed in dilute saline, and were resuspended before fumigation and/or extraction. Soil fungal populations were cultured by adding 1 cm³ of a 10¹ soil dilution to 750 cm³ solution containing (per dm³) 660 mg NaNO₃, 330 mg KH₂PO₄, 165 mg KCl, 165 mg MgSO₄7H₂O, 6.6 mg FeSO₄, 165 mg yeast extract, 10 g sucrose, 100 mg streptomycin sulphate and 5 mg tetracycline hydrochloride. Organisms were cultured in the dark for 5 d at 20°C in unshaken flasks and harvested by vacuum filtration through 2.5 µm Nytrel Ti fabric. After being thoroughly mixed and washed in dilute saline the fungal mat was blotted dry before fumigation and/or extraction. A factorial design of 3(treatments)*4(extractants)*2(extraction periods) was used. The treatments were: unfumigated, chloroform fumigated, and hexanol fumigated. The extractants were 0.5M NaHCO₃ (pH 8.5), 0.1M NaHCO₃ (pH 8.5), 0.03M NH₄F + 0.1M HCl, and an anion exchage resin in the bicarbonate form. The extraction periods were 30 min and 16 h.

Total P in the bacterial or fungal cells was determined after digestion with concentrated nitric and perchloric acids. All other analyses were performed as described previously (Sections 3.1.1 and 3.2.1).

All treatments were replicated three times.

3.3.2 Results and discussion

The amounts of ³²P recovered by the different extractants from Roseworthy and Inman Valley soils are shown in Figure 12 and 13. Both type of extractant and biocide exerted
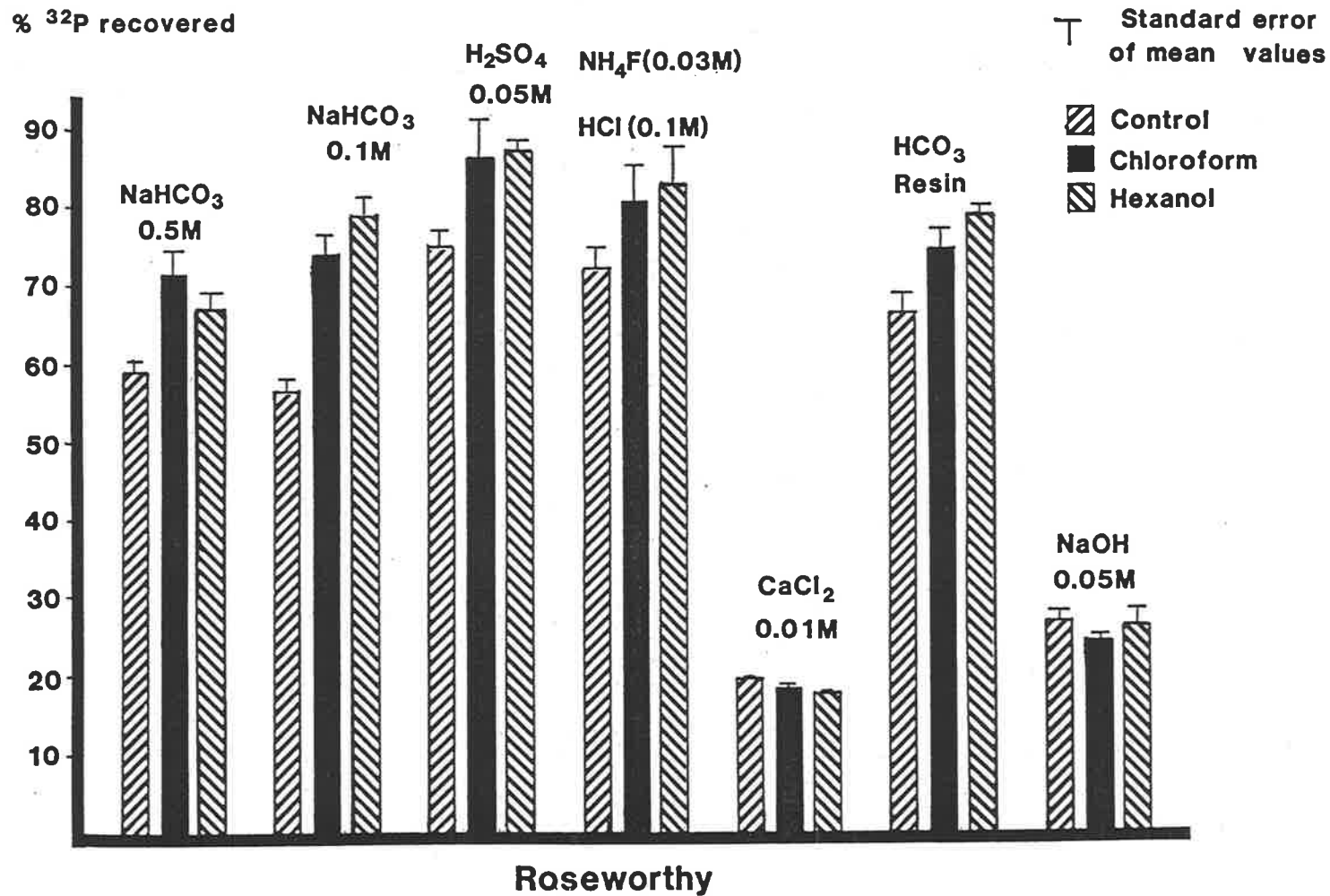
Figure 12. Effect of extractants on the recovery of ^{32}P from Roseworthy soil
amended with ^{32}P-labelled tryptic soy broth and incubated until
microbial activity reached a maximum.
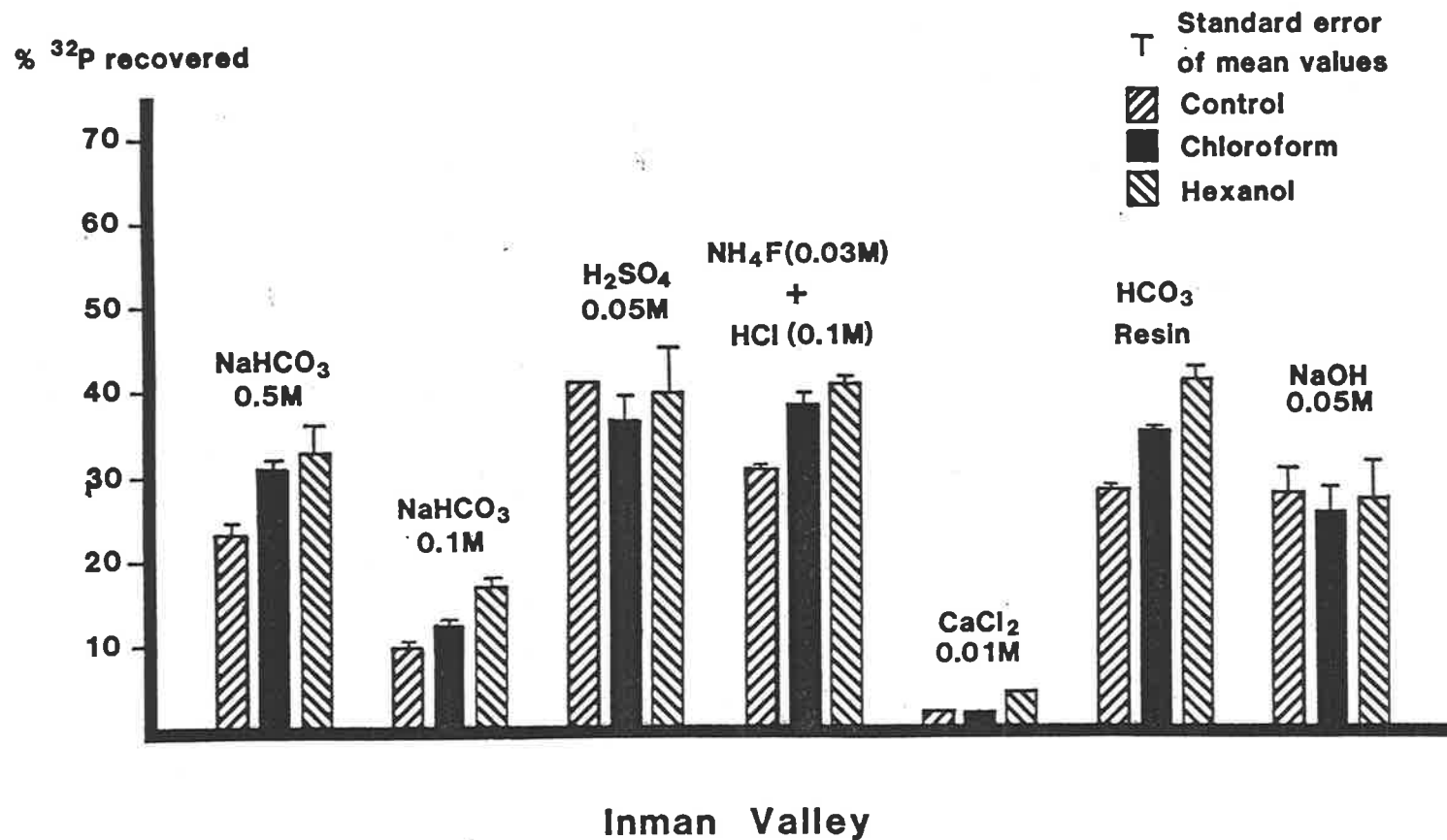
Figure 13. Effect of extractants on the recovery of ^{32}P from Inman Valley soil amended with ^{32}P-labelled tryptic soy broth and incubated until microbial activity reached a maximum.

significant effects (P<0.05) on recovery of ^{32}P from both
soils. There was a significant interaction between effects due
to biocide and extractant for the Roseworthy soil. Observed
^{32}P flushes using $0.01\underline{M}$ $CaCl_2$ were small or insignificant.
Williams and Sparling (1984) recently found $CaCl_2$ to be a poor
extractant for detecting P flushes from fumigated mineral
soils. Microbial P released by the biocidal treatment could
not be detected in the $0.05\underline{M}$ NaOH extracts of either soil or
the $0.05\underline{M}$ H_2SO_4 extracts of the Inman Valley soil. This may
have been due either to poor extraction of P from killed cells
or to too vigorous extraction of P from living organisms in the
control treatments. In view of the strength of these
extractants the latter explanation seems more likely. Sparling
et al, (1985b) found $1\underline{M}$ H_2SO_4 to be lethal for bacterial cells,
and the $0.05\underline{M}$ H_2SO_4 used in this study may have had the same
effect. This extractant also suffered from the disadvantage of
extracting large amounts of non-microbial P (i.e. high control
values) thus decreasing the precision of microbial P
measurement. The extractants $0.03\underline{M}$ NH_4F + $0.1\underline{M}$ HCl, $0.5\underline{M}$ and
$0.1\underline{M}$ $NaHCO_3$ (pH8.5), and HCO_3^- resin all gave relatively low
control values and large increases in P extracted on treatment
with chloroform or hexanol. Brookes et al (1982) tested four
extractants for their ability to extract P released from soil
by chloroform vapour, and concluded that $0.5\underline{M}$ $NaHCO_3$ (pH 8.5)
was the most suitable reagent. Hedley and Stewart (1982) also
recommend $0.5\underline{M}$ $NaHCO_3$ (pH 8.5), although they used a 16 h
extraction with HCO_3^- resin (Sibbesen, 1977) prior to the $0.5\underline{M}$
$NaHCO_3$ (pH 8.5) extraction. However, the resin extraction
technique has drawbacks as microbial activity

during extraction could decrease the amounts of P removed from the unfumigated sample, and increase the amounts of microbial P measured. This could be a significant error in soils which had recently received inputs of C, due to the stimulated demand for P and other nutrients.

The effect of biocide, extractant, and shaking period on release of P from live bacterial and fungal cells is shown in Tables 11 and 12. Analysis of variance (ANOVA) results indicated all treatments (biocide, extractant and period of extraction) and interactions to be significant at the 0.01% level for both bacteria and fungi. A considerable increase in the release of P from living organisms occurred where longer shaking periods were used. Increasing the shaking period from 30 min to 16 h, for example, increased the recovery of P_t from live fungal cells from 19.2±2.0% to 30.8±2.5% of total cell P (using 0.5$\underline{M}$ NaHCO$_3$ (pH 8.5)). Similarly for bacteria the increase was from 20.9±6.9% to 50.7±2.2%. The effect of shaking period on extraction of P from dead cells (i.e. hexanol or chloroform treated) was insignificant. Published methods for determining microbial P differ in the length of extraction period used. Hedley and Stewart (1982) found increased damage to live organisms at longer extraction times but this was offset by greater release of P from chloroform treated organisms and therefore recommended a 16 h extraction period. Brookes et al (1982) found no increase in P flush by extending the extraction time beyond 30 min. A 30 min extraction was used for all further investigations because: (a) longer times increased the damage and extraction of P from live cells;

Extractant	Period		Bacteria % Recovery		
			Control	+Hexanol	+Chloroform
0.5$\underline{M}$ NaHCO$_3$ pH 8.5	30 min	P$_i$	3.39	55.06	61.03
		P$_t$	20.87	93.36	99.37
	16 h	P$_i$	9.55	56.44	63.40
		P$_t$	50.70	92.70	92.93
0.1$\underline{M}$ NaHCO$_3$ pH 8.5	30 min	P$_i$	0	60.06	20.16
		P$_t$	3.18	89.99	85.49
	16 h	P$_i$	8.93	63.33	34.41
		P$_t$	52.16	88.45	91.31
0.03$\underline{M}$ NH$_4$F + 0.1$\underline{M}$ HCl	30 min	P$_i$	2.46	73.43	ND
		P$_t$	16.36	78.05	ND
	16 h	P$_i$	2.37	72.11	ND
		P$_t$	15.00	75.07	ND
Bicarbonate anion exchange resin	30 min	P$_i$	5.54	55.79	58.13
	16 h	P$_i$	3.92	52.69	57.29

SED 3.09

Coefficient of Variation 7.5%

Table 11 Release of P from soil bacteria (expressed as % total cell P) in the absence of soil as affected by 1) extractant, 2) shaking period, 3) biocide. Values represent the means of three replicates. ND = not determined. SED = standard error of difference between any two means.

Extractant	Period		Control	Fungi % Recovery +Hexanol	+Chloroform
0.5$\underline{M}$ NaHCO$_3$	30 min	P$_i$	0.99	13.10	27.85
pH 8.5		P$_t$	19.22	48.16	44.03
	16 h	P$_i$	3.20	17.76	28.34
		P$_t$	30.80	53.46	45.43
0.1$\underline{M}$ NaHCO$_3$	30 min	P$_i$	3.16	20.14	28.69
pH 8.5		P$_t$	23.94	50.83	45.53
	16 h	P$_i$	6.36	23.51	29.53
		P$_t$	35.75	51.91	49.64
0.03$\underline{M}$ NH$_4$F +	30 min	P$_i$	1.88	8.93	ND
0.1$\underline{M}$ HCl		P$_t$	20.46	27.98	ND
	16 h	P$_i$	2.18	9.84	ND
		P$_t$	22.86	32.89	ND
Bicarbonate anion exchange	30 min	P$_i$	3.06	15.76	26.54
resin	16 h	P$_i$	6.92	19.01	28.35

SED   1.69

Coefficient of Variation 8.4%

Table 12   Release of P from soil fungi (expressed as % total cell P) in the absence of soil as affected by 1) extractant, 2) shaking period, 3) biocide. Values represent the means of three replicates. ND = not determined.   SED = standard error of difference between any two means.

(b) longer times increase the background against which microbial P is measured (Bowman et al, 1978b) ; and (c) in freshly sieved field soils there is a greater risk of microbial activity (stimulated by sieving) influencing amounts of P extracted from control (unfumigated) treatments when longer extraction times are used.

The recovery of P from fumigated bacteria was considerably greater than from fumigated fungi. With a 30 min extraction period and hexanol as the fumigant, approximately 90% bacterial P and 50% fungal P was recovered as P_t by both $0.1\underline{M}$ and $0.5\underline{M}$ NaHCO$_3$ (pH 8.5). Flushes of P_t were considerably greater than flushes of P_i.

Hexanol produced greater ^{32}P flushes than chloroform although the differences were not consistently significant. As chloroform is carcinogenic and requires more care in handling than hexanol, the latter was chosen as the biocide for all further investigations.

3.4 Calibration for incomplete recovery of microbial P

The calculation of a calibration factor (K_p) is required, as extraction of microbial P by the fumigation technique is not complete.

3.4.1 Materials and methods

Bacterial and fungal populations from each soil were cultured as previously described (Section 3.3.1). Fresh bacterial cells were added to soils at a rate of approximately 17 μg P g^{-1} soil and fungal cells were added at rates varying

from 40-60 μg P g^{-1} soil. Preliminary experiments revealed that insignificant amounts of microbial P were released from unfumigated soil+organisms. The recovery of added microbial P was therefore calculated from the difference in P recovered from fumigated soil+organisms and that recovered from fumigated soil only. The four extractants identified in Section 3.3 as being suitable for recovery of P from soil organisms in situ were used for assessing the recovery from soil of added microbial P. All extractions and analyses were performed as described previously (Section 3.3.1). A factorial design of 2(soils) * 4(extractants) was used. All treatments were replicated three times.

3.4.2 Results and discussion

Percentage recoveries of added microbial P in the Roseworthy and Inman Valley soils are shown in Table 13. ANOVA results showed that all treatments and interactions were significant at the 0.1% level for both bacteria and fungi. With the mixed populations of soil organisms, 0.5$\underline{M}$ NaHCO$_3$ (pH 8.5) appeared to be the most efficient extractant for recovering added microbial P, if P$_t$ in the extracts was measured and not P$_i$. HCO$_3^-$ anion exchange resin gave poor recoveries of added microbial P as P$_i$, particularly in the strongly sorbing Inman Valley soil. P$_o$ desorbed and recovered by the resin was assumed to be negligible, as reported by Hedley et al (1982a). The 0.03$\underline{M}$ NH$_4$F + 0.1$\underline{M}$ HCl was also efficient in its recovery of microbial P, with most of the released P being inorganic. Using CHCl$_3$ and 0.5$\underline{M}$ NaHCO$_3$ (pH 8.5) the recovery of P$_i$ and P$_t$

from bacteria was 34.0±1.6% and 69.9±2.7% respectively in the Roseworthy soil, and 4.8±0% and 49.7±1.4% respectively for the Inman Valley soil. The recovery of P_i and P_t from fungi using $CHCl_3$ and 0.5$\underline{M}$ $NaHCO_3$ (pH 8.5) was 23.9±0.7% and 34.5±2.1% respectively in the Roseworthy soil, and 18.0±0.9% and 21.6±1.4% respectively in the Inman Valley soil.

It should be noted that Table 13 does not indicate the background values against which microbial P must be measured i.e. the amounts of P extracted by the various reagents from unfumigated soil. By considering the amounts of P released from unfumigated soil, the flush of P released by fumigation from soil microorganisms _in situ_, the recovery from those grown _in vitro_, and the recovery from those grown _in vitro_ and added to soil, it would appear that both 0.1$\underline{M}$ and 0.5$\underline{M}$ $NaHCO_3$ (pH 8.5) are the most suitable reagents for extracting microbial P released by fumigation. It was decided to use 0.5$\underline{M}$ $NaHCO_3$ (pH 8.5) as the extractant, as the P_i extracted from unfumigated soil is a measure of plant available P (Olsen et al, 1954) widely used in Australia and elsewhere for soil testing purposes.

The technique adopted in this study of using mixed populations of organisms divided broadly into bacterial and fungal groups differs from previous investigations where dead (lyophilised) organisms (Brookes et al, 1982), or individual species were chosen (Saggar et al, 1981; Hedley and Stewart, 1982; Strick and Nakas, 1984). The microflora of each soil were stimulated, harvested, and reintroduced to the soil. The use of a soil inoculum, broad spectrum nutrient media and low

Extractant		Bacteria		Fungi	
		Roseworthy	Inman Valley	Roseworthy	Inman Valley
		Percentage Recovery			
0.1 $\underline{M}$ NaHCO$_3$	P_i	31.6 ± 1.8	4.4 ± 0.3	19.7 ± 3.6	8.1 ± 0.3
pH 8.5	P_t	70.5 ± 3.5	66.1 ± 1.4	37.7 ± 6.8	9.5 ± 0.4
0.5 $\underline{M}$ NaHCO$_3$	P_i	36.4 ± 1.0	9.2 ± 0.5	32.4 ± 4.3	17.6 ± 1.0
pH 8.5	P_t	85.0 ± 3.4	61.8 ± 2.0	48.1 ± 6.1	19.4 ± 1.8
HCO$_3^-$ Resin	P_i	31.1 ± 0.6	3.9 ± 0.1	20.8 ± 3.0	16.3 ± 1.2
	P_t	ND	ND	ND	ND
0.03 $\underline{M}$ NH$_4$F	P_i	76.6 ± 3.0	20.1 ± 1.0	23.8 ± 0.2	33.5 ± 3.0
+ 0.1 $\underline{M}$ HCl	P_t	83.5 ± 2.3	26.2 ± 0.4	34.8 ± 0.8	32.1 ± 2.1

Table 13. Percentage recovery of P from bacterial and fungal populations added to soils and treated with hexanol. Values are the means of three replicates ± standard deviation and are not corrected for sorption. ND = not determined.

incubation temperatures encouraged a variety of organisms to flourish. For example, the bacteria harvested from the Roseworthy soil culture contained a mixture of both gram positive and gram negative rods and cocci. The fungi stimulated were identified as being predominantly _Mucor_ and _Penicillium_ spp. The proportions of each species in the population were unknown and it is assumed that the microbial population responded (qualitatively) in each soil as it would do in the field after inputs of nutrients such as plant residues or substrates released on rewetting dry soil. This is a more realistic approach than one which produces K_p values obtained from one or two species only or from arithmetic means of a large number of species, many of which may not be present in the soil in question. A disadvantage is that organisms from each soil must be cultured, and a separate K_p determined for each soil.

In calculating an overall K_p factor, a problem can arise from partition of soil organisms into bacterial and fungal groups. If recovery of P from the two groups is significantly different, as found with the soils used in this study, account must be taken of the relative contribution of each group to the total biomass. It is generally agreed that fungi are the dominant group in most soils in terms of total biomass (Clark and Paul, 1970). However, ratios of fungal to bacterial biomass (F:C ratios) can vary with both soil type and season (Clark and Paul, 1970; Shields _et al_, 1973; Faegri _et al_, 1977). Values ranging from 1:1 to 11:1 have been reported. Generally F:C ratios are approximately 2:1 or 3:1 (Clark and

Paul, 1970; Anderson and Domsch, 1975), with arable soils

having slightly higherF:C ratios than pasture or grassland

soils (A. West - personal communication). The F:C ratios used

for calculation of K_p factors were 3:1 for the Roseworthy soil

and 2:1 for the Inman Valley soil and the K_p factors thus

obtained for the Roseworthy and Inman Valley soils were 0.57

and 0.33 respectively.

3.5 Correction for sorption of released P

The K_p factors determined above included a correction for

sorption of released P_i and P_o. Sorption of P_i and P_o released

from microbial cells by fumigation will decrease the recovery

of P_i and P_t by the extracting solution. Brookes et al (1982,

1984) maintained that a large proportion (>80%) of the P_t

released is P_i, and recommended that a correction be made for

P_i sorbed. They maintained that the degree of sorption of P_i

from the $0.5M$ NaHCO$_3$ solution was independant of P

concentration in the solution i.e. the sorption isotherm was

linear. Thus P_i sorbed could be assessed from the recovery of

a single addition of P_i added to the unfumigated soil before

extraction. Hedley and Stewart (1982) recommended that a

correction for sorption of both P_i and P_o be included in the

K_p factor, which must then be determined separately for each

soil. A study was therefore undertaken to determine the most

effective method of correcting for sorption of released P.

3.5.1 Materials and methods

Three methods to correct for sorption of released P_i were

tested. In the first, a "spike" of 20 μg P g^{-1} soil was added to the soil immediately before extraction with 0.5$\underline{M}$ NaHCO$_3$, and the percentage recovery of added P determined (Brookes et al, 1982). In the second, a range of P additions (0.3 - 20.0 μg P g^{-1} soil) was used and a sorption isotherm constructed. In the third method, 10 kBq ^{32}P both with and without carrier ^{31}P (0.2 μg cm^{-3}) were added to 0.5$\underline{M}$ NaHCO$_3$ suspensions and labile ^{31}P$_i$ (solid+solution) calculated (Talibudeen, 1957). To minimise microbial uptake of P in unfumigated soils a 2 h shaking period was used with the isotope being added 1 h after shaking began. Appropriate controls were prepared to correct for possible sorption of ^{32}P to the plastic bottles, funnels and filter papers when ^{32}P was used without carrier ^{31}P. A 30 min extraction period was used for the experiments measuring recovery of added ^{31}P. As microbial P is measured against a background of unfumigated soil, only unfumigated soil was used for this study. A soil:solution ratio of 1:20 was used throughout. All other analyses were performed as described previously (Section 3.2.1).

3.5.2 Results and discussion

Attempts to correct for sorption of released ^{31}P$_i$ using ^{32}P dilution were unsuccessful. At the short equilibration times used (1 h), ^{32}P$_i$ was found to have a greater affinity for the sorbing surface than ^{31}P$_i$. This was evidenced by overestimates of labile ^{31}P$_i$ using measured recoveries of added ^{31}P$_i$ standards. For example, the recovery of a "spike" of 20 μg P g^{-1} soil added to the Inman valley soil calculated by the

isotope dilution equation was 113.9%. This may be due to ^{32}P being initially held at a higher specific activity on the solid surfaces and not in equilibrium with the surrounding solution as discussed by White (1976). Longer equilibration times could not be used because of the risk of microbial uptake of ^{32}P in unfumigated soils leading to overestimates of labile $^{31}P_i$ and hence overestimates of microbial P.

There was no significant advantage in constructing a sorption isotherm to correct for sorbed $^{31}P_i$ in these soils. The relationships between P added and P recovered for the Roseworthy and Inman Valley soils were found to be linear.

$$Y = 1.234(X-6.66) + 7.95 \quad R^2 = 0.999^{***} \quad \text{Roseworthy}$$

$$Y = 1.715(X-3.92) + 6.80 \quad R^2 = 0.999^{***} \quad \text{Inman valley}$$

where Y = P added, X = P recovered and *** denotes significance at the 0.1% level.

In soils where a large proportion of the released P_t is in organic forms, as found in this study, this correction can be regarded only as a minimum value. Sorption of P_o depends on the amounts and nature of the organic compounds which themselves can be expected to vary depending on the microbial population present in each soil. Sorption of released P_o can best be accomplished by including this unknown into the proportional recovery factor (K_p) as suggested by Hedley and Stewart (1982). As each soil must be calibrated for K_p, this causes no extra inconvenience.

3.6 Release of P from soil P_o by fumigation

As fumigation might alter the extractability of non-microbial P_o from soil, this aspect was investigated.

As pointed out by Brookes et al (1982), the effect of P_o compounds in soil on measurement of microbial P cannot be simply evaluated by studying the effect of fumigation on particular P_o compounds, as a large proportion of the P_o compounds in soils have yet to be identified. Indirect approaches are therefore necessary.

3.6.1 Materials and methods

Roseworthy, Inman Valley and a third soil from Mallala, South Australia (Table 18, page 97) were used. The soils were wetted to -66 kPa and incubated for 14 d at 20°C. The soils (5 g samples) were then sealed in air-tight containers and sent as surface freight to Melbourne. Half of the soils were sterilised by γ-irradiation from a ^{60}Co source (50 kGy), before all the soils were returned to Adelaide. Total time in transit was 7 d for both sterilised and non-sterilised soils. The soils were then extracted with $0.5\underline{M}$ $NaHCO_3$, or fumigated with hexanol or chloroform, and extracted, except that the suspensions were treated to a mild ultrasonic dispersion (75 W, 60 s) before extraction. All other analyses were performed as described previously (Section 3.1.1).

3.6.2 Results and discussion

Recovery of P_t from the three soils when hexanol was used as the fumigant are shown in Table 14. The results for

chloroform-fumigated soils were similar (data not presented).
Hexanol caused a significant increase in the extraction of P_t
from γ-irradiated soil only in the Inman Valley soil.

As discussed by Brookes et al (1982) amounts of P_t extracted
from γ-irradiated soils may increase because (a) fumigation
solubilises non-microbial P, or (b) γ-irradiation does not
effect the complete lysis of soil organisms. Brookes et al
(1982) argue that (b) is the more likely explanation, as
γ-irradiation kills cells by damaging their DNA but leaves their
cellular structure intact. Indeed, seven years earlier Labeda
et al (1975) directly observed the fine structure of
γ-irradiated soil microorganisms in situ, and found that
γ-irradiation did not alter cell structure. Fumigation with
chloroform, on the other hand, has recently been shown to

Table 14. Amounts of $^{31}P_t$ extracted from γ-irradiated and/or
fumigated soils.

Soil	Treatments		P_t	P_t flush
	γ-irradiation	Hexanol	μg g^{-1} soil	
Roseworthy	−	−	4.6	−
	−	+	7.9	3.3ᵃ
	+	−	7.6	3.0ᵃ
	+	+	8.0	3.4ᵃ
Inman	−	−	16.8	−
Valley	−	+	23.2	6.4ᵃ
	+	−	24.4	7.6ᵃ
	+	+	29.2	12.6ᵇ
Mallala	−	−	13.9	−
	−	+	24.0	10.1ᵃ
	+	−	22.4	8.5ᵃ
	+	+	24.3	10.4ᵃ

Within soils, values in a column followed by different letters
are significantly different at the 5% level ($P \le 0.05$).

cause complete lysis of microbial cells in soil (Martin and Foster, 1985). Thus it might be expected that fumigation would release P from γ-irradiated soils by completing the lysis of the microorganisms. It was assumed that the mild ultrasonic dispersion of the soil suspensions would disrupt cell membranes in organisms killed by γ-radiation, yet leave intact the membranes of organisms in unsterilised unfumigated soils. If this assumption is correct, it is wrong to assume that the flush of P_t on fumigation of the Inman Valley soil came exclusively from the soil biomass. However, fumigation of autoclaved soil did not increase the amounts of P extracted (data not shown). Autoclaving of soil is known to disrupt microbial cells totally (Labeda et al, 1975). Air drying has also recently been shown to cause death of a large proportion of the soil biomass as measured by substrate induced respiration (Sparling et al, 1985b). Sparling et al, (1985b) also found good agreement between amounts of P_i released by air drying, and amounts of P present in the biomass before air drying as determined by fumigation. More recently Spier et al, (1986) showed that the amount of P_t released by fumigation of soil exposed to microwave radiation (2450 MHz), decreased with increasing time of exposure, until no significant increase was observed on fumigation after 50 s or more exposure to microwave radiation. The decrease in flush of P due to fumigation was closely related to decreases in microbial C determined by substrate induced respiration and numbers of organisms determined by plate counting. Thus it appears that fumigation, air drying, autoclaving, and microwave irradiation all cause

release of P from the same soil component. It seems unlikely that this source of P is anything other than the microorganisms in soil.

3.7 Release of P from plant material by fumigation

Birch (1961) was the first to recognise that fumigation could affect the extractability of P from living plant material. Hence if plant roots or (fresh residues) are present in a soil sample, errors in the determination of microbial P could result. As later experiments were to investigate changes in microbial P in soils which had recently received inputs of plant material (Chapters 5 and 6), the extent to which plant material might affect the values obtained for micobial P was investigated.

3.7.1 Materials and methods

Wheat seedlings were grown in a culture solution based on that of Johnson et al (1957) - Plate 1b, page 164. The solution supplied N (16 mmol), P (200 μmol), K (6.06 mmol), Ca (4 mmol), Mg (1 mmol), S (1 mmol) and all essential micronutrients. Continuous stirring was effected through aeration, and solution pH was adjusted daily to 6.0 with $Ca(OH)_2$. ^{32}P-labelled plant material was obtained by adding 925 kBq carrier free ^{32}P dm^{-3} to the solution at the start of the growth period to give a specific activity of 149 MBq ^{32}P g^{-1} ^{31}P. Plants were grown at a constant 18°C. Photosynthetically active radiation (PAR) was 500 μmol quanta m^{-2} s^{-1} for 15 h day^{-1}, and relative humidity was 75% (day) and

90% (night). The plants were harvested after 21 d growth.

After harvest, plants were separated into roots and shoots.
The shoot material was cut into 15 mm lengths, washed with
water for 5 min and blotted dry. The fresh material was either
fumigated according to the method of Brookes et al (1982), or
added to 5 g samples of field moist soil (Treatment 2, Section
3.1) at a rate of 20 mg g^{-1} soil before fumigation. When this
procedure was used for roots, the variability was large.
Accordingly, individual intact fresh roots were counted with
the roots suspended in deionised water (Martin and Cunningham,
1972). This method measured 90.1±4.3% of the total root ^{32}P
determined by normal wet digestion procedures. Thus the exact
amount of ^{32}P in each section of root was known. Immediately
after ^{32}P determination the portion of intact root was
fumigated or added to soil and fumigated as described above.

To determine the effect of drying, separate portions of
^{32}P-labelled root and shoot material were dried at 50°C for 24
h before being treated as described above for fresh shoots.
The rates of addition were 3 mg dry shoot, or 1 mg dry root
g^{-1} soil. These experiments were conducted with chloroform as
the fumigant: later work showed that hexanol behaved
identically.

Plant materials and amended soils were extracted for 30 min
or 16 h as described previously (Section 3.1.1).

3.7.2 Results and discussion

Fumigation increased 0.5M NaHCO₃ extractable P from both

fresh shoot and fresh root material regardless of the
extraction period used (Table 15). The absence of any release
of P from fumigated shoots using the 30 min extraction period
appeared to be due to the hydrophobic nature of the plant
cuticle, which prevented the extractant from effectively
penetrating the tissue. Longer extraction times seemed to
overcome the problem, as the plant tissue was fully "wetted"
after the 16 h extraction. Extending the extraction time from
30 min to 16 h caused a large increase in the amount of P
extracted from fresh unfumigated roots, probably due to damage
and rupture of cells. These results agree with those of
Sparling et al (1985a), who recently demonstrated that the
presence of plant roots can interfere with the determination of
microbial P and ATP. Martin and Foster (1985) used electron
microscopy to provide visual evidence of damage to root cells.
For field soils, the errors arising from the presence of plant
material are large enough to cause concern. Published values
for rooting densities under crops and forests vary from 0.1 to

Table 15. Effect of time on extraction of ^{31}P by $0.5\underline{M}$ NaHCO$_3$ (%
total plant P) from fumigated (CHCl$_3$) and unfumigated
freshly cut plant materials. Standard deviation of
mean values in parentheses.

Material	Treatment	Extraction period	
		30 min	16 h
Roots	Unfumigated	26.1(3.2)	69.5(8.5)
	Fumigated	64.6(9.3)	89.3(1.6)
Shoots	Unfumigated	0(0)	6.3(1.4)
	Fumigated	18.0(5.1)	82.0(5.2)

12.0 mg dry root g^{-1} soil (Lupton et al, 1974; Lynch and

Panting, 1980), but generally the values fall within the range

0.3 to 2.0 mg root g^{-1} soil (Troughton, 1957; Persson, 1983).

Assuming that plant roots contain 0.1% P, the upper limit for

root-bound P in soils would be approximately 10 μg P g^{-1} soil.

If we assume that a maximum of 40% of this is released by

fumigation, then 4 μg P g^{-1} soil would be the maximum error

involved. Published values of total P in soil biomass vary

from 5 to 100 μg P g^{-1} soil (Perrott and Sarathchandra, 1982;

Brookes et al, 1984), which equates with 2 to 40 μg P g^{-1} soil

for P released by fumigation (assuming a K_p factor of 0.4). If

P released by fumigation from microorganisms and plants is

sorbed to the same extent during extraction, then significant

errors in measured values of microbial P are likely in some

soils if plant material is included in the sample.

In the experiment using ^{32}P-labelled plant materials, drying

increased the P extractability of unfumigated materials. All

shoot ^{32}P and two-thirds shoot ^{32}P was extractable following

the drying treatment (Table 16). Recovery of ^{32}P from fresh

plant materials was slightly different from that obtained in

the previous experiment using unlabelled materials. Recovery

of ^{31}P from fresh roots was significantly higher than ^{32}P

recovery for both fumigated and unfumigated treatments. This

may have been due to the roots having been left intact for the

experiment with ^{32}P-labelled materials. There is also the

possibility that the plant material was not uniformly labelled

with ^{32}P. Nevertheless the effect of fumigation on increasing

Table 16. Effect of drying on extraction of ^{32}P by 0.5$\underline{M}$ NaHCO$_3$ (pH 8.5) (% total plant ^{32}P) from fumigated (CHCl$_3$) and unfumigated plant materials. A 16 h extraction period was used.

Treatment	Material			
	Fresh root (intact)	Fresh shoot (cut)	Dried root (cut)	Dried shoot (cut)
Unfumigated	48.7(2.2)	24.9(5.3)	66.5(4.2)	103.4(7.3)
Fumigated	61.9(3.2)	87.7(2.2)	61.8(6.1)	108.4(8.3)

the recovery of P from fresh plant material was consistent for both experiments. Fumigation had no significant effect on the release of P from dried plant materials. This result agrees with those of Martin and Cunningham (1972) who found desiccation to be the major factor influencing the water solubility of P in plant roots. Their results indicated approximately 50% root P became water extractable on drying. Similarly, Bromfield and Jones (1972) found water soluble P in shoots to be up to 80% of the total P in "hayed-off" pasture species. As 0.5$\underline{M}$ NaHCO$_3$ is a slightly stronger extractant than water, especially for labile organic P compounds, 0.5$\underline{M}$ NaHCO$_3$ would be expected to extract more P than water.

Recently Sparling $\underline{et}$ $\underline{al}$ (1985a) observed that the interference of plant roots on measured values of microbial P could be minimised by subjecting the soil to a 7 d incubation before fumigation. However, this is likely to alter the values obtained for microbial P as the organisms may respond during the incubation period both qualitatively and quantitatively to soil disturbance during sampling and sieving. To test if

incubation of freshly sampled soil before fumigation affected
the values obtained for microbial P, Mallala soil was fumigated
and extracted at varying times after sampling. The soil was
stored moist over soda lime in large sealed containers at 20°C.
Results are presented in Table 17. Significantly lower values
were obtained for hexanol-released P_t when the soil samples
were incubated prior to fumigation. This result agrees with
that of West et al (1986), who found that incubation of soils
altered measured values of soil respiration and biomass C, N, P
and ATP contents. These workers altered the water potential of
the soil before incubation. By incubating the soil at its
field water potential and at 20°C (field soil temperature at 50
mm at sampling was 14°C), it was hoped that the effect of
pre-incubation would be minimised. However, it appears that
the soil must be fumigated and extracted immediately after
sampling if the values obtained for microbial P are to reflect
those which pertain in the field at the time of sampling.

The drying, grinding and pre-incubation of soil before
measurement of microbial P, as recommended by Hedley and
Stewart (1982), will overcome errors associated with

Table 17. Effect of pre-incubation on the measured flush of
$^{31}P_t$ caused by fumigation of the Mallala soil.
Standard error of mean difference shown in
parentheses.

Time of incubation (d)	P_t flush $\mu g \ g^{-1}$
0	10.1(0.3)
12	6.9(0.2)
32	7.6(0.3)

inclusion of plant material in the sample, but leads to changes
in measured values of microbial P (Section 3.1). Although
extending the extraction period decreases the error due to root
material, it increases the error due to shoot material included
in the sample. Extending the extraction period to 16 h is also
not recommended for the reasons previously noted in Section
3.3. Physical removal of plant material before fumigation is
the preferred option. The magnitude of errors will then depend
on the efficiency with which plant material can be removed from
the sample before fumigation. Obviously, the greater the
amount of fine root material in the sample, as in grassland
soils, the greater will be the potential error.

3.8 Efficiency of handpicking to remove root material

Pot and field experiments with the Mallala soil were
undertaken to investigate changes in microbial P as a result of
the addition of fertiliser and plant residues (Chapters 5 and
6). The efficiency of handpicking as a method of removing the
interference of root material in the determination of microbial
P was therefore investigated.

3.8.1 Materials and methods

^{32}P-labelled wheat roots were obtained as described
previously (Section 3.7). Fresh ^{32}P-labelled wheat roots (470
mg) were intimately mixed with a weight of freshly sampled
Mallala soil equivalent to 100 g oven-dry weight. The
soil+roots was then sieved (<2 mm) and the roots picked out.
Subsamples were then extracted directly, or fumigated and

extracted as described previously in Section 3.7.

3.8.2 Results and discussion

Sieving and handpicking to remove roots from the soil eliminated errors associated with the inclusion of root material in the sample. The percentage recovery of ^{32}P from unfumigated soil ($1.7\pm0.8\%$) was not significantly different from the recovery of ^{32}P from fumigated soil ($2.2\pm0.6\%$). However, it should be remembered that organisms colonising the rhizoplane itself, e.g. endo- and ectotrophic mycorrhiza as well as populations of bacteria, will be removed by this treatment. Also, the labelled roots were added to soil and not grown in situ. Thus none of the labelled root material had penetrated aggregates less than 2 mm diameter which would normally contain (after sieving) root material impossible to remove by hand.

3.9 Summary and conclusions

From a wide range of biocides and extractants hexanol, in conjunction with $0.5\underline{M}$ $NaHCO_3$ (pH 8.5), was selected as the most effective combination for extracting microbial P from soils. A 30 min extraction period was chosen. Storage of moist soil or alteration of the soil water potential prior to fumigation had a large effect on measured values of microbial P. Calibration of each soil due to incomplete recovery of microbial P is recommended. The interference of plant material is best avoided by physical removal of the material from the sample prior to fumigation, but the efficiency with which this can be achieved must first be ascertained for the system under study.

CHAPTER 4

TRANSFORMATIONS AND MOVEMENT OF P IN THE RHIZOSPHERE

4.1 Introduction

As bacteria and fungi are 20 to 50 times more abundant in the rhizosphere than in bulk soil (Newman, 1978; Rovira, 1979), and as roots withdraw P from the soil solution and may also exude or leak P back into the soil (Federovski, 1958; Emmert, 1959; Woods and Brock, 1964; Harrison, 1978), the rhizosphere is a key site for P transformations. Plants have a large influence on reactions taking place through uptake of P from the soil solution, and the stimulation of microbial activity in the vicinity of the roots (Blair and Boland, 1978; Helal and Sauerbeck, 1984).

A limitation of the fumigation technique for measuring microbial P in soils containing actively growing plants is interference of live root material (Chapter 3). While the bulk of the roots can be removed by hand, there remains an undefined amount of fine root which may lead to overestimates of microbial P.

This chapter describes a technique to measure movement of P both into and out of plant roots, and the forms of P in soil, including microbial P, associated with them. The technique ensures that soil sampled from the rhizosphere is entirely free from live root material.

4.2 Materials and methods

^{33}P-labelled wheat plants were grown in thin layers of soil treated with ^{32}P-labelled fertiliser. Roots were separated

from the soil by growing them within porous membrane envelopes
(Brown and Anwar-Ul-Haq, 1984) centrally located in soil in
perspex boxes measuring 125 mm * 130 mm * 6 mm (Figure 14 and
Plate 1c, page 164). The membrane material was polyamide. It
had apertures of 5 μm: thus the root system, including root
hairs, was entirely confined within the envelope. However,
water and nutrients were free to move across the membrane.
Growth of plants in the membrane envelopes was similar to that
of plants with unconfined roots (Plate 1d, page 164). Each box
contained 120 g (oven-dry equivalent) of air dry, sieved (<2
mm) soil (0-100 mm layer) amended with ^{32}P-labelled fertiliser
(38.5 mg^{31}P kg^{-1}, 2.66 MBq^{32}P kg^{-1}). The Mallala soil was used
for this study (Table 18).

Table 18. Description of Mallala 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
Soil classification(USDA)	Calcixerollic xerochrept

After the soil was incubated for 7 d at 20°C and -66 kPa water
potential, germinated wheat seeds were planted within the
membrane envelope. The seedlings were labelled with ^{33}P
(carrier free) by foliar application at 9 and 13 d after
planting, and by injection into the stem at 19 and 21 d to give
a total application of 250 kBq ^{33}P plant^{-1} at harvest. Soil

^{33}P labelled tops
(by injection)

5 μm diameter
pore membrane

^{33}P labelled roots
(by translocation)

^{32}P labelled soil
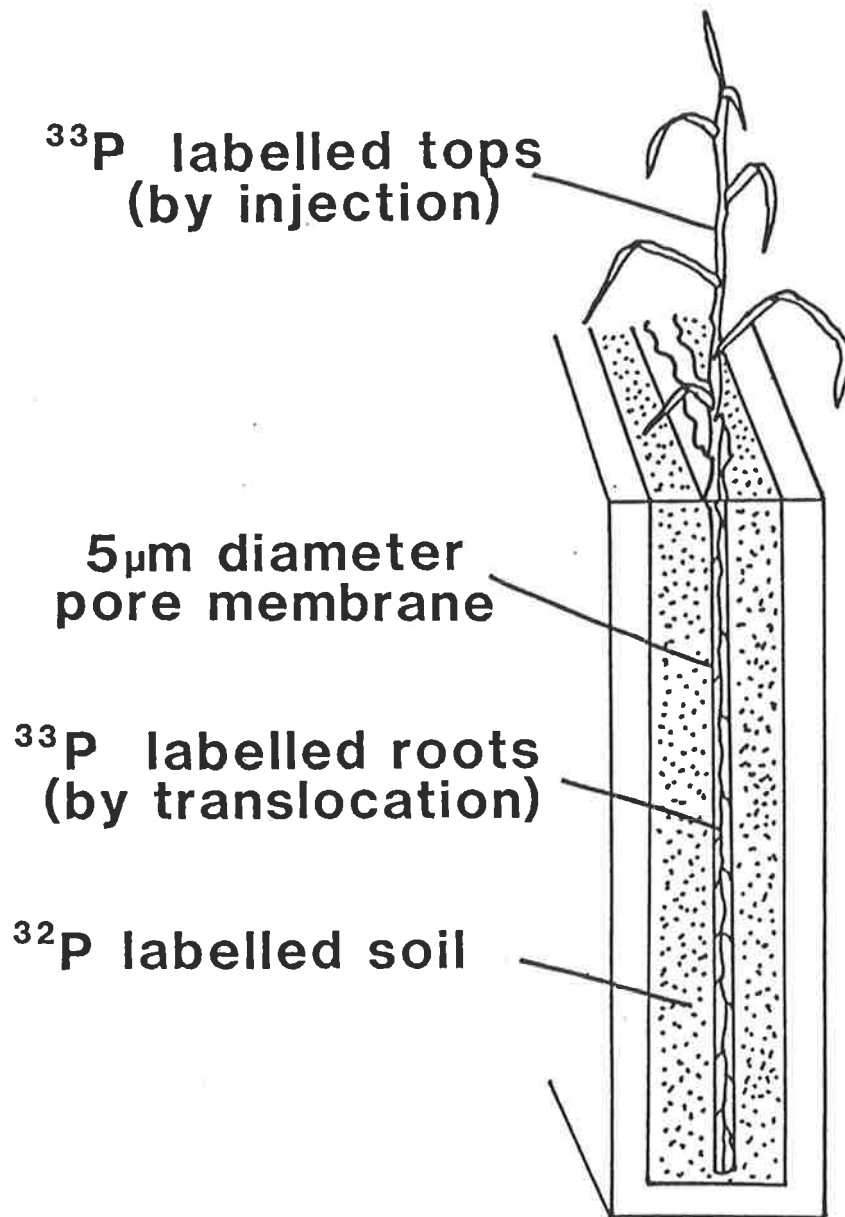
Figure 14. Design of perspex box and membrane technique
used.

water potential was adjusted to -66 kPa daily. Plants were grown for 22 d at 20°C (day), 15°C (night), PAR was 500 μmol quanta m^{-2} s^{-1} for 15 h day^{-1}, and relative humidity was 60% (day) and 90% (night).

The plants were harvested by dismantling the perspex boxes and removing the intact membrane envelope from the soil. The moist soil was fractionated without drying. After being soaked in deionised water for 2-3 min the soil-free root system was peeled away from the envelope. Plants were separated into roots and tops, and weights were determined after the material was dried for 48 h at 70°C. Amounts of ^{31}P, ^{32}P and ^{33}P in the plant materials were determined after wet digestion with nitric and perchloric acids.

Tops of plants were harvested from a second series of boxes and the box (including soil and intact roots) was then dried at 50°C for 48 h. The soil and roots were rewetted to -66 kPa water potential, and incubated at 20°C for 48 h. This treatment is subsequently referred to as the dried/rewetted treatment. Each treatment was replicated six times.

Duplicate subsamples of soil from each box were fractionated according to a modification of the method of Hedley et al (1982a). Microbial P was determined as described previously (Section 3.7). A K_p of 0.4 was obtained for the soil using mixed populations of soil organisms as described in Section 3.4. Suspensions were centrifuged for 10 min at 2010g before filtration (Whatman No.42). The amount of extract remaining in the residue was determined gravimetrically after each step in the procedure.

The 0.1$\underline{M}$ NaOH extracts were acidified to flocculate organic materials. Total ^{31}P ($^{31}P_t$) in the extracts was determined after digestion with perchloric acid (Brookes and Powlson, 1981).

The amounts of ^{32}P and ^{33}P were determined by liquid scintillation counting using the external standard channels ratio method (L'Annunziata, 1979). The 0.1$\underline{M}$ NaOH extracts were either acidified or digested prior to counting to minimise chemiluminescence. All counts were corrected for quenching and decay and converted to Bq.

4.3 Results and discussion

4.3.1 P flux from plant roots

A significant proportion of the ^{33}P applied to the plant tops was translocated to the root system (Table 19). Of the ^{33}P translocated to the roots, 0.9% to 4.9% was recovered in the soil. Of the ^{33}P recovered from the soil, the greatest proportion (63.0±5.8%) was found in the fumigated/NaHCO$_3$ fraction; the rest was recovered in the HCl fraction.

In studies of root exudation, solution culture has generally been used to facilitate sampling and analysis (Rovira, 1969). Reports of loss of P by roots (other than by translocation to the shoots) have also been confined mainly to qualitative studies of losses to aqueous media (Akhromeiko and Shestakova, 1958; Emmert, 1959; Rovira and Bowen, 1970; Smith, 1976), and to the use of plants with excised tops (Woods and Brock, 1964). Ritz and Newman (1985) showed indirectly that loss of ^{32}P by both intact and dying roots of pasture plants may be

Table 19. Distribution of ^{32}P and ^{33}P at harvest (before drying/rewetting). Values are the means of six replicates (standard deviation of mean values in parentheses).

	% recovered	
	^{32}p	^{33}p
Soil	88.0(1.2)	0.7(0.4)
Top	7.4(0.9)	71.5(6.7)
Root	4.6(0.8)	27.8(6.9)
Total	100.0	100.0
Microbial biomass	14.1(1.8)	0.1(0.1)

considerable (as shown by ^{32}P uptake of adjacent plants).
Newman and Ritz (1986) recently showed that mycorrhizas are
unimportant in facilitating the transfer of ^{32}P between plants.
Fedorovski (1958) used a split root technique with maize — one
half of the root system was supplied with ^{32}P-labelled
fertiliser — and found that 10 to 23% of the ^{32}P taken up was
exuded into the soil containing unlabelled fertiliser.
However, loss of ^{31}P by living roots cannot be readily
calculated from the loss of radioisotope, as an unknown
proportion of any measured loss of radioisotope will be due to
isotopic exchange (Weigl, 1968) rather than net loss from the
root. It seems likely that any inorganic ^{31}P leaking from a
root would be quickly reutilised by the root, and would not
diffuse away against the concentration gradient which usually
prevails in soil around active roots. Thus, any movement of

^{31}P away from active roots is likely to be confined to that contained in complex organic compounds in root exudates. There is the possibility that P may be lost through the extension of mycorrhizal fungi away from the roots, although the short period of growth used here makes this unlikely in this experiment. If mycorrhiza had caused significant amounts of P to move away from the root, significant amounts of ^{33}P would then have been detected in the soil biomass. In this study, the measured loss of ^{33}P by living wheat roots was small and the isotope lost was found predominantly in the $NaHCO_3$ extract. The difference between amounts of ^{33}P recovered from the unfumigated and fumigated soils was insignificant, so it can be assumed that mycorrhizal transport of P away from the roots in this study was insignificant. This suggests that most of the ^{33}P loss was due to isotopic exchange and that loss of P by the roots to soil through exudation was negligible. Any P in indiffusible exudates would have remained close to the root or inner surface of the membrane, and would have been included in root P. Hence losses of P from unrestricted roots might be greater than encountered with the technique used here.

The specific activity of ^{33}P in the $NaHCO_3$ extract of unfumigated soil (1.04 Bq ^{33}P μg^{-1} ^{31}P) was significantly ($P < 0.001$) greater than the $NaHCO_3$ extract of fumigated soil (0.77 Bq ^{33}P μg^{-1} ^{31}P). The proportion of ^{33}P recovered in the microbial biomass was insignificant. This indicates that most of the P in the microbial biomass originated from the soil, and was not derived from P being exuded from, or leaking from, the roots.

4.3.2 Distribution of ^{32}P activity

Recovery of ^{32}P by the soil fractionation procedure employed was $101.4\pm5.3\%$ of total soil ^{32}P. The latter was determined by perchloric acid digestion of the unplanted soil and represented $102.3\pm3.6\%$ of ^{32}P added. Of the ^{32}P labelled fertiliser applied, 7.4% was recovered in plant tops, 4.6% in plant roots and 14.1% in the microbial biomass ($K_p = 0.40$) (Table 19).

Care must be taken in interpreting the results of ^{32}P distribution, as ^{32}P activity is affected both by isotopic exchange, and by biological and chemical processes in the soil. All of the ^{31}P and ^{32}P in ^{32}P-labelled fertiliser would initially be present in water soluble form and hence extractable with $NaHCO_3$ solution. However ^{31}P would become adsorbed to surfaces and precipitated as complex solid phases. ^{32}P would undergo similar reactions and would also exchange with soil ^{31}P. These reactions would be most rapid in the first few days after initial soil-fertiliser contact (Russell et al, 1954). As isotopic exchange can occur with soil fractions which are not plant available (Machold, 1962), the proportion of fertiliser ^{31}P entering the plant will be underestimated by ^{32}P data (Russell et al, 1954). Thus the above mentioned values of 12.0% and 14.1% for fertiliser ^{32}P entering the plant and microbial biomass respectively indicate the minimum amount of fertiliser ^{31}P entering these pools. The value obtained for the proportion of ^{32}P labelled fertiliser found in the microbial biomass depends on the value of the calibration factor (K_p). Even allowing for some error in the

K_p value because organisms grown _in vitro_ have to be used in its determination (Section 3.4), it is clear that a considerable proportion of the applied ^{32}P was incorporated into the biomass. If it is assumed that the microbial biomass does not have access to soil P which is isotopically exchangeable but not available to plants (e.g. ^{31}P held within crystalline solids or in fissures in crystal lattices (Russell _et al_, 1954)), then the biomass and plants were equally effective in obtaining P from the applied fertiliser. These results support the hypothesis of Hedley _et al_ (1982a) that the microbial biomass is a major component and driving force of P cycling in soils. In this experiment the microbial biomass represented only 5.6±0.7% of the total P in the system, yet played a major role in the accumulation of fertiliser P. The results agree with those of Helal and Sauerbeck (1984), who noted an increase in the specific activity of organic P fractions close to roots growing in ^{32}P-labelled soil. They suggested this may be due to an increase in microbial P, or to an enhanced extractability of P_o compounds. The results presented above lend support to the hypothesis that the microbial biomass associated with roots is effective in accumulating fertiliser P. Brookes _et al_ (1984) have shown that a substantial quantity of P may be held in the biomass of both arable and pasture soils. From a knowledge of the turnover of microbial C in the soils studied, they were able to estimate the turnover of microbial P in the soils. They estimated fluxes of 2.2 to 40 kg ha^{-1} year^{-1}; the higher rates were associated with pasture soils. From Table 19, it can be

seen that the biomass assimilated approximately 14% of the
applied fertiliser P. This equates to an uptake of about 7 kg
P ha^{-1} over the 22 d growth period. If this uptake is extended
to the vegetative growth period of wheat (60-80 d), the flux of
P through the biomass is approximately 25 kg ha^{-1} year^{-1}. The
figures obtained above relate to rhizosphere soil only, and
were obtained under favourable conditions in a growth chamber.
Less fertiliser P would be expected to pass through the biomass
in the field.

4.3.3 P distribution following a drying/rewetting cycle

As the P content of the roots in each box just prior to
drying/rewetting could not be measured, it was calculated from
a knowledge of the P content of the tops and the mean root/top
ratio for P in the soils analysed before drying/rewetting.

$$^jP_{root} = {}^jP_{top} / R$$

where R = mean $^jP_{top} / {}^jP_{root}$ before drying/rewetting, and j =
atomic weight (31, 32 or 33). The percentage of root P lost to
the soil was calculated as follows:

$$\%Loss = (1-(^jP_{root(remaining)} / {}^jP_{root(calculated)})) * 100$$

As expected from the the results of Martin and Cunningham
(1972), the drying/rewetting treatment after harvest of the
tops caused considerable movement of P (67.6±4.3%) out of the
roots and into soil fractions, as evidenced by root ^{31}P data.
Similar results were obtained from ^{32}P data. However
calculations from ^{33}P data showed that 81.2±4.2% root P moved
out of the roots, indicating that root ^{31}P was not uniformly

Table 20. Concentrations of ^{33}P in soil before and after a
         drying/wetting treatment. (M = before
         drying/rewetting, D = dried/wetted)

	Bq ^{33}P μg^{-1} soil		
	M	D	LSD (P<0.05)
0.5M NaHCO$_3$	19.0	185.7	17.0
Hexanol released 0.5M NaHCO$_3$	2.2	-4.1	22.7
0.1M NaOH	0	127.1	21.5
Sonicate/0.1M NaOH	0	12.5	1.8
1.0M HCl	12.2	95.0	10.6
Residual	0	0	0

labelled with ^{33}P. The concentrations of ^{33}P in all soil P
fractions were significantly higher after drying/rewetting. In
the soils which had been dried and rewetted, the amounts of ^{33}P
in the fumigated NaHCO$_3$ extracts were slightly smaller than the
amounts in the unfumigated extracts, and gave rise to the
negative value in Table 20. The difference in ^{31}P
concentration due to fumigation was not significant however.
Of the ^{33}P released by the roots, the largest percentage (43%)
was found in the unfumigated NaHCO$_3$ extract. Although large
and rapid increases in microbial activity accompany rewetting
of soil, no incorporation of root derived P into the biomass
was observed (i.e. no ^{33}P was detected in the biomass). The
microbial P content of the soil following drying/rewetting was
7.1±4.1 μg P g^{-1} soil compared to 22.9±2.5 μg P g^{-1} before
drying/rewetting.

Distribution of ^{31}P and specific activity of ^{32}P in the soil

Table 21. Distribution of ^{31}P following a drying/wetting treatment (M = before drying/rewetting, D = dried/wetted).

Extractant	$\mu g \ g^{-1}$			
	$^{31}P_i$		$^{31}P_t$	
	M	D	M	D
0.5$\underline{M}$ NaHCO$_3$	16.5	23.4***	18.6	25.9***
Hexanol released 0.5$\underline{M}$ NaHCO$_3$xx	8.1	1.2***	9.2	2.8***
0.1$\underline{M}$ NaOH	33.6	33.2ns	74.6	72.3*
Sonicate/0.1$\underline{M}$ NaOH	4.6	3.7***	9.7	8.6**
1.0$\underline{M}$ HCl	nd	nd	98.2	111.6**
Residual	nd	nd	201.2	215.1ns

*, **, *** denotes significance at the 5, 1 and 0.1% level, ns = not significant, nd = not determined.

Table 22. Specific activity of ^{32}P following a drying/wetting treatment (M = before drying/rewetting, D = dried/wetted).

Extractant	Bq ^{32}P μg^{-1} ^{31}P		
	M	D	LSD (P<0.05)
0.5$\underline{M}$ NaHCO$_3$	8.67	4.72	0.18
Hexanol released 0.5$\underline{M}$ NaHCO$_3$	5.85	33.61	15.1xx
0.1$\underline{M}$ NaOH	4.14	3.50	0.13
Sonicate/0.1$\underline{M}$ NaOH	3.48	3.49	0.23
1.0$\underline{M}$ HCl	1.45	1.53	0.15
Residual	0.62	0.81	0.10

xx As hexanol-released P is itself a mean difference, a modified T-test was used (Appendix 1).

were also altered by the drying/rewetting cycle (Tables 21 and 22). The large increase in ^{31}P extracted by $NaHCO_3$ on drying/rewetting the soil was mostly inorganic. A proportion of this P would have originated from dying roots (Martin and Cunningham, 1972) as well as lysed microorganisms (Sparling et al, 1985a). That the microbial population was substantially decreased by the drying treatment and had not recovered by the time measurements were made is clear from the decrease in hexanol released ^{31}P. The observed decreases in aggregate protected (sonicate/NaOH) $^{31}P_i$ and $^{31}P_o$ on drying/rewetting can be attributed to the release of substrates previously protected within aggregates (Adu and Oades, 1978). Increases in the 1.0M HCl extractable $^{31}P_t$ and small increases in residual $^{31}P_t$ can be attributed to reversion of P to more stable forms.

That microorganisms are important in P cycling has often been inferred from observations of net changes in ^{31}P pools in soils subjected to incubations in the laboratory (Chauhan et al, 1981) or to cultivation (Hedley et al, 1982a). The use of radioisotopes offers advantages over this approach, as flows can be identified rather than inferred, although interpretation of the data is complicated by isotopic exchange reactions. In examining the data in Table 22, three possible causes can be considered for any change in specific activity of the P fractions due to drying/rewetting;

    (1) isotopic exchange with unlabelled soil ^{31}P,

    (2) increase of specific activity due to a flow of highly labelled P into the fraction, and

(3) decrease of specific activity due to a flow of

unlabelled (or low activity) P into the fraction.

Isotopic exchange, both during the drying/rewetting period and

during the extraction, would cause ^{32}P to be redistributed

among the soil fractions. Thus ^{32}P would tend to move from the

more labile fractions (0.5$\underline{M}$ NaHCO$_3$ and 0.1$\underline{M}$ NaOH) to the more

stable fractions (1.0$\underline{M}$ HCl and residual P). Decreases in

specific activity of ^{32}P in the former are therefore a

combination of (1) and (3) above, and increases in specific

activity in the latter are a combination of (1) and (2).

However, the large increase in specific activity of the hexanol

released fraction is unlikely to be due to (1). The reasons

for this increase are unclear. It is possible that (a) a pool

of very highly labelled P was used by the recolonising

organisms, or (b) a highly labelled fraction of the biomass

survived the drying/rewetting cycle. It was considered that

(a) could represent the dead root material, but the specific

activity of this material was not sufficiently great (10.4±0.9

Bq ^{32}P μg^{-1} ^{31}P) to account for the very high values of specific

activity of ^{32}P found in the hexanol-released fraction. The

latter explanation (b), seems more likely, and it could also

explain the large decrease in specific activity of the NaHCO$_3$

fraction. The organisms killed would have a low specific

activity, and would cause the large dilution of isotope in this

fraction. It may be concluded from the data that there is a

link between the survival and/or dynamics of growth of

microbial populations in the soil (due to wetting/drying

cycles), and their ability to take up P. Currently there is no

other evidence in the literature to support this hypothesis.

4.4 Summary and conclusions

Losses of P by intact, healthy growing roots of wheat through diffusible exudation were minimal under the conditions used in this study. Microorganisms in the rhizosphere were found to compete with plants for fertiliser P. Over the 22 d growth period, plants took up 12.0% and the microbial biomass 14.1% of the fertiliser ^{32}P applied to the soil at the start of the experiment. On drying and rewetting of the soil after the wheat tops were harvested, a large proportion of root P moved into soil fractions while ^{32}P appeared to accumulate in the biomass and stable forms of P.

CHAPTER 5

TRANSFORMATIONS OF P IN CROPPED SOIL - POT EXPERIMENT

5.1 Introduction

Wheat crops obtain their P from added fertiliser, and from organic and inorganic forms of P in the soil. As mentioned earlier, most of the research effort to date on the residual effect of added fertiliser has concentrated on reactions involving inorganic P in soils. However, where pastures in a cereal/pasture rotation are fertilised a considerable proportion of P may be carried over to the cereal phase in pasture residues. While there are many studies in which P mineralisation and decomposition of organic materials in soils have been examined e.g. Kaila (1954); Thompson et al (1964); Barrow (1960); Birch (1961); Floate (1970a); White and Ayoub (1983), most have not involved plants. The results of Blair and Boland (1978) and Helal and Sauerbeck (1984) have indicated the need to include growing plants in the system under study. Fertiliser P was not included as a treatment in the studies of Blair and Boland (1978) and Dalal (1979), in which uptake of P from residues by plants was examined. In the field, P is normally added with the cereal crop at sowing, and the crop and the soil microbial biomass can obtain P from both the residues and the fertiliser as well as from the soil. From the results of Chapter 4, it appears that the microbial biomass is capable of assimilating large amounts of fertiliser P, although the situation in the rhizosphere is likely to be different from the bulk soil where microbial populations and activity are lower. The aim of this experiment, therefore, was to evaluate the

- 112 -

relative importance of pasture residues and fertiliser as
sources of P for wheat plants. The role of the microbial
biomass in competing with plants for available P was also
assessed.

5.2 Materials and methods

The experiment was conducted in a growth chamber and
involved a short-term rotation sequence due to the short
half-lives of both radioisotopes of P. ^{33}P-labelled medic
(Medicago truncatula cv. Paraggio) residues and ^{32}P-labelled
monocalcium phosphate (MCP) were added to a soil previously
cropped to medic, and a crop of wheat (Triticum aestivum cv.
Warigal) was grown.

The Mallala soil was used for this experiment (Table 18).
MCP was added to the soil as a solution to bring the soil to
-66 kPa water potential and add P at 7.7 μg g^{-1} soil (equivalent
to 10 kg P ha^{-1}). The solution was thoroughly mixed into the
soil using a mechanical mixer to ensure uniform distribution of
fertiliser. Soil equivalent to 1300 g oven-dry weight was
added to each of 12 pots, and eight germinated medic seeds were
placed just below the soil surface in each pot. Plants were
thinned to 5 seedlings per pot after 7 days. Soil water
potential was adjusted daily to -66 kPa. Plants were grown for
53 days in a growth chamber at 20°C (day), 15°C (night),
relative humidity was 65% (day), 95% (night), and PAR was 500
μmol quanta m^{-2} s^{-1} for 15 h day^{-1}.

A second series of pots was similarly treated, except that
carrier free ^{33}P was added to the MCP solution to give a

specific activity of 3.48 GBq g^{-1} ^{31}P.

Medic tops were harvested by clipping at the soil surface.
Roots were separated from the soil by hand picking, as the
roots from the ^{33}P-labelled soil and the root-free unlabelled
soil were to be reused for the wheat phase of the rotation.
There was no difference in growth between unlabelled and
^{33}P-labelled plants. Plant materials were dried at 70°C and
ground to pass a 1.0 mm sieve.

Soil from the pots containing no ^{33}P (i.e. unlabelled medic)
was air dried to approximately 5% gravimetric water content
over a 48 h period. From the plant material grown in
^{33}P-labelled soil, a mixture of ^{33}P-labelled medic residues was
prepared from the tops and roots in the ratio 1:2. The medic
residues were then mixed into the unlabelled soil at a rate of
3.46 g kg^{-1} (oven-dry) soil, which would equate to a carryover
of 1.5 t tops + 3.0 t roots ha^{-1} at the end of the pasture
phase of the rotation. The residues had a P content of 0.11% P
and a specific activity of 89 MBq ^{33}P g^{-1} ^{31}P, and added 3.8 µg
P g^{-1} soil. The soil was wetted to −66 kPa water potential and
incubated for 7 days at 20°C. After being air dried for 48 h
at the end of the incubation period, the soil was rewetted to
−66 kPa water potential with a ^{32}P-labelled MCP solution which
supplied 15.4 µg ^{31}P g^{-1} (oven-dry) soil, and had a specific
activity of 598 MBq ^{32}P g^{-1} ^{31}P. Nitrogen (as NH$_4$NO$_3$) was
added at a rate of 30.8 µg N g^{-1} soil to all pots. Two residue
treatments (with and without residue addition) were combined
with two fertiliser treatments (with and without MCP addition)
in a 2*2 factorial design with threefold replication. The soil

in each pot was mixed thoroughly to ensure uniform distribution of isotopes throughout the soil. Five germinated wheat seeds were placed in pots containing 1 kg soil treated as described above. The pots were placed in the growth chamber maintained under the same conditions as given previously. After emergence was complete, plants were thinned to 4 per pot. After 7 days growth, plants growing in soil amended with residues showed signs of S deficiency. Accordingly all plants were harvested and a basal application of 7.7 mg S, 2.4 mg Mg and trace elements was added to all pots before resowing with wheat. All pots were harvested 34 days after resowing and plants separated into tops and roots. The roots were separated by gentle agitation under water, and washed free of soil with a spray of water over a fine sieve.

Plant materials were weighed after being dried over 24 h at 70°C. The plant material was ground and 0.1 g samples were digested with 6 cm^3 HNO$_3$ and 1 cm^3 70% HClO$_4$.

Subsamples of soil were taken for analyses both before planting and after harvest of the wheat tops. After harvest, but before washing to remove roots, subsamples of soil were removed from each pot with a 14 mm diameter tube. Roots were removed from this soil by hand picking. Soil samples were fractionated and other analyses carried out as described in Section 4.2, except that the fumigated soils were placed in a fume hood for 12 h instead of 24 h to allow evaporation of hexanol.

5.3 Results and discussion

5.3.1 Dry weight and ^{31}P uptake

Addition of fertiliser P increased both dry weight and ^{31}P uptake of wheat plants (Table 23). Addition of residues depressed both dry weight and ^{31}P uptake of wheat, both in the presence and absence of added fertiliser ^{31}P. Amounts of ^{31}P held in the microbial biomass were significantly increased by both residue and fertiliser P addition: the former had the larger effect.

Table 23. Dry weight and ^{31}P uptake of wheat tops and roots, and amounts of microbial P in the soil.

Treatment	Dry weight (g pot^{-1})		P uptake (mg pot^{-1})		Microbial P (μg g^{-1} soil)
	Tops	Roots	Tops	Roots	Soil
Control	1.56	2.13	2.69	2.52	19.2
+Residues	0.86	1.27	1.86	1.64	25.0
+Fertiliser	2.70	4.42	5.30	4.68	21.0
+Res.+Fert.	2.40	3.80	4.35	4.52	25.8
Significant effects (F test) (P<0.05)	Res. Fert. Res.*Fert.	Res. Fert.	Res. Fert.	Res. Fert.	Res. Fert.

The decrease in dry weight and P uptake of wheat in the presence of residues appeared to be due to competition between the plants and the soil biomass for available P. The S deficiency initially observed in wheat plants growing in soils to which medic residues had been added was the first evidence that a flush of microbial activity had occurred. S deficiencies are not normally encountered in the field on this soil type, presumably because P is usually added as

superphosphate which would alleviate both P and S limitations. While no measurements were made of the dynamics of microbial growth and P uptake during the course of the experiment, it can be seen from Table 23 that the amounts of P held in soil microorganisms at the end of the experiment were considerably higher in those soils which had received medic residues. Supporting evidence is provided by the radioisotope data.

5.3.2 ^{32}P and ^{33}P uptake

Of the total P in plants and microbial biomass, the proportion derived from the plant residues and fertiliser was calculated from radioisotope data as follows.

$$Z = SA_x \ / \ SA_y, \text{ where}$$

SA_x = specific activity of P in wheat top, root, or
microbial biomass, and

SA_y = specific activity of P in plant residue or fertiliser
added

Results are presented in Table 24. Addition of residues significantly decreased the proportion of fertiliser P in the plant tops, and increased the proportion of microbial P obtained from the fertiliser. Addition of fertiliser had no effect on the proportion of plant P taken up from the residues, but significantly increased the proportion of biomass P derived from residues.

Table 24. Percentages of total P contained in wheat tops and
roots, and in the biomass derived from plant residues
and fertiliser.

	+Fertiliser	+Residues	+Residues +Fertiliser	LSD P=0.05
		%		
Tops				
^{33}P(Residue)	−	6.7	7.1	0.7
^{32}P(Fertiliser)	40.1	−	35.3	0.6
Roots				
^{33}P(Residue)	−	7.0	6.5	1.4
^{32}P(Fertiliser)	33.9	−	29.6	7.3
Microbial biomass				
^{33}P(Residue)	−	8.1	9.4	0.3
^{32}P(Fertiliser)	16.9	−	17.9	0.9

The distributions at harvest of ^{32}P from the added
fertiliser, and ^{33}P from the residues, are shown in Table 25.
In the absence of residues, a similar amount of ^{32}P (from
fertiliser) was found in plants as was found in the soil
microbial biomass. With residue addition, the biomass appeared
to accumulate ^{32}P at the expense of the plants. By far the
greatest proportion of ^{33}P (from residues) was recovered in the
biomass, both in the absence and presence of fertiliser P.
Fertiliser P appeared to stimulate plant uptake of ^{33}P from the
residues.

Ghoshal (1975a) found that the addition of a C source to
soil decreased the uptake of fertiliser P by rye plants, and
concluded that an increase in microbial activity had caused
immobilisation of fertiliser P. Plant residues provide a
source of C, P and other nutrients.

Table 25. Distribution of isotopes at harvest expressed as percentages of the amounts applied.

		+Fertiliser	+Residues	+Residues +Fertiliser	LSD P=0.05
				%	
^{33}P	Tops	—	4.2	8.3	0.3
	Roots	—	3.9	7.9	1.0
	Microbial biomass	—	68.4	65.3	7.6
^{32}P	Tops	13.5	—	9.7	1.0
	Roots	10.0	—	8.4	0.6
	Microbial biomass	22.6	—	29.2	1.0

Previous studies have found that the P concentration of the residues may be one of the most important factors affecting the uptake of P by plants growing in soils amended with plant residues. Fuller et al (1956) found that native soil P may be immobilised when the added residues contain less than 0.2% P. They found that P from root residues was less readily available than that from tops. Dalal (1979), on the other hand, found the ^{32}P in labelled clover roots to be more available than P from tops as measured by ^{32}P uptake by oats. Dalal measured high rates of recovery (± 40%) of ^{32}P from residues by the plant, although the P content of the clover residues used was high (0.55% P). In this study, an attempt was made to simulate the conditions prevailing in the field under medic-wheat rotations. The fertiliser rate used approximates to that which would be added to a soil with medium-low P status. Roots and tops of medic residues were combined in a mixture, and added at

a rate, which would represent the approximate proportions, and amounts, of these plant parts remaining at the close of season in a grazed pasture. The medic from which the residues were obtained was grown in the same soil as the following wheat crop, and the residues had a P concentration (0.11%) similar to that found in the field (unpublished data). However, it should be remembered that because of the limitation imposed by rapid radioisotope decay, immature plants had to be used for residues in the experiment. In the field, mature plants remaining after grazing contribute the plant material which is carried over to the cereal phase of the rotation, and these may not influence the turnover of P in the system in exactly the same way as found here. The results presented indicate that only small amounts of P from the medic residues were utilised by the following wheat crop, even where no fertiliser P had been added. From Table 24, it can be estimated that the contribution of medic residues to the P nutrition of the wheat plant was about a fifth of that contributed by the fertiliser. This proportion is similar to the relative amounts of P applied to the soil by the two sources - medic residues supplied approximately one quarter of the amount of P applied as fertiliser.

Martin and Cunningham (1972) showed that the release of P from desiccated wheat roots was mainly through autolytic activity of the plant enzymes. Thus without any microbial activity a large amount of inorganic P could enter the plant-available pool when the dry soil was rewetted. Birch (1961), on the other hand, found rapid growth of microorganisms

on rewetted plant materials prevented the release of inorganic P to solution. Inorganic P was detected in significant quantities only after a decrease in the microbial population. The results presented here support those of Birch, as a large proportion of the P from residues was found in the microbial biomass even after 40 days in soil containing P fertiliser and actively growing plants. It is evident that a considerable proportion of the applied ^{32}P was incorporated into the biomass (Table 25), even in the absence of residues. Addition of residues caused the proportion of ^{32}P entering the biomass to increase, while the proportion entering the plant was decreased. These results support the conclusions drawn from simulation modelling (Blair et al, 1976; Cole et al, 1977), and laboratory studies (Hedley et al, 1982a). It remains to be determined whether similar results would be observed under field conditions, where environmental stresses such as desiccation (Sparling et al, 1985b) and predation (Elliott et al, 1984) would favour release of P from the biomass.

In previous studies where the turnover and plant uptake of P has been traced with radiophosphorus (Fuller et al, 1956; Blair and Boland, 1978; Till and Blair, 1978; White and Ayoub, 1983; Sharpley, 1986), isotopic exchange reactions appear to have been discounted during interpretation of the results. However, as soon as radiophosphorus is released in inorganic form from labelled plant material (regardless of the process involved), it becomes liable to isotopic exchange. Thus uptake of P by plants from both residue and fertiliser sources will be underestimated by radiotracer techniques, as discussed

previously in Section 4.3.2.

A large proportion of the ^{33}P recovered was found in the microbial biomass. While fumigation can cause significant release of P from fresh wheat roots and shoots, even a mild drying treatment (50°C for 24 h) causes the flush due to fumigation to disappear, due to extensive damage and release of P from unfumigated materials (Section 3.7). However, to check if fumigation causes significant release of P from dried and ground medic plants, 0.5 g samples of unfumigated and fumigated residues were extracted with 100 cm³ 0.5$\underline{M}$ NaHCO₃. As the fumigated samples must stand for a total of 48 h after being wetted with biocide (Section 5.1), release of P_o through plant enzyme activity is possible immediately after the dried material is wetted. Accordingly some unfumigated residue was treated similarly to fumigated samples, except that it was wetted with 2 cm³ de-ionised water instead of hexanol. All other analyses were as described previously (Section 5.1). Results are presented in Table 26.

Table 26. Percentages of total P in dried medic residues recovered by 0.5$\underline{M}$ NaHCO₃ as P_i and P_t.

Treatment	% total plant P	
	P_i	P_t
Unfumigated	73.8	72.5
Unfumigated (wetted)	81.8	80.0
Fumigated	74.1	77.0
LSD (P = 0.05)	8.0	6.8

Almost all of the P extracted from the plant material, whether
fumigated or unfumigated, was in inorganic form. The
observations that a large proportion of P in plant materials is
soluble in water or bicarbonate following drying support these
results (Bromfield and Jones, 1972; Martin and Cunningham,
1972; White, 1981). There was an increase in the amount of P_i
and P_t extracted from unfumigated residue which had been wetted
with water as described above. Activity of enzymes in the
plant material is a possible mechanism whereby compounds in the
dried material, formerly insoluble in $0.5\underline{M}$ $NaHCO_3$, could be
extracted by this reagent after wetting. There was no
significant increase in the recovery of P from either the dried
or wetted residues due to fumigation with hexanol. Thus it is
unlikely that the fumigation technique caused release of ^{33}P
from the plant residues in this study. The high proportion of
^{33}P found in the biomass may in part be a reflection of the
spatial location of substrates in the soil. ^{33}P would be
closely associated with sources of C and N used by the
microorganisms for growth, while ^{32}P would be physically
removed to some extent from these microsites. On a larger
scale, the competition between plants and the soil biomass for
available P may be affected by the spatial distributions of the
fertiliser and plant residues in the soil. In the experiment,
the P sources were mixed throughout the soil. In the field
plant residues would become mixed into the soil with
cultivation. However, fertiliser P is normally placed in a band
close to the surface during sowing, and this may decrease the
competitiveness of the soil microbial biomass for uptake of

fertiliser P.

5.3.3 Changes in ^{31}P distribution

As expected, addition of fertiliser P caused large increases in ^{31}P extracted by $0.5\underline{M}$ $NaHCO_3$, and subsequent plant growth decreased $0.5\underline{M}$ $NaHCO_3$ extractable P (Tables 27 and 28). While $^{31}P_i$ extracted by $0.5\underline{M}$ $NaHCO_3$ in the control and +residue treatments increased over the plant growth period, there were decreases in organic P ($^{31}P_o = ^{31}P_t - ^{31}P_i$). Small decreases due to plant growth in $^{31}P_i$ and $^{31}P_t$ extracted by $0.1\underline{M}$ NaOH and $0.1\underline{M}$ NaOH following sonification were observed, although the differences were not consistently significant.

Table 27. Changes in $^{31}P_i$ concentration in soil as a result of wheat growth. I = initial values, F = after wheat growth. Means followed by different letters indicate a significant (P<0.05) difference between initial and final values. P_i in $1.0\underline{M}$ HCl and residue fractions not determined.

Extractant	Control		+Fertiliser		+Residues		+Residues +Fertiliser	
	I	F	I	F	I	F	I	F
					μg g^{-1} soil			
$0.5\underline{M}$ $NaHCO_3$	0.3a	2.0b	9.0a	3.3b	0.3a	1.9b	8.4a	2.8b
Hexanol-released P	10.4a	7.2b	7.9a	7.9a	11.6a	8.0b	10.1a	9.5a
$0.1\underline{M}$ NaOH	15.6a	15.1a	20.2a	19.2b	16.3a	15.6b	20.5a	18.4b
Sonicated $0.1\underline{M}$ NaOH	2.2a	2.1a	2.7a	2.4b	2.2a	2.4b	2.6a	2.6a

Table 28. Changes in $^{31}P_t$ concentration in soil as a result of wheat growth. Symbols as for Table 27.

Extractant	Control		+Fertiliser		+Residues		+Residues +Fertiliser	
	I	F	I	F	I	F	I	F
				µg g^{-1} soil				
0.5M NaHCO$_3$	4.9[a]	2.5[b]	10.6[a]	3.8[b]	4.2[a]	2.6[b]	9.9[a]	3.5[b]
Hexanol released P	8.1[a]	7.7[a]	8.3[a]	8.4[a]	12.2[a]	10.0[a]	10.5[a]	10.3[a]
0.1M NaOH	56.3[a]	53.1[b]	63.5[a]	56.3[b]	62.2[a]	56.0[a]	61.4[a]	57.9[a]
Sonicated 0.1M NaOH	7.5[a]	6.7[b]	8.4[a]	7.1[b]	7.5[a]	7.8[a]	8.0[a]	7.4[a]
1.0M HCl	81.9[a]	85.7[a]	92.0[a]	78.6[b]	80.6[a]	79.3[a]	83.4[a]	84.8[a]
Residue	197[a]	189[a]	207[a]	188[b]	197[a]	199[a]	188[a]	196[a]

In soil which had received only fertiliser, there were significant decreases in the $^{31}P_t$ extracted by 1.0M HCl (no $^{31}P_o$ could be detected in this fraction), and in the residue fraction. The results in Tables 27 and 28 cast some doubt on the suitability of the terms commonly used to describe the relative availability of P fractions separated by the technique used in this and other studies. In soil which had received fertiliser only, plant growth depleted $^{31}P_t$ in the residue fraction and that extracted by 1.0M HCl. Hedley et al (1982b) also found that plants were able to draw P from the 1.0M HCl fraction, yet this fraction has been termed "more stable

inorganic phosphorus composed probably of apatites" (Stewart and McKercher, 1982). The basis for this interpretation stems from the results of Williams et al (1980). However, these workers used a slightly different extraction scheme to that proposed by Hedley et al (1982a) and used here, in that a mixture of sodium dithionite, sodium citrate, and sodium bicarbonate at 85°C was employed as the initial step in the fractionation scheme. This mixed reagent (DCB) is designed to extract dicalcium phosphate dihydrate (DCPD), $CaHPO_4.2H_2O$, and was also found to extract most of the P contained in hydroxyapatite (HA), $Ca_{10}(PO_4)_6(OH)_2$ (Williams et al, 1980). Cold $0.5\underline{M}$ $NaHCO_3$ (pH 8.5) is not so vigorous an extractant as DCB at 85°C however, and will extract only small amounts of P contained in DCPD or HA (de Bussetti et al, 1980). It is possible that a water + HCO_3^- anion exchange resin extraction preceding the $0.5\underline{M}$ $NaHCO_3$ extraction, as recommended by Hedley et al (1982a), would dissolve the more soluble Ca phosphates such as DCPD (Wagar et al, 1986). de Bussetti et al (1980) found that Cl^- anion exchange resin was effective in partially dissolving pure DCPD and HA. However, the initial pH of this unbuffered system was 3.5, and extensive dissolution would be expected. Anion exchange resin in the HCO_3^- form tends to increase the pH of acidic soil suspensions and buffer the pH of alkaline soil suspensions (Sibbesen, 1978), so it is unlikely to cause complete dissolution of DCPD or HA in suspensions of calcareous soil. The subsequent $0.1\underline{M}$ NaOH extractions would cause little dissolution of calcium phosphates due to the high pH of the extracting solutions. Thus the $1.0\underline{M}$ HCl solution

would extract some slightly soluble calcium phosphates such as DCPD (pK_{sp} = 6.56) or octocalcium phosphate (OCP; pK_{sp} = 46.91), $Ca_8H_2(PO_4)_6.5H_2O$, as well as sparingly soluble forms such as HA (pK_{sp} = 113.7) or fluorapatite (pK_{sp} = 118.4). DCPD, HA and OCP are all reaction products formed after MCP is added to soils (Sample et al, 1980), and there is evidence that some sparingly soluble phosphates have a high potential availability to plants (Olsen et al, 1983). Thus in the extraction scheme of Hedley et al (1982a) and that used here, the P extracted by 1.0M HCl is likely to contain forms available for plant uptake. Wagar et al (1986) have also used the fractionation scheme of Hedley et al, and found that large additions of P to a slightly alkaline soil resulted in large increases in the P extractable with 1.0M HCl. The increased amounts of P held in the HCl-extractable fraction remained stable even after 8 y of cropping and no further addition of fertiliser. Wagar et al (1986) did not investigate the possible formation of calcium phosphates during extraction of the soil, which contained both free Ca and very large amounts of P. However, they recognised that sparingly soluble, but plant available, forms of P could remain in the soil during fractionation until extracted by 1.0M HCl.

The depletion due to plant growth of forms of P solubilised in the laboratory only by boiling concentrated acids is more difficult to explain however. Hedley et al (1982b) also observed decreases in amounts of residue P due to plant growth. Further investigation by these workers revealed that a large proportion of the residue P is inorganic, and that plant growth

depleted this fraction by decreasing rhizosphere pH and causing dissolution of basic phosphates. Wagar et al (1986) found that wetting and incubation caused measurable changes in amounts of residue P extracted from soils which had been stored air dry, and suggested that microbial activity may have caused the redistribution of P. These workers also suggested that the residue P fraction consists largely of P_o, although no data were provided to substantiate this claim. It would appear therefore that the more chemically resistant fractions separated by this fractionation technique should not be regarded stable in a biological sense.

There is a possibility that basic phosphates may be precipitated during alkaline stages of the extraction process. Indeed, this was the reason Hedley et al (1982b) used a resin pretreatment in the extraction scheme. However it was found in this study that 91% of a spike of inorganic P (25 μg P g^{-1} soil) added to the soil before extraction, was recovered by $0.5\underline{M}$ $NaHCO_3$ (pH 8.5) and a further 6% was found in the $0.1\underline{M}$ NaOH extract. As stated by Jackson (1958), the carbonate ion added in the $0.5\underline{M}$ $NaHCO_3$ (pH 8.5) extract, by the solubility product of $CaCO_3$, maintains the Ca activity low enough in most soils to prevent reprecipitation of liberated phosphate as calcium phosphate.

5.4 Summary and conclusions

The results confirmed those of Chapter 4. The microbial biomass was found to assimilate a proportion of the labelled fertiliser similar to that taken up by plants. A large proportion of the P contained in medic residues was found in the microbial biomass. The results suggest that a large proportion of the available P in soil passes through microbial cells, particularly that P which is released from plant residues in the soil. The effectiveness of plant residues from a preceding pasture in providing P to a wheat crop was less than that of currently applied fertiliser. However, this effectiveness was roughly proportional to the total amounts of P added by the two sources.

CHAPTER 6

TRANSFORMATIONS OF P IN CROPPED SOIL - FIELD EXPERIMENT

6.1 Introduction

The results of Chapter 5 demonstrated that the microbial biomass assimilated a proportion of the applied fertiliser equal to plant uptake, and that a large proportion of the P contained in the medic residues entered and remained in the microbial pool. These results were obtained in a growth chamber where conditions were favourable for growth of both plants and microorganisms for the duration of the experiment. Fertiliser was uniformly mixed throughout the soil. In the field, both plants and microorganisms can suffer from environmental stresses, such as desiccation, excess heat, freezing and predation. Also, sources of P are not uniformly distributed throughout the soil. While plant residues, such as roots and ungrazed or unharvested tops, may be mixed into the soil during cultivation, P fertiliser is generally banded near the soil surface during the sowing of cereals. As the spatial location of P in soil affects plant P uptake (Kafkafi, 1964; Rudd and Barrow, 1973; Engelstad and Terman, 1980; Barry et al, 1985), it is likely that banding of fertiliser will also affect uptake of P by the soil biota. The dynamics of P uptake by the microbial biomass and wheat plants under field conditions was therefore studied.

6.2 Materials and methods

The experiment was conducted at Mallala, situated approximately 60 km north of Adelaide in South Australia (Plate

1e, page 164). The characteristics of the soil have been given previously (Table 18, page 97). The plot used was fallowed in 1985 and maintained weed-free up to the time of planting in 1986. ^{33}P-labelled medic residues and ^{32}P-labelled MCP were applied to soil cropped with wheat.

6.2.1 Preparation of ^{33}P-labelled medic residues

To obtain medic residues with a high specific activity of ^{33}P, plants were grown in sand which had been washed three times in deionised water. To closed pots containing 1.25 kg sand packed to a bulk density of 1300 kg m^{-3} was added a basal application of $CaCO_3$ (3 mg g^{-1}), $CaSO_4$ (200 μg g^{-1}), and $Ca(NO_3)_2.4H_2O$ (16.87 μg g^{-1}). Eight germinated medic (Medicago truncatula cv. Paraggio) seeds were placed 2 cm below the sand surface and a slurry of Rhizobium bacteria applied around the seed. The sand surface was covered to a depth of 20 mm with white polythene beads and the sand watered to 15% gravimetric water content (-50 kPa). Plants were grown for 42 days in a growth chamber at 20°C (day), 15°C (night), relative humidity was 60% (day), 95% (night), and PAR was 500 μmol quanta m^{-2} s^{-1} for 14 h day^{-1}. The water potential of the sand was adjusted daily to -50 kPa. At 0, 14, 23, 30, 35 and 39 days a solution supplying 20.83 mg KH_2PO_4, 8.33 mg $MgSO_4.7H_2O$, 833 μg $FeSO_4.7H_2O$, 833 μg $MnSO_4.4H_2O$, 500 μg $ZnSO_4.7H_2O$, 333 μg $CuSO_4.5H_2O$, 333 μg H_3BO_3, 33.33 μg $CoSO_4.7H_2O$, 33.33 μg H_2MoO_4, and 4.11 MBq (corrected to t = 0) carrier-free ^{33}P was added to each pot. Uniform labelling of the plant material was therefore encouraged by feeding the plants at each time with a

nutrient solution having a specific activity of ^{33}P equal to the specific activity of P already taken up by the plant. The time intervals chosen for application of nutrient solution were determined by the rate of growth of the medic plants (determined in a preliminary experiment).

Medic tops were harvested by clipping at the sand surface. Roots were separated from the sand by gentle agitation under water, and washed free of sand with a spray of water over a fine sieve. Plant materials were dried at 70°C and ground to pass a 1.0 mm sieve. A mixture of the residues was prepared from the tops and roots in the ratio 1:2. The residues had a ^{31}P content of 0.303% and a ^{33}P specific activity of 237.5 MBq g^{-1} ^{31}P (corrected to the time of sowing the wheat crop).

6.2.2 Wheat growth

Wheat plants were grown within open ended pots made from PVC cylinders 150 mm deep, having an inside diameter of 100 mm and driven into the soil to a depth of 100 mm. Soil within the pot was removed, sieved (< 2 mm), and ^{33}P-labelled medic residues added at a rate of 3.46 g kg^{-1} (oven-dry soil). The residues were thoroughly mixed throughout the soil by end over end mixing in a polythene bottle placed in a cement mixer. The soil, except for the last 40 mm topsoil, was then replaced within the PVC cylinder to a bulk density of 1400 kg m^{-3}. To the exposed soil surface was added evenly 40 cm^3 of a solution which supplied ^{31}P (7.69 μg P g^{-1} as $Ca(H_2PO_4)_2$, N (30.7 μg N g^{-1} as NH_4NO_3), S (7.69 μg S g^{-1} as $CaSO_4$), Mg (2.21 μg Mg g^{-1} as $MgSO_4.7H_2O$). The solution also contained ^{32}P to give an

application rate of 4.80 kBq g^{-1} soil, and a specific activity of ^{32}P of 624 MBq g^{-1} ^{31}P added. Trace elements were also applied at rates equivalent to half of the total amounts used for the medic growth phase (Section 6.2.1). Two germinated wheat (<u>Triticum</u> <u>aestivum</u> cv. Warigal) seeds were placed on the exposed surface and the remaining top 40 mm soil was packed into the cylinder. Thus the ^{33}P in the residues was evenly distributed throughout the soil, while ^{32}P-labelled fertiliser was concentrated in a band 40 mm below the soil surface (Figure 15).

A total of 27 cylinders was placed in the soil as described above, within a plot area of 1.6 m * 1.2 m. The cylinders were placed in groups of three as shown in Figure 16 and Plates 1 e, f and g (page 164). Soil outside the cylinders (guard areas) was also sown with wheat and received the same amounts of nutrients as soil within the cylinders. No radioisotopes were applied to soil in the guard areas. Soil temperature at 60 mm, weekly rainfall and evaporation at the site were continuously monitored during the experiment. Daily rainfall data were obtained from the nearest recording station approximately 1 km from the site. The plot was fenced to prevent rabbit, sheep or bird grazing, snail bait was laid, and the soil was maintained weed-free by hand weeding. The wheat plants were thinned to one per cylinder after emergence. Due to the very dry start of the 1986 season the plot was irrigated at 2, 5, 25 and 68 days after planting to prevent water stress.

At 0, 7, 18, 32, 46, 61, 81 and 95 days a group of 3 cylinders was removed from the plot and transported to the
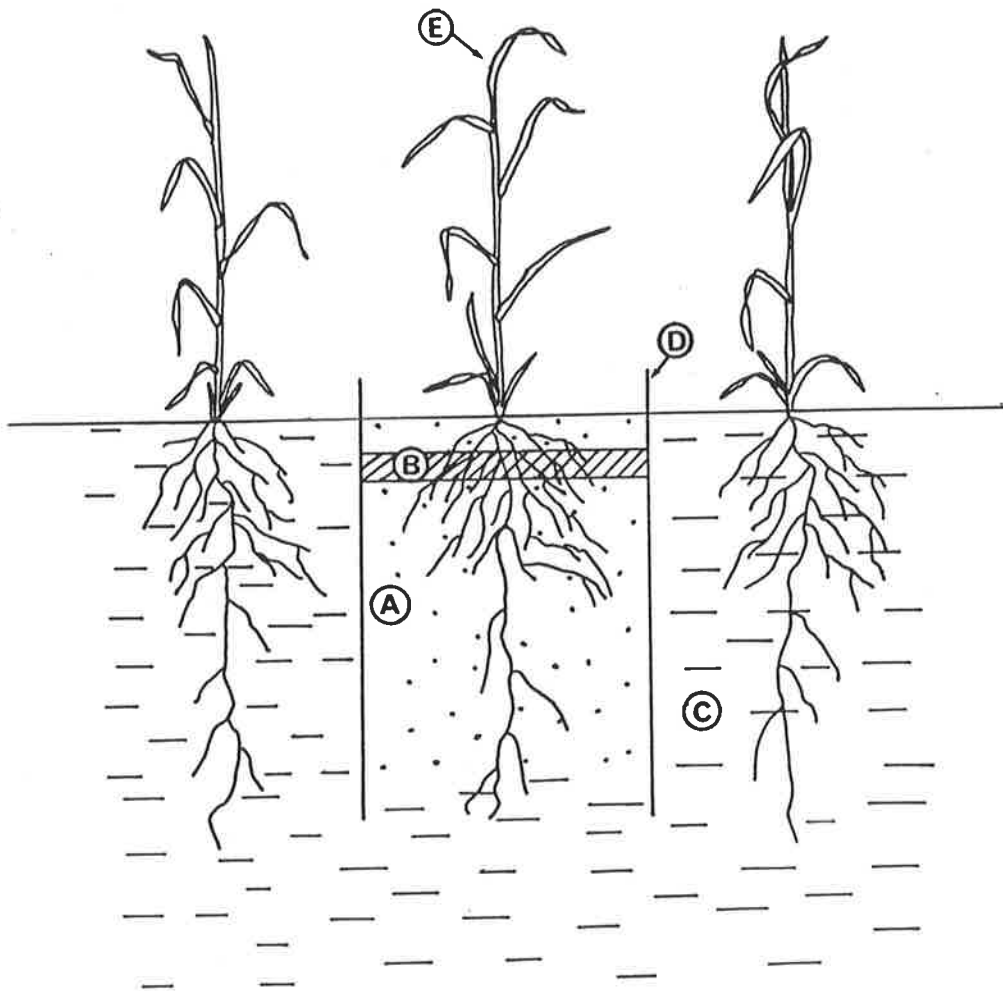
Figure 15. Cross section of open-ended pots used in the field experiment and distribution of isotopes. A = ^{33}P-labelled soil (+residues), B = ^{32}P-labelled soil (+fertiliser), C = unlabelled soil, D = PVC cylinder and E = wheat plant.
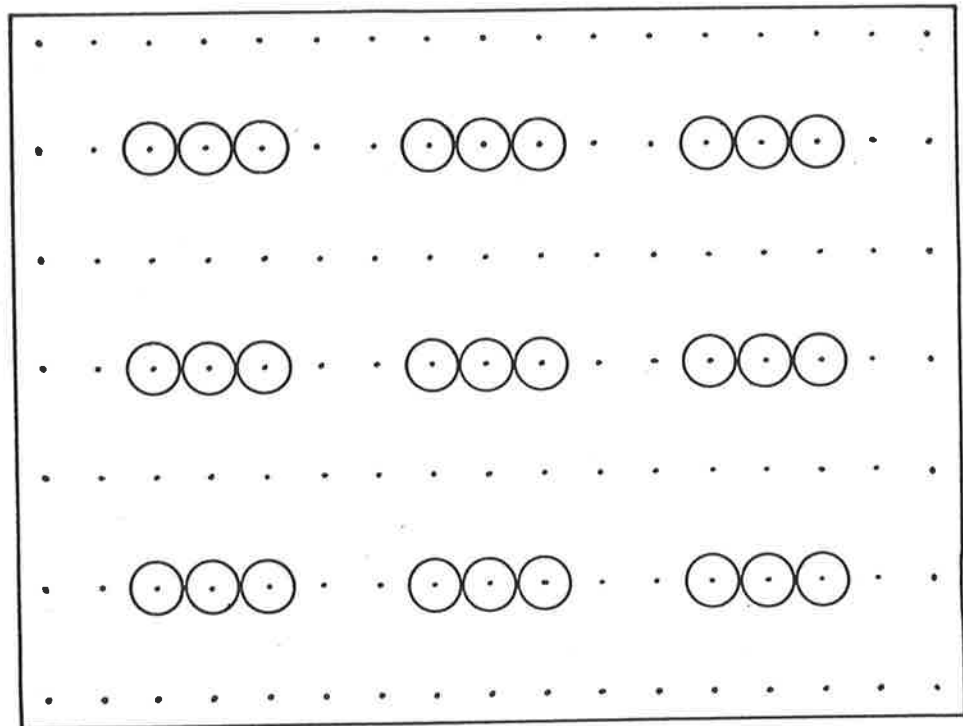
·   **Wheat plant**

⊙   **PVC cylinder**

20 cm

Figure 16. Plan of experimental plot.

laboratory for sampling and analysis. At each sampling time unlabelled soil was added to the holes created by removal of the cylinders so that soil conditions would not be drastically changed for the plants in the remaining cylinders.

6.2.3 Sampling and analyses

Wheat tops and roots were harvested, and soil samples to 100 mm depth were taken as described in Section 5.2. Microbial P was determined on the soil samples as described in Section 3.9, except that sorption of the $^{31}P_i$ and sorption and/or isotopic exchange of the ^{32}P and ^{33}P released by fumigation were measured. Sorption of $^{31}P_i$ was determined by adding a spike of 10 μg $^{31}P_i$ g^{-1} soil to an unfumigated portion of the sample before extraction, and calculating the proportional recovery of added P. An amount of 10 μg P g^{-1} soil was chosen for the P spike, as the P flush measured at this site during the 1985 season varied from 8 to 12 μg P g^{-1}. Correction for sorption and/or exchange of the isotopes was more complex. As the disappearance of isotope from solution (or the appearance of isotope in solution) depends not only on the isotopic concentration, but also on the specific activity of the solution P, correction for isotope recovery cannot be calculated from the disappearance of an added spike of isotope. For example, if a single spike of ^{33}P was added to the soil, a proportion of the isotope was not recovered by the extracting solution. However, if the same amount of ^{33}P was added, but with a quantity of ^{31}P also (i.e. lower specific activity), then desorption of ^{33}P from the soil was observed as ^{31}P

displaced ^{33}P already present on some of the exchange sites.
Thus if P released from the microbial biomass by fumigation has
a low specific activity this can cause desorption of isotope
from the exchange sites on the soil surfaces, increase the
observed concentration of isotope in the solution, and thus
overestimate the amount of isotope in the microbial pool. The
following experimental strategy was therefore adopted to
measure if appreciable errors existed due to adsorption or
desorption of isotope from exchange sites. To 5 g (oven-dry
weight) portions of unfumigated soil from each cylinder were
added 0.5 cm^3 solution containing 50 μg ^{31}P and a range of ^{32}P
and ^{33}P concentrations. This produced a series of simulated P
flushes with a range of specific activities from 0 to 410 Bq
μg^{-1} ^{31}P for ^{32}P, and from 0 to 96 Bq μg^{-1} ^{31}P for ^{33}P. The
difference (in Bq g^{-1} soil) between the recovery of isotope
from the unfumigated soil only and the unfumigated soil plus
added isotope (Δ^{32}P or Δ^{33}P) could then be related to the
specific activity of the released P.

Total and inorganic P were measured by the method of Walker
and Adams (1958). Organic P was calculated from the difference
between total and inorganic P.

6.3 Results and discussion

6.3.1 Plant growth and P uptake

Dry weight, and ^{31}P, ^{32}P, and ^{33}P uptake by the plant roots
and tops are shown in Table 29. At the first harvest the
plants were too small to be separated into roots and tops and
therefore the whole plant was digested.

Time (d)	Dry matter yield (mg pot^{-1})		^{31}P uptake (µg pot^{-1})		^{32}P uptake (kBq pot^{-1})		^{33}P uptake (kBq pot^{-1})	
	R	T	R	T	R	T	R	T
7	22 (3)		83 (13)		0.7 (0.3)		0.1 (0)	
18	22 (2)	22 (0)	38 (3)	76 (3)	4.1 (1.7)	8.1 (4.4)	0.8 (0.2)	1.3 (0.6)
32	102 (4)	72 (7)	125 (10)	132 (9)	18.9 (2.7)	22.2 (5.7)	1.9 (0.2)	2.0 (0.3)
46	278 (16)	267 (7)	-#	-	-	-	-	-
61	341 (43)	333 (31)	479 (57)	720 (58)	57.8 (6.8)	136.9 (6.9)	10.3 (1.7)	16.8 (3.2)
81	439 (11)	524 (25)	794 (8)	1510 (118)	89.0 (4.3)	226.0 (5.1)	25.3 (2.3)	50.0 (5.2)
95	1137 (204)	1527 (299)	2046 (215)	4285 (386)	129.3 (22)	485.0 (83.7)	47.3 (8.3)	101.0 (18.4)

Table 29 Dry matter yield, ^{31}P, ^{32}P and ^{33}P uptake by wheat roots and tops. Values are the means of three replicates. Standard errors of mean values in parentheses. T = tops. R = roots. # = an accident during digestion caused the loss of all P data for this harvest.

Dry weight of roots during the early stages of growth equalled
that of the tops, but growth of the tops exceeded that of the
roots after 61 days. This agrees with the recently published
results of Roemer and Schilling (1986). When total dry weight
and ^{31}P uptake are plotted against time, it can be seen that
both these increased greatly towards the end of the experiment
(Figure 17), by which time the plants had reached a stage of
growth equivalent to Feekes scale 7 (Large, 1954).

Uptake of ^{32}P was much more variable than ^{33}P uptake,
especially during the early stages of growth. This was
expected as the ^{33}P was uniformly distributed throughout the
soil, while the ^{32}P was applied as a solution to the soil and
completely uniform distribution across the cylinder could not
be ensured. Thus in the early stages of growth ^{32}P uptake by
the plant would be influenced to a large extent by root
distribution. As the plants matured and root mass in the pots
increased, the plant roots effectively integrated the uneven
isotope distribution, thus decreasing variability between
plants. The trend of radioisotope uptake by whole plants was
similar to ^{31}P uptake (Figure 18a). At 95 days after planting
11.6% and 5.4% of the applied ^{32}P and ^{33}P had entered the wheat
plants. These figures are smaller than those obtained in the
growth chamber experiment (after 34 days growth) where 18.1%
and 19.1% of the ^{32}P and ^{33}P applied had entered the plants.
Uptake of P from the residues in the field was lower than
observed in the growth chamber — the P concentration of the
residues used in the field experiment was almost 3 times that
used in the pot experiment, yet uptake of ^{33}P by the
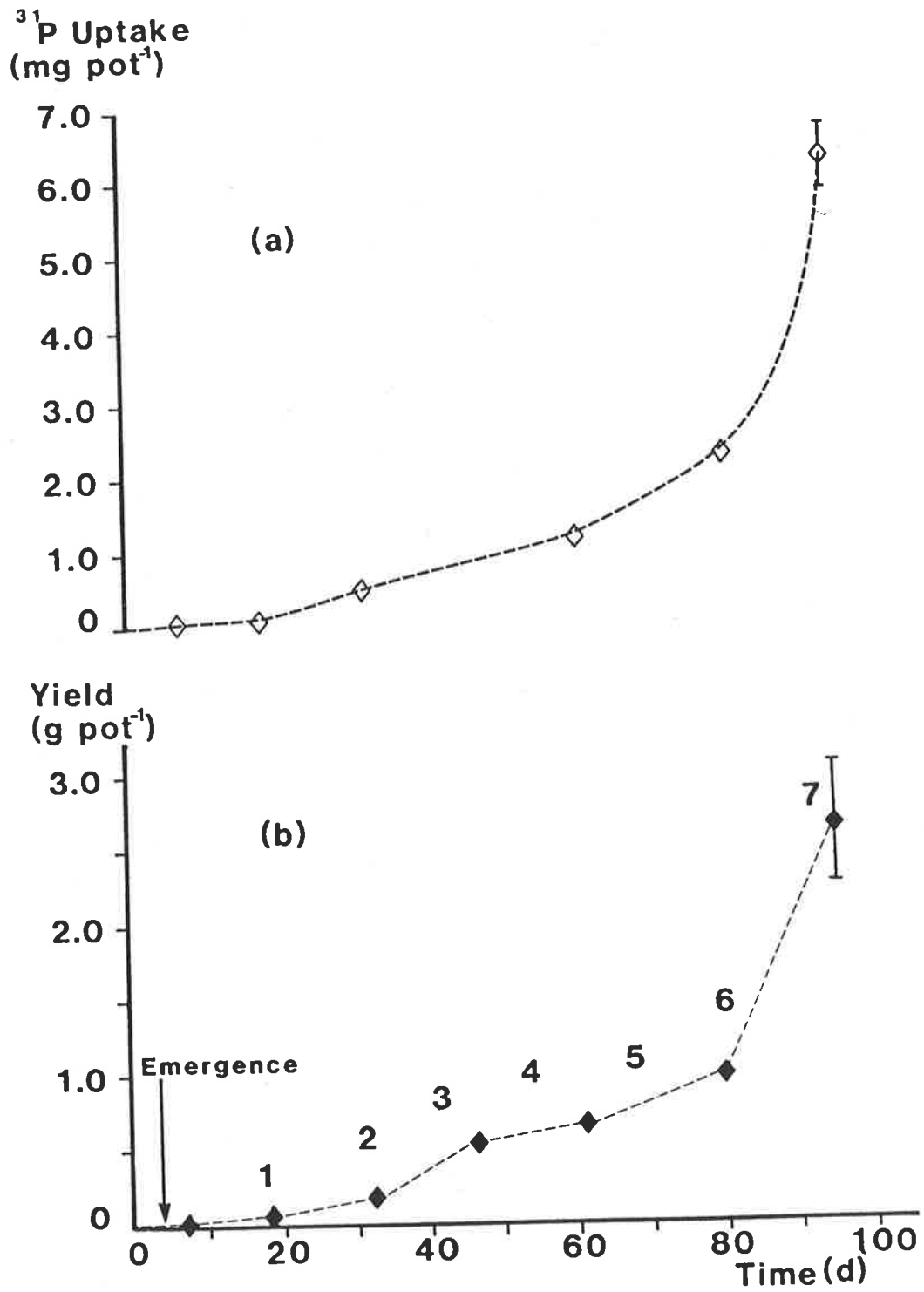
Figure 17. (a) ^{31}P uptake by wheat.
(b) Dry matter yield of wheat.
I = SE of mean values in this and subsequent figures (not shown where SE is less than area covered by the symbol).
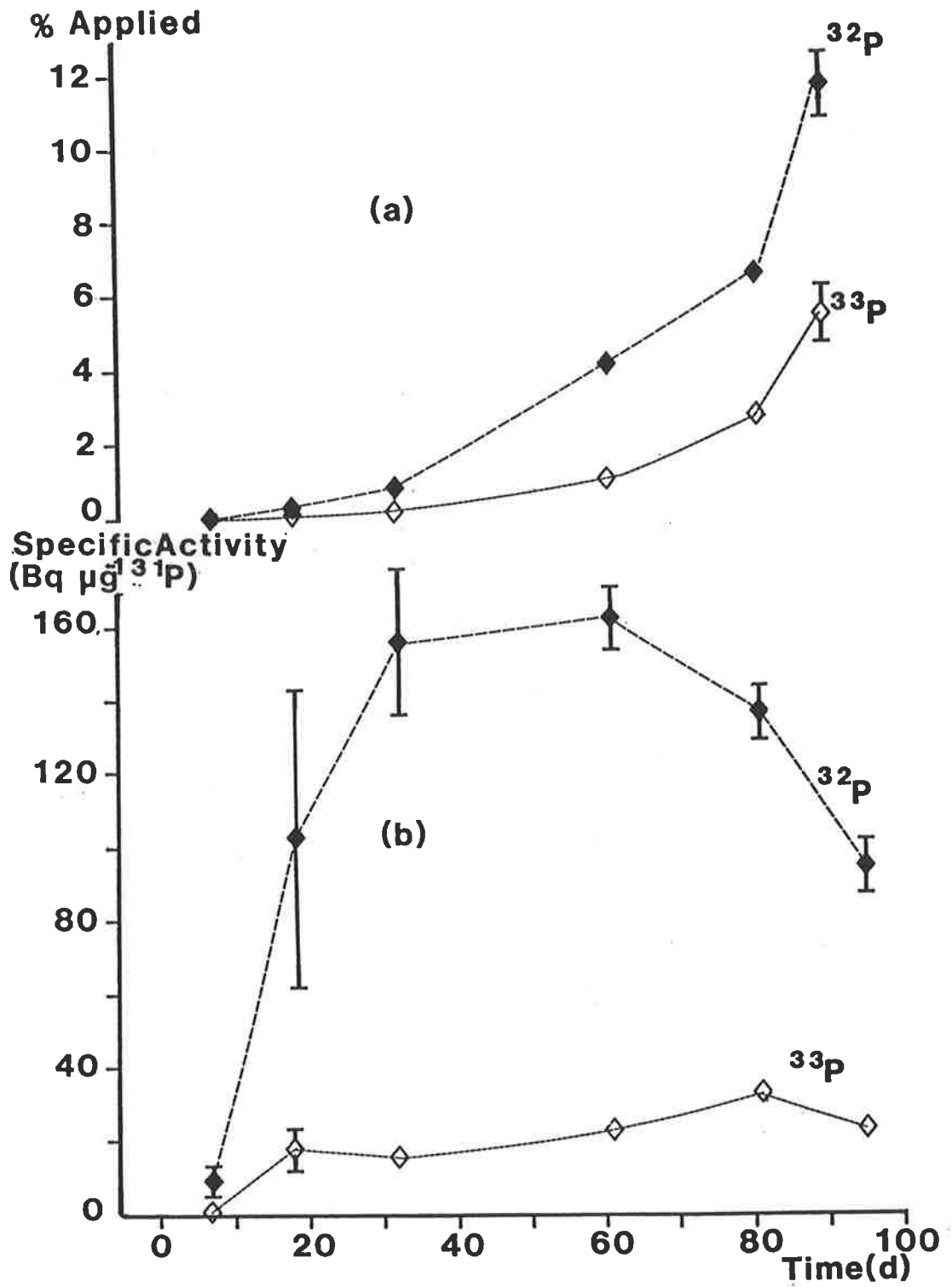
Figure 18. (a) Percentages of applied isotopes
recovered in the wheat plants.
(b) Specific activities of ^{32}P and ^{33}P in
the wheat plants.

MEASUREMENT OF PHOSPHORUS IN THE SOIL MICROBIAL BIOMASS: A MODIFIED PROCEDURE FOR FIELD SOILS

M. J. McLaughlin and A. M. Alston

Department of Soil Science, Waite Agricultural Research Institute, The University of Adelaide,
Glen Osmond, South Australia 5064

and

J. K. Martin

C.S.I.R.O., Division of Soils, Glen Osmond, South Australia 5064

Summary—A range of gaseous, liquid and vapour biocides was tested in combination with seven extractants for their ability to release P from soil microorganisms *in situ*. The biocides tested were chloroform, ethanol, propan-1-ol, hexan-1-ol, β-propiolactone, formaldehyde, glutaraldehyde, ethylene oxide and methyl bromide. The extractants tested were 0.5 M NaHCO$_3$ (pH 8.5), 0.1 M NaHCO$_3$ (pH 8.5), 50 mM NaOH, 10 mM CaCl$_2$, 50 mM H$_2$SO$_4$, 30 mM NH$_4$F + 0.1 M HCl, and an anion exchange resin in the bicarbonate form suspended in distilled water. An incubation technique using ^{32}P ensured that only microbial P was measured.

Chloroform and hexanol were the most effective biocides: the latter is preferred because of its less hazardous nature. The best extractant was 0.5 M NaHCO$_3$ (pH 8.5). Mixed populations of soil organisms were used for calibration, and the K_p factors obtained with hexanol and 0.5 M NaHCO$_3$ (pH 8.5) were 0.33, 0.40 and 0.57 for the three soils studied.

Since microflora differ from soil to soil, as do the amounts and form of P released, calibration is necessary for each soil. Incubation is not recommended as a prior treatment for samples used to measure biomass P: the soils should be treated with hexanol or extracted immediately after sampling to avoid quantitative or qualitative changes in the biota or biomass. Errors associated with the inclusion of roots in the sample can be minimized by removing the bulk of the roots before fumigation.

INTRODUCTION

That microorganisms are important in the transformations of organic and inorganic forms of phosphorus (P) in soils has long been recognized. Renewed interest in the role of microorganisms in P cycling developed through the use of simulation models (Halm *et al.*, 1972; Blair *et al.*, 1976; Cole *et al.*, 1977; Chapin *et al.*, 1978). Subsequent indirect estimates of P held in soil microorganisms (biomass P) showed that relatively large amounts could be held in this form (Anderson and Domsch, 1980). Direct estimates of biomass P were made possible with the development of techniques to extract and measure this pool in soils (Brookes *et al.*, 1982; Hedley and Stewart, 1982). The techniques utilize chloroform as the biocidal agent—a feature common to many methods designed to measure nutrients held in the biomass (Jenkinson, 1976; Jenkinson and Powlson, 1976). However, unlike measurements of biomass C and N by the fumigation–incubation technique, where incubation of soil with inoculated organisms is required after fumigation, measurements of biomass P do not require that all traces of biocide be removed from the soil. Indeed, as Brookes *et al.* (1982) point out, any biocide remaining in the soil will help prevent microbial growth during the subsequent extraction. Thus a wide range of highly efficient clinical and industrial biocides could be used for measure-

ments of biomass P (or C, N and S) in soils by fumigation–extraction methods.

A disadvantage of current techniques for biomass P measurement when applied to field soils is that an incubation period is used before fumigation ("pre-incubation"). The method of Hedley and Stewart (1982) uses dried, ground, rewetted and incubated (21 days) soil while that of Brookes *et al.* (1982) uses a 10 day incubation of sieved field-moist soil. Both of these incubation procedures may cause qualitative and quantitative changes in the soil biota and hence affect biomass P values.

We have tested a wide range of biocides and extractants and describe a modified procedure for measurement of biomass P in field soils.

MATERIALS AND METHODS

Soils

Three soils of different texture, pH and moisture characteristics were chosen (Table 1). As biomass P in field soils varies with time and stable values were required for comparative studies in the laboratory, the soils were treated initially according to the method of Brookes *et al.* (1982) and incubated at a water potential of −66 kPa for 21 days at 20°C before use.

Table 1. Description of soils

	Roseworthy	Inman Valley	Mallala
Sand (%)	88	51	55
Silt (%)	7	28	24
Clay (%)	5	21	21
pH_w (1:5)	8.5	6.0	8.3
Gravimetric water			
at -66 kPa (%)	7.6	22.4	23.8
Organic C (%)	0.73	2.80	1.50
Organic N (%)	0.09	0.25	0.17
P^1 extracted by			
0.5 M NaHCO$_3$ (pH 8.5) P$_i$	4.6	2.0	16.6
P$_t$	7.0	19.0	21.4
Land use	Cultivated wheat–pasture	Native pasture	Cultivated wheat–pasture

[1]Air dry ground soil, pre-incubated for 14 days at 20°C and -66 kPa water potential. The soil:solution ratio was 1:20 and 16 h extraction was used.

Biocides

A range of vapour, liquid and gaseous biocides was used—chloroform (CHCl$_3$), ethanol (C$_2$H$_5$OH), propan-1-ol (C$_3$H$_7$OH), hexan-1-ol (C$_6$H$_{13}$OH), β-propiolactone (C$_2$H$_4$O$_3$), formaldehyde (CH$_2$O), glutaraldehyde (C$_5$H$_8$O$_2$), ethylene oxide (C$_2$H$_4$O) and methyl bromide (CH$_3$Br). Ethylene oxide and methyl bromide, being gases at room temperature, were introduced into evacuated desiccators containing the soils to be treated. Gas pressure was adjusted to 90 kPa. Glutaraldehyde was combined with NaHCO$_3$ extractant as an 0.25% aqueous solution (Borick *et al.*, 1964). The remaining biocides were used both in vapour form according to the method of Brookes *et al.* (1982), and as liquids (2 ml per 5 g oven-dry soil equivalent). The water miscible alcohols (ethanol and propan-1-ol) were diluted to give 70% aqueous solutions—this being the most effective concentration against organisms in their natural or dried states (Hamilton, 1971). All soils were fumigated for 36 h at 20°C before extraction. Vapour and gaseous biocides were removed by repeated evacuation of the desiccator, and liquid biocides were allowed to evaporate for 24 h in a fume hood.

Extractants

Seven extractants were used—0.5 M NaHCO$_3$ (pH 8.5), 0.1 M NaHCO$_3$ (pH 8.5), 50 mM NaOH, 10 mM CaCl$_2$, 50 mM H$_2$SO$_4$, 30 mM NH$_4$F + 0.1 M HCl (Bray and Kurtz, 1945) and an anion exchange resin in the bicarbonate form suspended in distilled water (Sibbesen, 1977). A 1:20 soil solution ratio was used throughout, and soils or organisms were shaken (end-over-end) for 30 min or 16 h at 20°C.

Isotopic incubation technique

An incubation technique using ^{32}P was adopted to ensure that any "flushes" of P measured by a particular combination of biocide and extractant could be attributed to P held only within soil microorganisms. Isotopically-labelled Tryptic Soy Broth (TSB) was added to field-moist soils at a rate which supplied (g^{-1} oven-dry soil) 680 μg C, 225 μg N, 60 μg P, 5 kBq ^{32}P and water sufficient to bring the water potential to -66 kPa. TSB was chosen as it is similar to soil extract media in terms of microbial response (Martin, 1975). When microbial activity, as determined by CO$_2$ evolution, had reached a peak (24–36 h

at 20°C) the soils were fumigated and extracted. Any increase in recovery of ^{32}P from fumigated treatments over controls could be attributed solely to lysis and extraction of P from soil microorganisms *in situ*.

Calibration—K_P determination

Soil bacterial populations were cultured by adding 1 ml of a 10^4 soil dilution to 750 ml 0.3% TSB containing 100 mg cycloheximide. Organisms were cultured in the dark for 5 days at 18°C in unshaken flasks and harvested by centrifugation (10,000 g) for 20 min at 4°C. The organisms were repeatedly washed in dilute saline, and were resuspended for introduction into soils. Soil fungal populations were cultured by adding 1 ml of a 10^1 soil dilution to 750 ml solution containing (l^{-1}) 660 mg NaNO$_3$, 330 mg KH$_2$PO$_4$, 165 mg KCl, 165 mg MgSO$_4$ 7H$_2$O, 6.6 mg FeSO$_4$, 165 mg yeast extract, 10 g sucrose, 100 mg streptomycin sulphate and 5 mg tetracycline hydrochloride. Organisms were cultured in the dark for 5 days at 20°C in unshaken flasks and harvested by vacuum filtration (2.5 μm Nytrel Ti fabric). The fungal mat was thoroughly mixed and washed in dilute saline, and blotted dry before subsampling and introduction into soils.

Soil bacterial suspensions (1 ml) or fresh fungal hyphae (approx. 70 mg) were added to soil, fumigated and extracted as described above. Separate subsamples were digested using nitric and perchloric acids, and were analysed for total P. Percentage recovery of microbial P was calculated after subtracting the P released from fumigated soil without added organisms (Brookes *et al.*, 1982).

Correction for sorption

Three methods to correct for sorption of released inorganic P (P$_i$) were tested. In the first, a standard "spike" of 20 μg P g^{-1} soil was added and the percentage recovery of added P determined (Brookes *et al.*, 1982). In the second, a range of P additions (0.3–20.0 μg P g^{-1} soil) was used and a sorption isotherm constructed. In the third method, which involved isotopic dilution, 10 kBq ^{32}P both with or without carrier ^{31}P (0.2 μg ml^{-1}) were added to suspensions and labile ^{31}P$_i$ (solid + solution) calculated (Talibudeen, 1957). To minimize microbial uptake of P in control soils a 2 h shaking period was used and the isotope was added after 1 h of shaking.
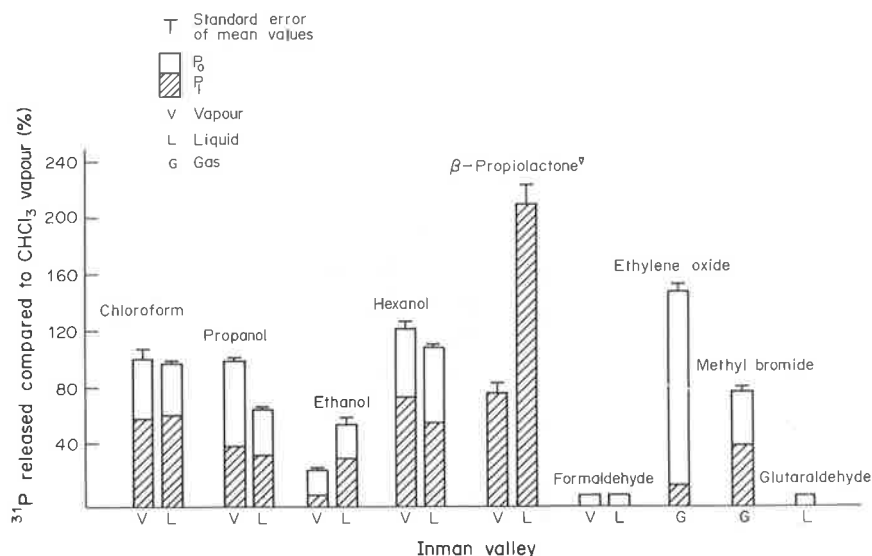
Fig. 1. Effect of biocides on the release of $^{31}P_i$ and $^{31}P_t$ from the Inman Valley soil compared to chloroform vapour (= 100%). The extractant was 0.5 M $NaHCO_3$ (pH 8.5). $\nabla = {}^{31}P_t$. $^{31}P_i$ not determined due to intense coloration of the extract.

Influence of plant root material

In previous studies, plant root and shoot materials were found to influence the values obtained for microbial P using the chloroform fumigation technique (McLaughlin and Alston, 1985). To determine if biocides other than chloroform had the same effect, ^{32}P-labelled wheat roots were fumigated according to the methods of McLaughlin and Alston (1985). The effectiveness of hand-picking of samples to remove roots, and the associated errors in microbial P measurements were also determined. Fresh ^{32}P-labelled wheat roots (470 mg) were intimately mixed with a weight of freshly sampled Mallala soil equivalent to 100 g oven-dry weight before the soil was sieved (<2 mm) and the roots picked out. Subsamples were then extracted directly, or were fumigated and extracted, and the ^{32}P activities in the extracts determined by Cerenkov counting.

Influence of prior incubation

Freshly sampled, sieved (<2 mm) and root-free Mallala soil was either immediately fumigated and extracted as above or stored moist at 20°C in large sealed containers over soda lime for up to 32 days before fumigation and extraction.

Analyses

The ammonium molybdate–ascorbic acid method of Murphy and Riley (1962) was used to determine $^{31}P_i$ in the filtered (Whatman No. 42) extracts. Extracts containing HCO_3^- ions were first treated to remove CO_2 and those containing fluoride ions were treated with boric acid before colorimetric analysis (John, 1970). Total ^{31}P ($^{31}P_t$) in the extracts was determined colorimetrically after digestion with perchloric acid in the presence of $MgCl_2$ (Brookes and Powlson, 1981).

Amounts of ^{32}P in the extracts were determined by Cerenkov counting. All counts were corrected for quenching and decay.

RESULTS AND DISCUSSION

Effectiveness of biocides

Flushes of $^{31}P_i$ and $^{31}P_t$ following treatment of the Inman Valley soil with the various biocides and 0.5 M $NaHCO_3$ (pH 8.5) are shown in Fig. 1.

Results for the Roseworthy soil (not shown) were similar except that β-propiolactone vapour was much more effective than the liquid form on this soil. Liquid biocides were generally more effective than vapour forms in the Roseworthy soil with no such trend being evident for the Inman Valley soil. The proportion of $^{31}P_i$ as a percentage of $^{31}P_t$ varied from 2% for ethanol vapour to 72% for hexanol vapour in the Inman Valley soil. Chloroform released 57% of $^{31}P_t$ as $^{31}P_i$ in this soil which is lower than the average values found by Brookes et al. (1984).

Glutaraldehyde, formaldehyde and ethanol vapour treatments all produced small flushes of P. Formaldehyde has been found to be an effective sterilant in soils (Dalton and Hurwitz, 1948) although Phillips (1977) warns that formaldehyde does not penetrate well into porous surfaces. Formaldehyde gas also hydrolyzes readily in water and hence its penetration into moist soils could be poor. Glutaraldehyde is twenty times as effective as formaldehyde as a bactericidal and sporicidal agent (Hamilton, 1971). However, glutaraldehyde may leave cell membrane and wall structures intact (Labeda et al., 1975) thus protecting the cell contents against release by the extractant. This could explain the very small P flushes observed in both soils when glutaraldehyde was used as the biocide.

In both soils hexanol and β-propiolactone produced flushes of P which exceeded that of chloroform. Hexanol has not been widely used as a bactericide or fungicide although it is recognised that the effectiveness of aliphatic alcohols increases with chain length and reaches a maximum at 5–8 C atoms (Hamilton, 1971). This trend in effectiveness was
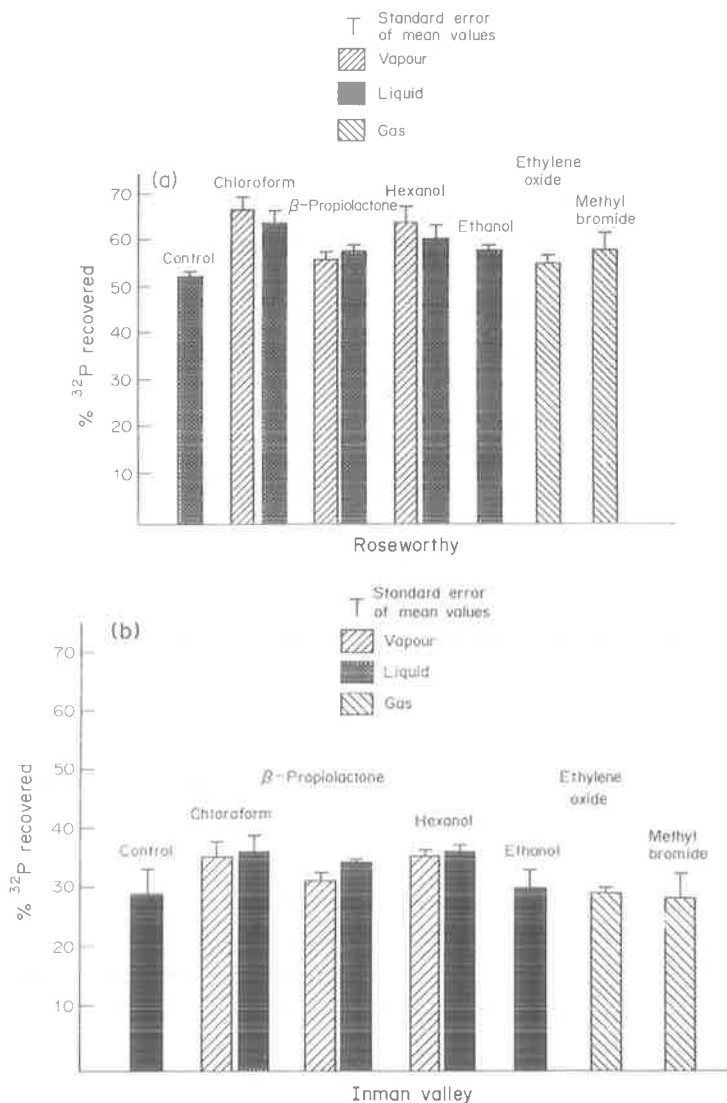
Fig. 2. Effect of biocides on the recovery of ^{32}P from (a) Roseworthy and (b) Inman Valley soils amended with labelled tryptic soy broth and incubated until microbial activity peaked. The extractant was 0.5 M NaHCO$_3$ (pH 8.5).

evident in the data: observed P flushes increased in the order—ethanol < propanol < hexanol. Hedley and Stewart (1982) tested pure ethanol and propanol as fungicides in soil and found them to be less effective than chloroform. β-propiolactone is highly active against vegetative bacteria, bacterial spores, fungi and viruses (Hoffman, 1971; Phillips, 1977), but it can also react with many complex compounds so that non-biomass P could also be released. The incubation technique with ^{32}P labelling was adopted to ascertain which of the biocides was most effective in releasing biomass P only. The results for the Roseworthy and Inman Valley soils are shown in Fig. 2.

Recoveries of ^{32}P were lower from the Inman Valley than from the Roseworthy soil. This can be attributed to the greater ^{31}P content and finer texture of the former permitting greater isotopic exchange and sorption respectively. In contrast to the data for ^{31}P no large ^{32}P flushes occurred when β-propiolactone was used. This suggests that a large proportion of the observed ^{31}P flush came from sources other than the added biomass. As β-propiolactone hydrolyzes rapidly in water to form hydracrylic acid, a proportion of the ^{31}P released may have originated in basic phosphates and been released by dissolution. Ethylene oxide and methyl bromide, both commonly used for soil sterilization, were ineffective in rendering biomass P extractable. Hexanol and chloroform gave the largest flushes of ^{32}P: differences between these two biocides and between the vapour and liquid forms were insignificant. The liquid forms of chloroform and hexanol were therefore chosen as the biocidal treatments for evaluation of extracting agents.

Effectiveness of extractants

The recoveries of ^{32}P by the different extractants from the Inman Valley soil are shown in Fig. 3. Results for the Roseworthy soil were very similar
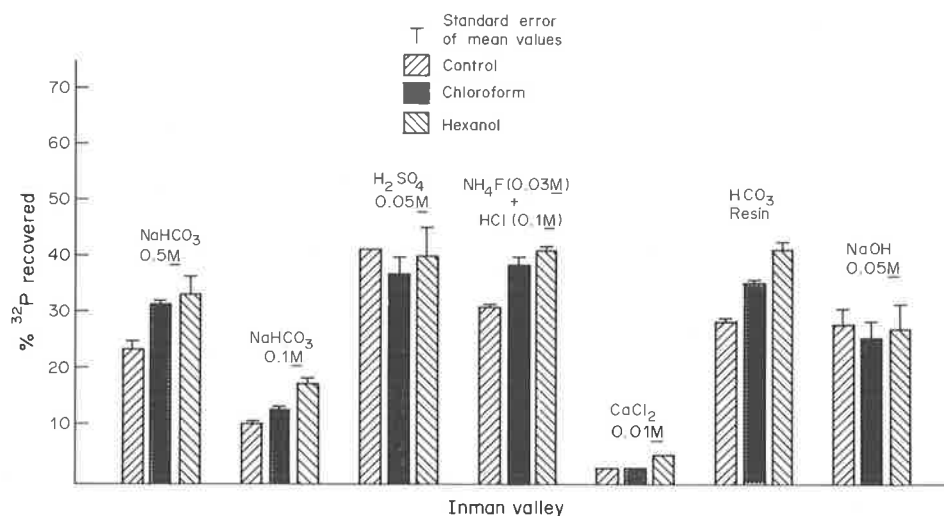

Fig. 3. Effect of extractants on the recovery of ^{32}P from Inman Valley soil amended with labelled tryptic soy broth and incubated until microbial activity peaked. Both hexanol and chloroform were used as biocides.

except that recoveries were higher for the reasons already noted.

The ^{32}P flushes extracted with 10 mM CaCl$_2$ were small or insignificant. Williams and Sparling (1984) also found CaCl$_2$ to be a poor extractant for detecting P flushes from fumigated mineral soils. Both 50 mM NaOH and 50 mM H$_2$SO$_4$ were also unsatisfactory extractants for detecting biomass P released by the biocidal treatment.

The other extractants—30 mM NH$_4$F + 0.1 M HCl, 0.5 M and 0.1 M NaHCO$_3$ (pH 8.5) and HCO$_3^-$ resin— all gave relatively low control values and large increases in extractable P on treatment with chloroform or hexanol. Hexanol produced greater ^{32}P flushes than did chloroform although the differences were generally not significant. As chloroform is carcinogenic and requires more care in handling than hexanol, the latter was chosen as the biocide for all further investigations.

Effect of extraction time

In initial studies, prolonged shaking times resulted in large releases of P from live bacterial and fungal cells. For example, increasing the shaking time from 30 min to 16 h increased the recovery of ^{31}P$_t$ by 0.5 M NaHCO$_3$ (pH 8.5) from live fungal cells from 19.2 $\pm$ 2.0% to 30.8 $\pm$ 2.5% of total cell P. The corresponding increase for bacteria was from 20.9 $\pm$ 6.9% to 50.7 $\pm$ 2.2%. The effect of shaking period on extraction of P from dead cells (i.e. those treated with hexanol or chloroform) was insignificant. The two published methods for measuring biomass P (Brookes *et al.*, 1982; Hedley and Stewart, 1982) differ in the duration of extraction used. Hedley and Stewart (1982) found increased damage to live organisms after prolonged extractions but this was offset by greater release of P from chloroform-treated organisms and they therefore recommended a 16 h extraction. Brookes *et al.* (1982) found no increase in P flush by prolonging extraction beyond 30 min. We decided to use a 30 min extraction in this study because: (a) longer times of extraction increased the

damage and extraction of P from live cells; (b) longer times increase the background against which biomass P is measured (Bowman *et al.*, 1978); and (c) in freshly-sieved field soils there is a greater risk of microbial activity (stimulated by sieving) influencing amounts of P extracted from unfumigated (control) treatments when longer extraction times are used.

Correction for sorption

Attempts to correct for sorption of released ^{31}P$_i$ using ^{32}P dilution were unsuccessful. There was no significant advantage in constructing a sorption isotherm to correct for sorbed ^{31}P$_i$. In soils where a large proportion of the released ^{31}P$_t$ is in organic forms, as found in this study, this correction can be regarded only as a minimum value.

Calibration—K$_P$ determination

Percentage recoveries of added microbial P from the Roseworthy and Inman Valley soils are shown in Table 2.

No reagent was clearly superior to the others for extracting organic and inorganic P, but 0.5 M NaHCO$_3$ (pH 8.5) appeared to be the most effective overall for the mixed populations of soil organisms used.

Recovery of ^{31}P$_t$ from fumigated bacterial cells was generally much greater than from fungal cells. For example, the recovery of bacterial P by the combination of hexanol and 0.5 M NaHCO$_3$ (pH 8.5) was 85.0 $\pm$ 3.4, 61.8 $\pm$ 2.0 and 75.2 $\pm$ 7.4% for the Roseworthy, Inman Valley and Mallala soils respectively. The corresponding recoveries of fungal P were 48.1 $\pm$ 6.1, 19.4 $\pm$ 1.8 and 29.0 $\pm$ 3.3%. In the absence of soil 93.4% and 48.2% of P in the Roseworthy bacterial and fungal populations respectively was recovered.

The technique adopted in this study of using mixed populations of organisms divided broadly into bacterial and fungal groups differs from previous investigations where individual species were chosen (Brookes *et al.*, 1982; Hedley and Stewart, 1982;

Table 2. Percentage recovery of P from bacterial and fungal populations added to soils and treated with hexanol. Values are the means of three replicates and are not corrected for sorption

| | | Bacteria | | Fungi | |
| | | Roseworthy | Inman Valley | Roseworthy | Inman Valley |
Extractant			Percentage recovery		
0.1 M NaHCO₃	P_i	31.6 ± 1.8	4.4 ± 0.3	19.7 ± 3.6	8.1 ± 0.3
(pH 8.5)	P_t	70.5 ± 3.5	66.1 ± 1.4	37.7 ± 6.8	9.5 ± 0.4
0.5 M NaHCO₃	P_i	36.4 ± 1.0	9.2 ± 0.5	32.4 ± 4.3	17.6 ± 1.0
(pH 8.5)	P_t	85.0 ± 3.4	61.8 ± 2.0	48.1 ± 6.1	19.4 ± 1.8
HCO₃⁻ resin	P_i	31.1 ± 0.6	3.9 ± 0.1	20.8 ± 3.0	16.3 ± 1.2
	P_t	ND	ND	ND	ND
30 mM NH₄F	P_i	76.6 ± 3.0	20.1 ± 1.0	23.8 ± 0.2	33.5 ± 3.0
+0.1 M HCl	P_t	83.5 ± 2.3	26.2 ± 0.4	34.8 ± 0.8	32.1 ± 2.1

(ND = not determined).

Strick and Nakas, 1984). The use of a soil inoculum, broad-spectrum nutrient media and low incubation temperatures encourages a response by a variety of organisms. For example, the Roseworthy soil culture contained a mixture of both Gram-positive and Gram-negative bacteria (rods and cocci), while the fungi were dominantly *Mucor* and *Penicillium* spp. The proportions of each species in the population were unknown but we assume that the microbial population responded (qualitatively) in each soil as it would do in the field after inputs of nutrients, e.g. plant residues or substrates released on rewetting dry soil. We feel this approach provides the best estimate of K_p factors until techniques are developed which isolate live undamaged, uncontaminated organisms from soil in sufficient quantities to allow accurate calibration.

However, account must be taken of the relative contributions which fungi and bacteria make to the microbial biomass in calculating K_p factors for soils where recoveries of P from the two groups of organisms are significantly different. Fungi dominate the microbial biomass in most soils, and although ratios of fungal to bacterial biomass (F:B ratios) may range between 1:1 and 11:1, values in the order of 2:1 to 3:1 are common (Clark and Paul, 1970; Shields *et al.*, 1973; Anderson and Domsch, 1975; Faegri *et al.*, 1977). Arable soils have been found to have slightly lower F:B ratios than soils under pasture or grassland (A. West, personal communication) therefore the ratios used for calculating K_p were 3:1 for the Roseworthy and Mallala soils, and 2:1 for the Inman Valley soil. The K_p factors were for Roseworthy 0.57, for Inman Valley 0.33, and for Mallala 0.40.

Influence of plant root material

A significant increase in recovery of root ³²P was observed on treatment with hexanol (Table 3), al-

though the magnitude of the effect was smaller than found previously with chloroform (McLaughlin and Alston, 1985). Subsequent studies with chloroform revealed that similar amounts of root P were released by both biocides, so that the lower values obtained in the present study may have been due to the younger age of root material used (14 day vs 21 day).

Sieving and handpicking to remove roots from root-amended soil eliminated errors associated with the inclusion of root material in the sample. It should be remembered however that organisms colonizing the rhizoplane itself, e.g. endo- and ectotrophic mycorrhiza as well as populations of bacteria, will be removed by this treatment. Also, the labelled roots were added to soil and not grown *in situ*. Thus none of the labelled root material had penetrated aggregates less than 2 mm diameter which would normally contain (after sieving) root material impossible to remove by hand.

Although prior incubation (Sparling *et al.*, 1985) or air-drying (McLaughlin and Alston, 1985) can also decrease errors associated with the inclusion of root material this may lead to changes in measured values of biomass P.

Influence of prior incubation

Microorganisms respond quickly to changes in environmental conditions so that the use of an incubation period after sampling can greatly change any indices of microbial activity (West *et al.*, 1986).

In this study, significantly lower values were obtained for biomass P when the soil samples had been incubated (Table 4). This result agrees with that of West *et al.* (1986), although these workers also altered the water potential of the soils before incubation. By incubating the soil at its field water potential and at 20°C (field soil temperature at sampling was 14°C), we had hoped to minimize the effects of prior incubation. It appears however, that the soil must be fumigated and extracted immediately after

Table 3. Effect of hexanol on percentage recovery of ³²P_t from wheat roots, and from soil amended with roots. The soil was sieved and the roots removed by handpicking before the determination

| Treatment | Roots only | Soils from which roots were removed |
		Percentage recovery
Control	12.4 ± 2.2	1.7 ± 0.8
Hexanol-treated	33.4 ± 4.6	2.2 ± 0.6
³²P_t flush	+21.0***	+0.5^NS

*** = Significant at $P < 0.001$. NS = not significant.

Table 4. Effect of prior incubation on measured biomass P in the Mallala soil ($K_p = 0.40$). Standard error of mean difference shown in parentheses

Time of incubation (days)	Biomass P (μg g⁻¹ oven-dry soil)
0	25.63 (0.78)
12	17.30 (0.48)
32	19.12 (0.87)

sampling if the values obtained for biomass P are to reflect those which pertain in the field at the time of sampling.

CONCLUSIONS

In the procedure finally adopted for measuring biomass P, the soils were sampled, sieved (<2 mm, hand picked to remove roots), and treated with hexanol in the field. Control (non-fumigated) treatments were shaken for 30 min at 20°C with 0.5 M NaHCO₃ (pH 8.5) as soon as possible after sampling (<4 h) using a soil:solution ratio of 1:20. Hexanol treated soils were extracted after 36 h fumigation and 24 h standing in a fume hood to allow evaporation of hexanol. Total $^{31}P_t$ in filtered extracts was determined and the P flush related to microbial P using a separate K_P factor determined for each soil using mixed microbial populations.

We believe the method described above gives a more realistic measurement of P held in soil microorganisms at the time of sampling than methods which use an incubation period before fumigation.

REFERENCES

Anderson J. P. E. and Domsch K. H. (1975) Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. *Canadian Journal of Microbiology* **21**, 314–322.

Anderson J. P. E. and Domsch K. H. (1980) Quantities of plant nutrients in the microbial biomass of selected soils. *Soil Science* **130**, 211–216.

Blair G. J., Till A. R. and Smith R. C. G. (1976) The phosphorus cycle—what are the sensitive areas? In *Reviews in Rural Science III—The Efficiency of Phosphorus Utilisation* (G. J. Blair, Ed.), pp. 9–19. University of New England Press, Armidale, NSW.

Borick P. M., Dondershine F. H. and Chandler V. L. (1964) Alkalinated glutaraldehyde, a new antimicrobial agent. *Journal of Pharmacological Science* **53**, 1273–1276.

Bowman R. A., Olsen S. R. and Watanabe F. S. (1978) Greenhouse evaluation of residual phosphate by four phosphorus methods in neutral and calcareous soils. *Soil Science Society of America Journal* **42**, 451–454.

Bray R. H. and Kurtz L. T. (1945) Determination of total, organic and available forms of phosphorus in soils. *Soil Science* **59**, 39–45.

Brookes P. C. and Powlson D. S. (1981) Preventing phosphorus losses during perchloric acid digestion of sodium bicarbonate soil extracts. *Journal of the Science of Food and Agriculture* **32**, 671–674.

Brookes P. C., Powlson D. S. and Jenkinson D. S. (1982) Measurement of microbial biomass phosphorus in soil. *Soil Biology & Biochemistry* **14**, 319–329.

Brookes P. C., Powlson D. S. and Jenkinson D. S. (1984) Phosphorus in the soil microbial biomass. *Soil Biology & Biochemistry* **16**, 169–175.

Chapin F. S., Barsdate R. J. and Barel D. (1978) Phosphorus cycling in Alaskan coastal tundra: a hypothesis for the regulation of nutrient cycling. *Oikos* **31**, 189–199.

Clark F. E. and Paul E. A. (1970) The microflora of grassland. *Advances in Agronomy* **22**, 375–435.

Cole C. V., Innis G. I. and Stewart J. W. B. (1977) Simulation of phosphorus cycling in semi-arid grasslands. *Ecology* **58**, 1–15.

Dalton F. H. and Hurwitz C. (1948) Effect of volatile disinfectants on survival of microflora in soil. *Soil Science* **66**, 233–238.

Faegri A., Torsvik V. L. and Goksoyr J. (1977) Bacterial and fungal activities in soil: Separation of bacteria and fungi by a rapid fractionated centrifugation technique. *Soil Biology & Biochemistry* **9**, 105–112.

Halm B. J., Stewart J. W. B. and Halstead R. L. (1972) The phosphorus cycle in a native grassland ecosystem. In *Isotopes and Radiation in Soil Plant Relationships Including Forestry*, pp. 571–586. SM151/7, IAEA, Vienna.

Hamilton W. A. (1971) Membrane active antibacterial compounds. In *Inhibition and Destruction of the Microbial Cell* (W. B. Hugo, Ed.), pp. 78–93. Academic Press, London.

Hedley M. J. and Stewart J. W. B. (1982) Method to measure microbial phosphate in soils. *Soil Biology & Biochemistry* **14**, 377–385.

Hoffman R. K. (1971) Toxic gases. In *Inhibition and Destruction of the Microbial Cell* (W. B. Hugo, Ed.), pp. 225–258. Academic Press, London.

Jenkinson D. S. (1976) The effects of biocidal treatments on metabolism in soil—IV. The decomposition of fumigated organisms in soil. *Soil Biology & Biochemistry* **8**, 203–208.

Jenkinson D. S. and Powlson D. S. (1976) The effects of biocidal treatments on metabolism in soil—V. A method for measuring soil biomass. *Soil Biology & Biochemistry* **8**, 209–213.

John M. K. (1970) Colorimetric determination of phosphorus in soil and plant materials with ascorbic acid. *Soil Science* **109**, 214–220.

Labeda D. P., Balkwill D. L. and Casida L. E. (1975) Soil sterilisation effects on *in situ* indigenous microbial cells in soil. *Canadian Journal of Microbiology* **21**, 263–269.

Martin J. K. (1975) Comparison of agar media for counts of viable soil bacteria. *Soil Biology & Biochemistry* **7**, 401–402.

McLaughlin M. J. and Alston A. M. (1985) Measurement of phosphorus in the soil microbial biomass: influence of plant material. *Soil Biology & Biochemistry* **17**, 217–220.

Murphy J. and Riley J. P. (1962) A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta* **27**, 31–36.

Phillips C. R. (1977) Gaseous sterilisation. In *Disinfection, Sterilisation and Preservation* (S. S. Block, Ed.), pp. 592–610. Lea & Febiger, Philadelphia.

Shields J. A., Paul E. A. and Lowe W. E. (1973) Turnover of microbial tissue in soil under field conditions. *Soil Biology & Biochemistry* **5**, 753–764.

Sibbesen E. (1977) A simple ion-exchange procedure for extracting plant–available elements from soil. *Plant and Soil* **46**, 665–669.

Sparling G. P., West A. W. and Whale K. N. (1985) Interference from plant roots in the estimation of soil microbial ATP, C, N and P. *Soil Biology & Biochemistry* **17**, 275–278.

Strick J. E. and Nakas J. P. (1984) Calibration of a microbial sulphur technique for use in forest soils. *Soil Biology & Biochemistry* **16**, 289–291.

Talibudeen O. (1957) Isotopically exchangeable phosphorus in soils. I. Factors influencing the estimation of labile phosphorus. *Journal of Soil Science* **8**, 86–96.

West A. W., Ross D. J. and Cowling J. C. (1986) Changes in microbial C, N, P and ATP contents, numbers and respiration on storage of soil. *Soil Biology & Biochemistry* **18**, 141–148.

Williams B. L. and Sparling G. P. (1984) Extractable N and P in relation to microbial biomass in UK acid organic soils. *Plant and Soil* **76**, 139–148.

MEASUREMENT OF PHOSPHORUS IN THE SOIL MICROBIAL BIOMASS: INFLUENCE OF PLANT MATERIAL

M. J. McLaughlin and A. M. Alston

Department of Soil Science, Waite Agricultural Research Institute, The University of Adelaide, Glen Osmond, South Australia 5064

Summary—The effect of plant material on measurement of P in the soil microbial biomass was evaluated using ^{32}P-labelled and unlabelled wheat plants. Fresh root and shoot material released significant amounts of P to 0.5 N $NaHCO_3$ (pH 8.5) following $CHCl_3$ fumigation. Treatment with $CHCl_3$ released 13–20% root P and 68–75% shoot P where a 16 h extraction period was used. With a 30 min extraction, release was 39% for roots and 18% for shoots. Calculations based on published values for rooting densities of field crops, show that significant errors in determination of soil biomass P are likely to occur where the $CHCl_3$ technique is used. Errors from inclusion of root material in the sample can be minimized by extending the extraction time from 30 min to 16 h.

INTRODUCTION

The concept that the soil microbial biomass is a labile component of soil organic matter (Jenkinson and Ladd, 1981) has stimulated the development of biochemical techniques to measure nutrients held in this pool. Phosphorus held in soil microorganisms is usually estimated by equating the increase in amount of some soil P fraction following the application of a biocidal treatment, to that held within the cells. Since Birch (1961) first proposed the use of $CHCl_3$ as a biocidal agent, a number of workers have utilized $CHCl_3$ fumigation to estimate biomass P (Chauhan *et al.*, 1979; Cole *et al.*, 1978; Stewart *et al.*, 1980; Williams and Sparling, 1984). Two standardized methods have been proposed (Brookes *et al.*, 1982; Hedley and Stewart, 1982), in which microbial P released after $CHCl_3$ fumigation is extracted using 0.5 N $NaHCO_3$ (pH 8.5). It is assumed that only microbial P is released by the $CHCl_3$ treatment. However, Birch (1961) appreciated that plant material may also be affected by $CHCl_3$ treatment, and hence overestimates of soil biomass P could result. We have investigated the extent to which the presence of plant material might affect the values obtained for biomass P in soils.

MATERIALS AND METHODS

Soils

The soils used are described in Table 1. Surface

soils (0–10 cm) were hand picked to remove large pieces of plant material and earthworms, and were sieved (<2 mm). They were held for 16 days at field water content in drums containing soda lime, and then stored for 4 weeks at 2°C as recommended by Brookes *et al.* (1982). Before use, stored soils were maintained at 20°C for 24 h.

Plant materials

Wheat seedlings were grown in solution culture using a solution based on that of Johnson *et al.* (1957). The solution supplied N (16 mmol), P (200 μmol), K (6.05 mmol), Ca (4 mmol), Mg (1 mmol), S (1 mmol) and all essential micronutrients. Continuous stirring was effected through aeration, and solution pH was adjusted daily to 6.0 using $Ca(OH)_2$. Isotopically-labelled plant material was obtained by adding 925 kBq carrier free ^{32}P l^{-1} to the solution giving a specific activity of 149 MBq ^{32}P g^{-1} ^{31}P. Plants were grown at a constant 18°C, photosynthetically active radiation was 500 μE $m^{-2} s^{-1}$ for 15 h day^{-1}, and relative humidity was 75% (day) and 90% (night).

Fumigation treatments

The plants were harvested after 20 days and separated into roots and shoots. The shoot material was cut into 1.5 cm lengths, washed with water for 5 min and blotted dry. The fresh material was either immediately fumigated according to the method of Brookes *et al.* (1982), or added to field moist soil

Table 1. Description of soils

	Roseworthy	Inman Valley
Sand (%)	88	51
Silt (%)	7	28
Clay (%)	5	21
pH_w (1:5)	8.5	6.0
Gravimetric water content at −66 kPa (%)	7.6	22.4
Organic C (%)	0.73	2.8
Organic N (%)	0.09	0.25
P_i extracted by 0.5 N $NaHCO_3$, pH 8.5 (μg g^{-1})	5.5	2.9
Land use	Cultivated wheat-fallow rotation	Native pasture

(20 mg g^{-1} oven-dry soil) before fumigation. When this procedure was used with roots variability in ^{32}P among replicates was large. Accordingly, individual intact fresh roots were counted with the roots suspended in deionized water (Martin and Cunningham, 1973). This method measured $90.1 \pm 4.3\%$ of the total root ^{32}P determined by the normal wet-digestion procedure. The intact root was then immediately fumigated or added to the soil and fumigated as above. Thus the exact amount of ^{32}P in each section of root added was known.

To determine the effect of drying, separate portions of root and shoot material were dried at 50°C for 24 h before being treated as described above for fresh shoots. The rates of addition were 3 mg dry shoot or 1 mg dry root g^{-1} oven-dry soil.

Extraction

Plant materials and amended soils (equivalent to 5 g oven-dry soil) were extracted by 0.5 N $NaHCO_3$ (pH 8.5) for 16 h at 20°C using a soil-to-solution ratio of 1-to-20. A 16 h extraction was chosen, rather than the 30 min period used by Brookes et al. (1982), as the longer time has been reported to increase the efficiency of microbial P extraction from soils (Hedley and Stewart, 1982). As absolute values for biomass P were not required, no correction for P fixation was performed. Results are therefore expressed as chloroform-released P rather than microbial-biomass P. All determinations were performed in triplicate.

Analysis

The contents of ^{31}P and ^{32}P in the plant materials were determined after wet digestion with nitric and perchloric acids in the presence of $MgCl_2$ to prevent volatile losses of P or its conversion to forms other than orthophosphate (Brookes and Powlson, 1981). Inorganic ^{31}P ($^{31}P_i$) was measured in the extracts using the automated method of Colwell (1965). The amounts of ^{32}P were determined by Cerenkov count-

ing. All counts were corrected for quenching and decay, and were converted to Bq.

Effect of fumigation on recovery of plant ^{32}P

$CHCl_3$ fumigation increased bicarbonate-extractable P from both fresh root and fresh shoot materials (Table 2). The percentage increase in plant ^{32}P recovery following $CHCl_3$ treatment was greater for shoot ($+62.8 \pm 3.3\%$) than for root ($+13.2 \pm 2.2\%$) material. Almost 90% of the total P in fumigated shoot material was extractable with bicarbonate solution. The amount of P extractable from dried material was not significantly increased by $CHCl_3$ fumigation; all shoot P ($103.4 \pm 7.3\%$) and two thirds of root P ($66.5 \pm 4.2\%$) was extractable in bicarbonate solution.

Effects of fumigation on recovery of plant ^{32}P from soils

$CHCl_3$ fumigation significantly increased the recovery of ^{32}P from incorporated fresh shoot material in both the Roseworthy ($+48.6 \pm 6.3\%$) and Inman Valley ($+30.0 \pm 1.9\%$) soils (Table 3).

When dried plant material was used, the $CHCl_3$ treatment again had no effect on recovery of ^{32}P.

Effect of added plant material on $CHCl_3$-released ^{31}P

Analyses for bicarbonate-extractable $^{31}P_i$ confirmed the results obtained with radiophosphorus (Table 4). Addition of fresh root material increased the net release of $^{31}P_i$ by $CHCl_3$ fumigation but differences were not significant ($P \leqslant 0.05$). Addition of fresh shoot material however produced large increases in extractable P in soils at the rates of addition used. Drying of the materials before addition again reduced the effect of $CHCl_3$ to insignificant levels (results not shown).

Table 2. Percentage recovery by bicarbonate extraction of total ^{32}P from unfumigated and $CHCl_3$-fumigated plant materials

| Treatment | Material | | | |
	Fresh roots (intact)	Fresh shoots (cut)	Dried roots (cut)	Dried shoots (cut)
Unfumigated	48.7	24.9	66.5	103.4
	**	***	NS	NS
Fumigated	61.9	87.7	61.8	108.4

NS = Not significant.
*, **, *** Differences between fumigated and unfumigated materials significant at $P \leqslant 5$, 1 and 0.1% respectively.

Table 3. Percentage recovery by bicarbonate extraction of ^{32}P from unfumigated and $CHCl_3$-fumigated plant materials added to soils

| Soil | Treatment | Material | | | |
		Fresh roots (intact)	Fresh shoots (cut)	Dried roots (cut)	Dried shoots (cut)
Roseworthy	Unfumigated	39.6	23.6	59.8	85.2
		NS	**	NS	NS
	Fumigated	47.0	72.2	52.0	83.7
Inman Valley	Unfumigated	20.9	6.9	24.0	45.2
		NS	***	NS	NS
	Fumigated	22.1	36.9	26.2	42.8

NS = Not significant.
*, **, *** Differences between fumigated and unfumigated materials significant at $P \leqslant 5$, 1 and 0.1% respectively.

Table 4. Extractable $^{31}P_i$ and percentage ^{31}P recovery from unamended (control) soils, and soils to which fresh root or shoot material was added

Soil	Treatment		$^{31}P_i$ (μg g^{-1})	Recovery of $^{31}P_t$ added (%)	$^{31}P_i$ released[1] by fumigation (μg g^{-1})
Roseworthy	Unfumigated:	control	5.5		
		+roots	8.6	35.8	
		+shoots	15.2	26.7	
	Fumigated:	control	7.2		
		+roots	11.0	43.4	
		+shoots	35.2	77.1	
	Control				1.7a
	+Roots				2.4a
	+Shoots				20.0b
Inman Valley	Unfumigated:	control	2.9		
		+roots	3.9	11.4	
		+shoots	4.2	3.7	
	Fumigated:	control	7.2		
		+roots	9.6	27.0	
		+shoots	23.4	44.3	
	Control				4.3a
	+Roots				5.7a
	+Shoots				19.2b

[1]As CHCl$_3$-released P is itself a mean difference, a modified t-test was used. Means followed by different letters are significantly different at the 0.1% level of probability.

Effect of extraction time

In a separate experiment with fresh unlabelled materials, the effect of extraction time on release of P from plants following CHCl$_3$ fumigation was determined. With a 16 h extraction, release of plant $^{31}P_i$ by CHCl$_3$ fumigation was similar to that found using ^{32}P-labelled materials: roots $+19.8 \pm 4.9\%$, shoots $+75.7 \pm 3.1\%$. However a shorter extraction (30 min) caused greater release of root P ($+38.5 \pm 5.7\%$) and smaller release of shoot P ($+18.0 \pm 2.9\%$).

DISCUSSION

It is evident that values obtained for P in the "microbial biomass" using the CHCl$_3$ fumigation technique can be affected by the presence of plant materials in the soil. The errors introduced were greater with plant shoots than with roots when a 16 h extraction was used, while the converse was true when the extraction time was 30 min. The smaller release of P from shoots after CHCl$_3$ treatment using the 30 min extraction appeared to be due to the hydrophobic nature of the plant cuticle, which prevented the extractant from effectively penetrating the tissue. Longer extraction times seemed to overcome this problem, as the plant tissue was fully "wetted" after 16 h extraction. The comparatively small P release from fresh roots by fumigation where the 16 h extraction was used can be attributed to greater release of P from the unfumigated controls.

Birch (1961) noted that plant materials exposed to CHCl$_3$ vapour exhibited greater P extractability than untreated controls and direct evidence recently obtained by Martin and Foster (1985) confirms that chloroform completely disrupts root cells by lysing cytoplasmic membrane systems. Birch (1961) utilized moistened dried materials, and non CHCl$_3$-treated controls were extracted immediately following the addition of plant material. The increase in P extractability on fumigation was attributed to plant enzyme

activity or diffusion of P$_i$ during the 24 h of the CHCl$_3$ treatment. In our study, untreated controls were kept moist in a desiccator for the same period as CHCl$_3$-treated samples (24 h). Therefore any increase in P extractability must be due to the effect of CHCl$_3$ alone on the plant cells. The only assumption made is that P held in microorganisms (and therefore susceptible to CHCl$_3$), situated both in and on the plant root or shoot surface, is negligible compared to total P within the plant material itself. Although no figures are available in the literature to verify this assumption, the vigorous washing of the materials before use would have minimized this source of error.

The effect of drying of the plant materials prior to fumigation increased the P extractability of unfumigated controls, with all shoot P and two-thirds root P being extractable following the drying treatment. This result agrees with those of Martin and Cunningham (1973) who found desiccation to be the major factor affecting the water solubility of P in plant roots. Their results indicated approximately 50% root P became extractable (in water) on drying. Similarly Bromfield and Jones (1972) found water-soluble P in shoots to be up to 80% total P in "hayed off" pasture species. As 0.5 N NaHCO$_3$ is a slightly stronger extractant than water, especially for labile organic P species, we would expect greater extraction of P, as indeed was found in this study.

In terms of measuring biomass P in soils, therefore, the prior treatment of the soil has an important effect. The drying, grinding and preliminary incubation of soils recommended by Hedley and Stewart (1982) before CHCl$_3$ treatment should prevent errors arising from inclusion of plant material in the sample, but may cause large qualitative and quantitative changes in the biomass. The method of Brookes et al. (1982) utilizes undried soils, so that errors may arise from the presence of fresh plant material. The size of the error will depend on the amount of plant material in the soil and the efficiency with which the material can be removed before fumigation.

In terms of field soils, the errors are large enough

to warrant concern. Published values for rooting densities under crops and forests vary widely from 0.1 to 12.0 mg dry root g^{-1} soil (Lupton *et al.*, 1974; Lynch and Panting, 1980), but generally values fall within the range 0.3 to 2.0 mg root g^{-1} soil (Troughton, 1957; Persson, 1983). Assuming that plant roots contain 0.1% P, the upper limit for root bound P in soils would be approximately 10 μg P g^{-1} soil. If we assume that a maximum of 40% of this is released by CHCl$_3$ treatment then 4 μg P g^{-1} soil would be the maximum error involved. Published values of total P in soil biomass vary from 5 to 100 μg P g^{-1} soil (Brookes *et al.*, 1984) which relate to values of 2 to 40 μg P g^{-1} soil for CHCl$_3$-released P if $k_p = 0.4$. If we assume that P released from plants and micro-organisms is sorbed to a similar extent during the extraction process, significant errors in measurement of P in soil biomass are likely when plant material is included in the sample. Longer extraction times will reduce the error involved for roots, but increase that for shoots. Complete removal of all plant material from a soil sample is virtually impossible without resorting to destructive techniques (e.g. washing). Hand sorting of samples will remove a large population of microorganisms associated with the plant materials (Martin and Foster, 1985). It appears therefore that errors due to plant material in soil biomass P measurements are inevitable using current methodology. As amounts of fresh shoot material in soils are generally low (except under special circumstances such as green manuring or incorporation of residues) a 16 h extraction period as proposed by Hedley and Stewart (1982) is recommended to minimize errors due to included root material.

REFERENCES

Birch H. F. (1961) Phosphorus transformations during plant decomposition. *Plant and Soil* **15**, 347–366.

Bromfield S. M. and Jones O. L. (1972) The initial leaching of hayed-off pasture plants in relation to the recycling of phosphorus. *Australian Journal of Agricultural Research* **23**, 811–824.

Brookes P. C. and Powlson D. S. (1981) Preventing phosphorus losses during perchloric acid digestion of sodium bicarbonate soil extracts. *Journal of the Science and Food and Agriculture* **32**, 671–674.

Brookes P. C., Powlson D. S. and Jenkinson D. S. (1982) Measurement of microbial biomass phosphorus in soil. *Soil Biology & Biochemistry* **14**, 319–329.

Brookes P. C., Powlson D. S. and Jenkinson D. S. (1984) Phosphorus in the soil microbial biomass. *Soil Biology & Biochemistry* **16**, 169–175.

Chauhan C. S., Stewart J. W. B. and Paul E. A. (1979) Effect of carbon additions on soil labile inorganic, organic and microbially held phosphate. *Canadian Journal of Soil Science* **59**, 387–396.

Cole C. V., Elliott E. T., Hunt H. W. and Coleman D. C. (1978) Trophic interactions in soils as they affect energy and nutrient dynamics. V. Phosphorus transformations. *Microbial Ecology* **4**, 381–387.

Colwell J. D. (1965) An automatic procedure for the determination of phosphorus in sodium hydrogen carbonate extracts of soils. *Chemistry and Industry*, May 1965, 893–895.

Hedley M. J. and Stewart J. W. B. (1982) Method to measure microbial phosphate in soils. *Soil Biology & Biochemistry* **14**, 377–385.

Jenkinson D. S. and Ladd J. N. (1981) Microbial biomass in soil: measurement and turnover. In *Soil Biochemistry*, Vol. 5 (E. A. Paul and J. N. Ladd, Eds), pp. 415–471. Dekker, New York.

Johnson C. M., Stout P. R., Broyer T. C. and Carlton A. B. (1957) Comparative chlorine requirements of different plant species. *Plant and Soil* **8**, 337–353.

Lupton F. G. H., Oliver R. H., Elliss F. B., Barnes B. T., Howse K. R., Welbank P. J. and Taylor P. J. (1974) Root and shoot growth of semi dwarf and taller winter wheats. *Annals of Applied Biology* **77**, 129–144.

Lynch J. M. and Panting L. M. (1980) Variations in the size of the soil biomass. *Soil Biology & Biochemistry* **12**, 547–550.

Martin J. K. and Cunningham R. C. (1973) Factors affecting the release of phosphorus from decomposing wheat roots. *Australian Journal of Biological Science* **26**, 715–727.

Martin J. K. and Foster R. C. (1985) A model system for studying the biochemistry and biology of the root–soil interface. *Soil Biology & Biochemistry* **17**, 261–269.

Persson H. A. (1983) The distribution and productivity of fine roots in boreal forests. *Plant and Soil* **71**, 87–101.

Stewart J. W. B., Hedley M. J. and Chauhan B. S. (1980) The immobilisation, mineralisation and redistribution of phosphorus in soils. In *Proceedings of the Western Canada Phosphate Symposium* (J. T. Harapiak, Ed.), pp 276–306. Alberta Soil Science Society, Edmonton.

Troughton A. (1957) The underground organs of herbage grains. Bulletin of the Commonwealth Bureau of Pasture and Field Crops No. 44. Commonwealth Agricultural Bureau.

Williams B. L. and Sparling G. P. (1984) Extractable N and P in relation to microbial biomass in UK acid organic soils. *Plant and Soil* **76**, 139–148.