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Measurement of microbial biomass phosphorus in rhizosphere soil

J.K. MARTIN and R.L. CORRELL
CSIRO Division of Soils, Glen Osmond, South Australia, 5064 and CSIRO Division of Mathematical Statistics, Glen Osmond, South Australia, 5064

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Abstract

^{32}P-labelled monocalcium phosphate solution was supplied by point injection to the root system of wheat plants grown in soil cores in a controlled environment. There was no detectable incorporation of ^{32}P into organic P fractions in the soil remaining after roots were removed, confirming field observations. The techniques used to measure organic P (including biomass P) could detect an incorporation of ^{32}P into soil microbial biomass equivalent to 0.3 $\mu gP.g^{-1}$ soil, compared to a total soil biomass P content estimated to be ca. 6.5 $\mu gP.g^{-1}$ soil. The limited incorporation of the added P into microbial biomass in the root-free soil may be due partly to a limited diffusion of ^{32}P into the non-rhizosphere soil and partly to the removal of ^{32}P-labelled microbial biomass adhering to or in very close association with the root surface. It is proposed that in studies of soil nutrient status, total soil biomass P (roots + soil flora + microfauna) should be measured, rather than attempting an estimate of microbial P. A sequential extraction procedure using a single soil sample, where a biocide is added to the extracting solution, is proposed as an alternative to the conventional procedure for measuring soil biomass P where two soil samples, one treated with a biocide, are extracted simultaneously.

Introduction

A field study (Martin, 1985) measured the distribution of ^{32}P-orthophosphate, supplied to the roots of individual wheat plants, between plant components and inorganic and organic P fractions present in the soil freed from roots. Contrary to expectations, there was no evidence for the incorporation of ^{32}P into soil organic P fractions in the wheat rhizosphere in the period between germination and mid-tillering. Carbon derived from the wheat roots (Martin, 1977) would be expected to support the growth of a large microbial population in the rhizosphere containing ^{32}P-labelled phosphate with specific activity comparable to that of the plant tissues.

These results led to the proposal (Martin, 1980) that the labelled microbial biomass which would have grown up in response to the root-derived C, would be in such intimate association with the roots that it would be removed from the soil together with the roots. However, there were alternative explanations, including (i) an influence of soil factors such as P status and placement of the ^{32}P and (ii) limitations to the techniques used to measure $^{32}P_0$.

This paper presents the detailed results of experiments reported briefly (Martin, 1980) and additional experiments to determine the factors influencing the measurement of P in microbial biomass present in rhizosphere soils.

Materials and methods

Soils

Undisturbed soil cores (contained within PVC pipe, 100 mm internal dia., driven into the ground to a depth of 150 mm) collected from a field site at

Avon, South Australia were used for the first experiment. High P status soil cores were collected from the previous experimental site (Martin, 1985) while low P status soil cores were taken from a fenced headland which had not been cultivated for crop or pasture production, with less than 20 metres separating the two collection points. The bicarbonate extractable P values were 28.8 and 8.6 μgP.g^{-1} for the high and low P soils respectively.

Soil from cores collected from the low P status site was used in all the subsequent experiments. The soil was stored between experiments in the air-dry state until ^{32}P activity from the previous experiment had decayed below detectable limits. For experiments using repacked soil cores, the soil was adjusted to a water content of 15–16% by weight with a P-deficient nutrient solution (Robbins, 1946) and 2 kg amounts of moist soil were packed into polythene bags contained within the 100 mm dia.X 150 mm PVC tubes. In the soil incubation experiments, where a defined C source was added to soil together with ^{32}P to produce a labelled microbial biomass, 100 g of air-dry soil was mixed in a polythene bag with 15 ml of a solution containing the C source and ^{32}P-orthophosphate.

Plant culture

Single germinated wheat seedlings (*Triticum aestivum* L. cv. Halberd) were planted in each moistened core and placed in a growth chamber (Martin, 1977), with a 16 h photoperiod (quantum flux density 320 μE.m^{-2}.s^{-1}) and a temperature of 15°C. The periods of plant growth were 34 days and 55 days for experiments I and III respectively, while there were two harvests in experiment IV, at 18 and 42 days. At harvest, the tops were clipped at soil level and air-dried while the roots were separated from the soil as completely as possible by hand picking, with minimum mechanical disturbance of the soil.

Soil incubation experiments

Experiment IIa. 100 g air-dry soil was mixed with a solution containing ^{32}P at a concentration and specific activity identical to that used in the planted experiments and an amount of glucose equivalent to 15 or 30 μg C.g^{-1} soil. The moist soils were incubated within a polythene bag in the dark at 15°C for 28 days.

Experiment IIb. This experiment used repacked soil cores (150 mm × 100 mm dia.) amended with dehydrated Bacto-tryptic soy broth (Difco), added to soil in quantities equivalent to 30 μg C.g^{-1} soil. ^{32}P was injected into twelve cores from which six cores were kept as controls with no C addition. The solution containing the tryptic soy broth was added to the tops of the remaining cores in three batches at 7 day intervals, supplying 10%, 30% and 60% of the total C at each addition. The cores were held in the dark at 15°C for a period of 36 days from the ^{32}P addition.

Application of ^{32}P

a. Point injection. (Experiments I, IIb, III and IV). One week after seedling emergence, a multi-dose veterinary syringe (Hauptner, West Germany), was used to inject ^{32}P-labelled monocalcium phosphate solution into the soil, contained in polythene bags within the PVC cylinders, at a depth of 3 cm at five points around the enclosed plants. The total volume injected into the soil was 5 ml containing approximately 1.8 MBq ^{32}P and a carrier level of calcium orthophosphate equivalent to a P addition of 5 kgP ha^{-1} (the ^{32}P dosage was equivalent to approx. 1000 Bq.g^{-1} soil with a specific activity of 300 Bq. μg^{-1} P).

b. Uniform distribution. (Experiment III). Five ml of ^{32}P solution were added to soil from each core with enough distilled water to raise the water content of the air-dry soil to 15% by weight. The rewet soil was mixed by shaking vigorously within the polythene bag, prior to placement within the PVC cylinders and planting a germinated wheat seed.

Analysis of plant material and soil

The methods for the measurements of total P, ^{32}P activity and specific activity of P in plant material were as previously described (Martin, 1985). Soil organic P (^{32}P and ^{31}P) was measured as the dif-

ference in 1 *M* H_2SO_4 extracts of soil ignited at 550°C for 1 h and non-ignited soil. The ^{31}Pi content of the extracts was measured by colorimetry and the ^{32}Pi activity was measured either with a planchet counter or by measuring Cerenkov radiation in the acid extract with a liquid scintillation counter.

Biomass P was measured as the difference in P extracted from soil fumigated with $CHCl_3$ vapour (Brookes *et al.*, 1982) and untreated soil (ΔP). The extractants and the extractant:soil ratio (ml:g dry wt.) used in each of the experiments are listed below—

Planted I—0.5 *M* $NaHCO_3$ pH 8.5, 50:1
III—0.1 *M* $NaHCO_3$ pH 8.5, 40:1
IV—0.5 *M* $NaHCO_3$ pH 8.5, 0.1 *M* $NaHCO_3$ pH 8.5, 0.1 *M* H_2SO_4, 20:1
Incubated IIa, b—0.5 *M* $NaHCO_3$ pH 8.5, 1.0 *M* H_2SO_4, 50:1
V—0.5 *M* $NaHCO_3$ pH 8.5, 0.1 *M* $NaHCO_3$ pH 8.5, 0.01 *M* EDTA + 0.15 *M* NaCl pH 8.0, 20:1

^{32}P activity in the extracts was measured by Cerenkov radiation using a quench curve prepared for each extractant to correct for colour quenching. ^{31}Pi was measured by colorimetry. The significance of the difference between the P extracted from the fumigated and untreated soils (ΔP) was measured by a paired t-test for each extractant. ΔP values, obtained with different extractants in the same or different experiments, could not be compared with standard statistical methods but a procedure was developed (A.G. Constantine and R.L. Correll, personal communication) which provided an estimate of the sensitivity of the different extractants using a common fumigation technique. Sensitivity was defined as the ability of a particular method to detect a small change in biomass P.

Estimation of biomass P by a sequential extraction procedure

Soil biomass P was determined from measurements of P in solution before and subsequent to the addition of a biocidal concentration of liquid chloroform (Paul and Johnson, 1977). A ^{33}P-labelled soil microbial biomass, evenly dispersed through the soil and containing the bulk of the radioisotope, was obtained by incubating 10 g quantities of moist soil, mixed with 100 mg of finely-ground material from medic (*Medicago truncatula* cv. Paraggio) uniformly labelled with ^{33}P (McLaughlin and Alston, 1986), at 25°C for 28 days. The soils, contained within capped 250 ml polythene bottles, were then extracted for 2 h with 200 ml of one of three extractants—0.1 *M* $NaHCO_3$, 0.5 *M* $NaHCO_3$ or 0.01 *M* EDTA. 0.01 *M* EDTA was included since this extractant was preferred for the extraction of ^{14}C-labelled organic material from soil microbial biomass killed by exposure to chloroform vapour (Merckx and Martin, 1987). An aliquot of each extract was removed for analysis, after which 40 ml of liquid chloroform was added to the soil suspensions and a second set of samples removed for analysis after a further 2 h extraction. A second set of soils was treated identically, except that there was no addition of liquid chloroform.

Results and discussion

A number of factors were identified which could have been responsible for the failure to measure the incorporation of ^{32}Pi into soil organic P fractions (Martin, 1985) and several experiments were designed to determine the effect of each factor, singly or in combination with other factors, on this process. The results for each of these experiments are presented below.

I. Confirmation of field observations

The results from experiment I (Table 1) show that there was no significant incorporation of ^{32}P into organic P fractions (increase in ^{32}P activity extractable with 1 *N* H_2SO_4 following ignition) in either the high P or low P status soil. Air-drying had no significant effect in either soil in increasing the amount of ^{32}P activity extractable from non-ignited soil with 1 *N* H_2SO_4. Attempts to measure ^{32}P in microbial biomass by a fumigation technique using 0.5 *M* $NaHCO_3$ as extractant (Brookes *et al.* 1982) also gave negative results for both the high and low P soils. This confirmed the previous results and suggested that they were not influenced by

Table 1. Effect of soil P content and soil treatment on the amount of ^{32}P-activity extracted from rhizosphere soil by 1.0 *M* H_2SO_4 or 0.5 *M* $NaHCO_3$

Soil treatment	Soil P content	
	Low	High
	(Bq ^{32}P.g^{-1} dry soil)[a]	
H_2SO_4 extraction		
Fresh	302	451
Ignited	290	499
Air-dry	323	419
S.E.D.	15.5	24.7
$NaHCO_3$ extraction		
Fresh	189	321
Fumigated	197	307
t-value	1.750 (9)	−0.483 (9)

[a] Values are means of 10 replicates, SED is standard error of the difference between means, t-value is for the degrees of freedom shown in brackets.

climatic conditions, the very high P status of the soil or by the fact that the soils were air-dried before analysis.

II. Sensitivity of procedures for measuring biomass P

An obvious explanation for the negative results in experiment I was that the analytical techniques were not sufficiently sensitive to detect the amount of ^{32}P incorporated into the microbial biomass resulting from utilization of the root-derived C. This was estimated to be of the order of 0.2 μgP.g^{-1} soil, based on the assumptions that 60% of the rhizosphere CO_2 (Martin, 1977) came from microbial respiration, that C incorporated into microbial biomass represented no more than 25% of the respired CO_2 and a C:P ratio in the microbial cells of 30:1.

Two experiments were set up to estimate the lower limits at which the conversion of inorganic ^{32}P into organic components could be detected, using conventional methods.

Experiment IIa. There was a significant ($P < 0.001$) amount of $^{32}P_{org}$, presumably in microbial cells, measured with both the ignition and fumigation techniques (Table 2), showing that the negative results in the plant experiments, at least with the low P soil, were unlikely to be due to limitations of the analytical methods ($\Delta^{32}P$ = difference of ^{32}P activity in extracts from treated and untreated soils).

Experiment IIb. Similar results were obtained in this experiment, which simulated the conditions used in the planted experiments, both with respect to the banding of the ^{32}P and an uneven distribution of the freshly-added C within the soil cores. No significant incorporation of ^{32}P into microbial biomass could be detected by either the ignition or the fumigation techniques in the control soil but there was a significant ($P < 0.01$) incorporation of isotope into organic P components in the soil cores with added C (Table 2).

Efforts were made to increase the sensitivity of biomass P measurements by achieving a higher K_p factor than was obtained using 0.5 *M* $NaHCO_3$ as extractant. Ideally, there should be a minimal extraction of P from viable cells in the non-fumigated control and the maximum extraction of P from killed cells. A series of experiments (not presented)

Table 2. Effect of C addition and soil treatment on the amount of ^{32}P activity extracted from incubated soil by 1.0 *M* H_2SO_4 or 0.5 *M* $NaHCO_3$ solutions.

Soil treatment	C addition (μgC.g^{-1} dry soil)		
	Nil	15	30
	(Bq ^{32}P.g^{-1} dry soil)		
A. Loose soil			
H_2SO_4 extraction			
Fresh	–	580	592
Ignited	–	723	781
$\Delta^{32}P$[a]	–	143	189
		(7.732)	(7.734)
$NaHCO_3$ extraction			
Fresh	–	356	340
Fumigated	–	423	411
$\Delta^{32}P$[a]	–	67	71
		(13.586)	(7.303)
B. Repacked soil core			
H_2SO_4 extraction			
Fresh	1360	–	1299
Ignited	1490	–	1558
$\Delta^{32}P$[a]	130	–	259
	(1.898)	–	(4.741)
$NaHCO_3$ extraction			
Fresh	1010	–	957
Fumigated	1000	–	1050
$\Delta^{32}P$[a]	−10	–	93
	(−0.187)	–	(3.304)

[a] t-values for differences between biocidal treatments with 5 degrees of freedom are shown in brackets.

with a soil microbial biomass labelled *in situ* with ^{32}P compared $\Delta\,^{32}P$ values obtained with a range of extractants. 0.1 *M* $NaHCO_3$ consistently gave higher values for $\Delta\,^{32}P$ than 0.5 *M* $NaHCO_3$ (cf. Table 4). However, a comparison of the sensitivity of the different extractants (A.G. Constantine and R.L. Correll, personal communication) showed that no extractant was consistently superior to others in its ability to detect small changes in biomass P. Thus it seems preferable to continue to use the 0.5 *M* $NaHCO_3$ extractant, as recommended in several recent studies (Brookes *et al.*, 1982; McLaughlin *et al.*, 1986). In specific circumstances (*e.g.* van Veen *et al.*, 1987) 0.1 *M* H_2SO_4 may have an advantage as an extractant since (a) resorption of extracted P was less than for other extractants, (b) the extracts have very little colour, in contrast to the $NaHCO_3$ extracts, (c) the solution will contain Pi almost exclusively, in contrast to the alkaline extracts which contain a variable mixture of inorganic and organic P. The K_p factor for 0.1 *M* H_2SO_4 is uncertain and would be about 0.1 for newly killed cells but could be >0.6 for cells exposed to chloroform for 18 h or more, since there is evidence of extensive phosphatase activity in cells killed by chloroform vapour releasing the greater part of the cell P as acid-soluble Pi (Brookes *et al.*, 1984).

III. Effect of placement of P within the soil

An alternative explanation for the low degree of incorporation of ^{32}P, supplied with fertiliser P in the field experiments, into microbial biomass is the limited mobility of P in soils. The soil volume containing the radioisotope, introduced into the soil as five 1 ml injections, would be small compared with the soil volume explored by the wheat roots. ^{32}P absorbed by the roots from the injection zone would be translocated throughout the root system but it was conceivable that the amounts of ^{32}P released from roots away from the injection zone would be too small to produce a ^{32}P-labelled biomass which could be detected by the experimental techniques used in this study.

Experiment III. The results (Table 3) showed a significant ($P < 0.01$) incorporation of ^{32}P into microbial biomass in both planted treatments, as well as in the unplanted soil when the ^{32}P was uniformly distributed. This contrasts with the non-significant results obtained with unplanted soil when the ^{32}P was point-injected (Table 2b). There was an increase in the values for $\Delta\,^{32}P$ from the uniform distribution unplanted, to the point injection planted, to the uniform distribution planted treatments. These results provide evidence for the proposition that organic C released from the plant roots would increase the incorporation of ^{32}P into microbial biomass compared with that occurring in unplanted soil, and also support the proposal that the point injection technique could be limiting the incorporation of the labelled fertiliser P into the rhizosphere microbial biomass. A comparison of the $\Delta\,^{32}P$ values for the unplanted and planted cores from the uniform ^{32}P distribution treatment allows an assessment of the effect of root-derived C on the incorporation of ^{32}P into microbial biomass. There is no established value for K_p using 0.1 *M* $NaHCO_3$ as extractant, but a value of 0.5 seems reasonable from the comparative values for $\Delta\,^{31}P$ for 0.1 *M* and 0.5 *M* $NaHCO_3$ (Table 4). Using a K_p of 0.5, the proportion of the ^{32}P added to the soil initially, which was present in microbial biomass was 10.3% for the unplanted soil and 20.8% for the planted

Table 3. Effect of distribution of ^{32}P-labelled fertiliser within soil cores on ^{32}P contained within soil microbial biomass

Soil treatment	^{32}P distribution within soil (Bq $^{32}P.g^{-1}$ dry soil)		
	Point application	Uniform application	
	Planted	Planted	Unplanted
Fresh	57.0	63.5	229.1
Fumigated	109.5	140.1	267.5
$\Delta\,^{32}P$[a]	52.5	76.6	38.4
	(4.858)	(7.889)	(5.164)

[a] t-values for differences between biocidal treatments with 4 degrees of freedom are shown in brackets.

Table 4. Effect of plant age on ^{32}P and ^{31}Pi incorporation into soil microbial biomass and comparison of extractants

Plant age	Soil extractant		
	0.1 *M* H_2SO_4	0.1 *M* $NaHCO_3$	0.5 *M* $NaHCO_3$
18 days			
	Bq $^{32}P.g^{-1}$ soil		
Fresh soil	817.2	414.8	–
Fumigated soil	874.7	541.7	–
$\Delta\,^{32}P^a$	57.5	126.9	–
	(0.883)	(2.509)	–
	$\mu g\,^{31}P_i.g^{-1}$ soil		
Fresh soil	18.36	5.02	–
Fumigated soil	20.72	7.88	–
$\Delta\,^{31}Pi^a$	2.36	2.86	–
	(10.015)	(11.419)	
42 days			
	Bq $^{32}P.g^{-1}$ soil		
Fresh soil	573.8	193.4	384.6
Fumigated soil	594.0	314.2	387.6
$\Delta\,^{32}P^a$	20.2	120.8	3.0
	(0.372)	(7.754)	(0.155)
	$\mu g\,^{31}P_i.g^{-1}$ soil		
Fresh soil	18.25	4.12	8.23
Fumigated soil	20.47	7.04	10.71
$\Delta\,^{31}Pi^a$	2.22	2.92	2.48
	(5.549)	(27.532)	(13.775)

[a] t-values for differences between biocidal treatments with 4 degrees of freedom are shown in brackets.

treatments, corresponding to an incorporation of 0.3 $\mu gP.g^{-1}$ soil and 0.6 $\mu gP.g^{-1}$ soil for the respective treatments.

IV Effect of plant age on soil biomass P

If the postulate was correct, that P would be incorporated into rhizosphere microorganisms growing in response to organic C released from plant roots, plant age could be expected to influence the amount of biomass ^{32}P present in the rhizosphere of wheat plants grown in soil containing ^{32}P-orthophosphate.

Experiment IV. The much greater mass of roots at 42 days compared with that at 18 days (mean values for dry weight of roots per core were 347 mg and 77 mg respectively) implies a substantial input of root-derived C into the soil between 18 and 42 days, yet there was no corresponding increase in the $\Delta\,^{32}P$ values measured at the two harvest times (Table 4). This implies either the ^{32}P released from dead or senescent root tissue did not diffuse into the fraction of the soil which could be separated from the plant roots or there was a very rapid turnover of labelled biomass so that the net incorporation of ^{32}P into microbial biomass stayed relatively constant despite a large flow of C through the microbial pool.

There were much higher values for $\Delta\,^{32}P$ obtained with 0.1 *M* $NaHCO_3$ than with 0.1 *M* H_2SO_4 in the first harvest samples and than with either H_2SO_4 or 0.5 *M* $NaHCO_3$ in the second harvest. Similar trends were also observed for $\Delta\,^{31}P$ values obtained from measurements of inorganic P on extracts from the fumigated and untreated soils.

V Comparison of procedures for estimating biomass P

The estimation of ^{32}P incorporation into biomass by the conventional techniques was very unreliable. A major source of error was associated with the uneven distribution of the ^{32}P within small subsamples of soil from any particular experimental treatment, resulting, not infrequently, in negative values

for $\Delta^{32}P$ when the values for ^{32}P activity in extracts from non-fumigated soil were subtracted from those for the corresponding fumigated soils. A possible solution to the errors associated with the uneven distribution of the added radioisotope would be to make two sequential extractions on the same soil suspension—first without any biocidal treatment followed by a second extraction after a biocidal treatment.

Experiment V. The comparison of the double extraction procedure with the conventional procedure showed a highly significant ($P < 0.001$) release of ^{33}P following the addition of the liquid chloroform to the extracts (Table 5) but the $\Delta^{33}P$ values obtained with each of the extractants using the double extraction procedure were appreciably lower than the $\Delta^{33}P$ values obtained with the conventional procedure. The ratio of the $\Delta^{33}P$ values obtained with the double extraction: $\Delta^{33}P$ values obtained with the conventional procedure were 0.58, 0.84 and 0.47 for the 0.01 *M* EDTA, 0.1 *M* bicarbonate and 0.5 *M* bicarbonate extracts respectively.

General conclusions

The results presented here confirmed the earlier observations (Martin, 1985) of a very low degree of incorporation of fertilizer P into the rhizosphere soil biomass during the period of active wheat growth. The present techniques employing ^{32}P appear to be sufficiently sensitive to detect an incorporation of $< 0.3\,\mu g P.g^{-1}$ soil, compared with a total soil biomass P content ca. $6.5\,\mu g\,P.g^{-1}$ soil (Table 4), making it unlikely that a lack of sensitivity of the methods for measuring the incorporation of the ^{32}P into soil biomass was responsible for the negative results. Similar results were obtained by McLaughlin *et al.* (1988) who found only 3–5% of fertiliser P supplied to wheat in field experiments, was incorporated into the microbial biomass present in the root-free soil.

Two factors could be responsible for the very small degree of incorporation of the added P into soil biomass contained in the root-free soil—(1) a limited availability of the ^{32}P to the soil micoorganisms present in the root-free soil or (2) the newly formed microbial population, which would be expected to contain ^{32}P with a specific activity comparable to the plant tissue, would be in such close association with the roots that the bulk of the labelled organisms would be removed together with the roots. Limited diffusion of P into the root-free soil must be an important factor since McLaughlin *et al.* (1987) showed that < 5% of ^{33}P translocated to wheat roots was recovered from soil separated from the roots by a polyamide membrane. However, in experiment III where the ^{32}P was uniformly distributed through the soil, the additional P incorporation into soil biomass in the planted cores

Table 5. Comparative values, using different extractants, for ^{33}P incorporation into soil microbial biomass in soils incubated for 28 days, measured with a conventional procedure and a sequential extraction procedure

Fumigation	Extractant		
	0.01 *M* EDTA	0.1 *M* $NaHCO_3$	0.5 *M* $NaHCO_3$
	(Bq $^{33}P.g^{-1}$ dry soil)		
$CHCl_3$ vapour			
Fresh soil	44.9	13.1	35.3
Fumigated soil	198.3	157.7	205.0
$\Delta^{33}P$	153.4	144.6	169.7
t-value	28.826	24.182	13.514
$CHCl_3$ liquid			
Difference (4 h-2 h extraction)			
+ $CHCl_3$	101.0	117.2	95.1
− $CHCl_3$	11.6	−4.3	15.6
Corrected $\Delta^{33}P$	89.4	121.5	79.5
t-value	12.505	31.583	4.801

t-values for the differences between biocidal treatments are for 4 degrees of freedom.

compared with the unplanted treatment was 0.3 μgP.g^{-1} soil. The calculated biomass P resulting from root-derived C when the wheat plants were at the heading stage, as in this experiment, was ca. 0.8 – 1.0 μgP.g^{-1} soil. Thus it seems probable that the greater part of the microbial biomass growing in response to root-derived carbon was removed with the roots. This is not unexpected in view of the very close association between the rhizosphere microbial population and the root surface (Foster, 1986).

It is extremely difficult to obtain separate, quantitative values for the P content of root tissue and the associated rhizosphere microbial population, because of the similar chemical composition of the P components and the susceptibility to biocidal agents of both types of cellular material. Errors in the estimation of soil microbial biomass P due to the presence of root material have been recognised (McLaughlin and Alston, 1985; Sparling *et al.*, 1985), particularly in soils from permanent pastures where root densities can be in excess of 6 mg.g^{-1} soil. Treatments to reduce errors from roots include pre-incubation of the soil sample or physical removal of as much root material as possible by sieving and hand-picking. However, such treatments have been recognised as altering the microbial population present in the original soil sample. The soil microbial biomass is considered to be an important reservoir of potentially available plant nutrients and it seems probable that most measurements of microbial P have been made either to assess the potential nutrient status of a soil or to estimate fluxes of P within particular plant-soil associations. Roots can be regarded equally with microbial biomass as a labile reservoir of P, since the epidermal and cortical tissue, which would contain most of the root P, has a comparatively short life (20–40 days for cereals—Henry and Deacon, (1981) and more than 90% of the P in dead root tissue has been identified as inorganic P (Martin and Cunningham, 1973). In studies where the intention is to provide data relating to the potential P status of a soil-plant system rather than estimating P fluxes, it would seem preferable to measure total biomass P (*i.e.* roots + microbial biomass) on fresh field samples without removal of roots rather than attempting an incomplete estimate of microbial P. This would allow an accurate measurement of the proportion of the total soil P present in living tissue compared with the various inorganic and organic fractions of the soil matrix at different times in a growing season or in assessing the effects of different agronomic treatments on soil P components.

The results in Table 5 show that the sequential extraction procedure can provide a reliable estimate for biomass P, although a different K_p factor may be required to that used in the conventional procedure. The even distribution of the radioisotope through the soil and the small proportion present in the inorganic pool, and so extractable from the non-fumigated soil, eliminated the source of the high variability observed in experiments I, III and IV. Hence it was not possible to determine from experiment V if the sequential extraction procedure would be superior to the conventional procedure in samples where there was a substantial pool of inorganic P unevenly distributed through the soil. However, conceptually, the sequential extraction procedure can be expected to minimise errors associated with an uneven distribution of inorganic P, such as can occur in heavily fertilized arable and pasture soils, and can be considered as an alternative to the conventional procedure for measuring soil biomass P in such soils.

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