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Quantifying microbial biomass phosphorus in acid soils

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Abstract This study aimed to validate the fumigation-extraction method for measuring microbial biomass P in acid soils. Extractions with the Olsen (0.5 M $NaHCO_3$, pH 8.5) and Bray-1 (0.03 M NH_4F–0.025 M HCl) extractants at two soil:solution ratios (1:20 and 1:4, w/v) were compared using eight acid soils (pH 3.6–5.9). The data indicated that the flushes (increases following $CHCl_3$-fumigation) of total P (P_t) and inorganic P (P_i) determined by Olsen extraction provided little useful information for estimating the amount of microbial biomass P in the soils. Using the Bray-1 extractant at a soil:solution ratio of 1:4, and analysing P_i instead of P_t, improves the reproducibility (statistical significance and CV) of the P flush in these soils. In all the approaches studied, the P_i flush determined using the Bray-1 extractant at 1:4 provided the best estimate of soil microbial biomass P. Furthermore, the recovery of cultured bacterial and fungal biomass P added to the soils and extracted using the Bray-1 extractant at 1:4 was relatively constant (24.1–36.7% and 15.7–25.7%, respectively) with only one exception, and showed no relationship with soil pH, indicating that it behaved differently from added P_i (recovery decreased from 86% at pH 4.6 to 13% at pH 3.6). Thus, correcting for the incomplete recovery of biomass P using added P_i is inappropriate for acid soils. Although microbial biomass P in soil is generally estimated using the P_i flush and a conversion factor (k_P) of 0.4, more reliable estimates require that k_P values are best determined independently for each soil.

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Introduction

The microbial biomass mediates the transformation of biogenic nutrients (N, P and S) between inorganic and organic components and as such has an important role in their cycling in soils and availability to plants. Chloroform ($CHC1_3$) fumigation followed by extraction (at a soil:solution ratio of 1:4 or 1:2 w/v) has been used routinely for measuring microbial biomass C, N and S in a range of soils (Jenkinson 1988; Wu et al. 1994). For measuring biomass P, Olsen extractant (0.5 M $NaHCO_3$, pH 8.5) has usually been used for all soil types (Brookes et al. 1982; Hedley and Stewart 1982; Sarathchandra et al. 1984; Srivastava and Singh 1988). However, this extractant gives unreliable results for soils with a strong P-sorbing ability (Olsen and Sommers 1982; Kuo et al. 1988; Shuman et al. 1988). Specifically, Potter et al. (1991) showed that the flush of P determined using Olsen extractant was erratic in acid soils. In a more recent study, Oberson et al. (1997) recommended the use of the Bray-1 extractant (0.03 M NH_4F–0.025 M HCl) to measure biomass P from tropical soils with a high P-sorbing ability. Furthermore, the procedures proposed by different workers for measuring biomass P in soil were quite different.

In the procedure used by Brookes et al. (1982), soil was fumigated for 24 h in gaseous $CHCl_3$ then extracted using the Olsen extractant at a soil:solution ratio of 1:20 (w/v) for 30 min. Biomass P was calculated from the increase in inorganic P (the P_i flush) in the fumigated soil using a conversion factor (k_P). The value of k_P (0.4) was considered to represent the proportion of microbial biomass P extracted following fumigation. Brookes et al. (1982) suggested that, of the biomass P extracted, up to 80% was determined as inorganic P (P_i) and as such biomass-P figures should be corrected for sorption by the soil during extraction using values

for the recovery of added P_i. Hedley and Stewart (1982) adopted the same extractant but used a shorter fumigation time (30 min) with liquid $CHCl_3$, an extended extraction time of 16 h, and a larger soil:solution ratio (1:50). They also used a k_P value of 0.4 to calculate the amount of biomass P from the increase in total extractable P (the P_t flush) following fumigation, but did not correct the P value for the efficiency of P_i recovery. In subsequent work, McLaughlin et al. (1986) recommended the use of gaseous fumigation and a shorter extraction time, as proposed by Brookes et al. (1982), but supported Hedley and Stewart (1982) in recommending that total extractable P should not be corrected for the recovery of added P_i.

In this study, the methodology for measuring biomass P in acid soils was examined. The effectiveness of the Olsen and Bray-1 extractants was compared at soil:solution ratios of 1:4 and 1:20 (w/v), respectively. Additional investigations were made using ^{32}P-labelled bacterial and fungal biomass added to soil to compare the behaviour of P derived from the biomass during extraction with that of added P_i to determine whether it was appropriate to correct for the sorption of released biomass P in acid soils using added P_i. The purpose was to optimize the fumigation-extraction method for measuring biomass P in acid soils.

Materials and methods

Soils and sampling

The soils listed in Table 1 were from the University of Newcastle, Cockle Park Farm, Northumberland, UK. They were selected to provide a range of soil characteristics (pH, total P, C:P ratio, and microbial biomass C) and different management regimes. Soils 1 and 2 were sandy loams and had been maintained permanently under a ley-arable rotation. Soils (3–8) were from the Palace Leas Hay Meadow experiment and were clay loams. Different fertilizer treatments at this site have been continued since the establishment in 1896 (Pawson 1960). Soil cores (0–20 cm) from each plot were bulked, sieved (<2 mm), and mixed thoroughly to give a combined sample for each soil. Plant debris and living tissues, and any visible soil animals, were carefully removed. Moisture contents were adjusted to 40% of field water-holding capacity using distilled water. Samples were then conditioned for 10 days at 21 °C and 100% humidity.

Fumigation, sterilization and extraction

Portions of moist soils (6×5.0 g and 6×25.0 g, on an oven-dry weight basis) were weighed into glass jars and exposed to $CHCl_3$-vapour for 24 h in a vacuum desiccator at room temperature (Powlson and Jenkinson 1976). After residual $CHCl_3$ was removed, fumigated portions were transferred into polypropylene bottles (150 ml). To each half of these bottles (three containing 5.0 g portions and three containing 25.0 g portions) 100 ml of the Olsen (0.5 M $NaHCO_3$) or Bray-1 (0.03 M NH_4F–0.025 M HCl) extractants was added separately. All bottles were rotary shaken for 30 min. Suspensions were then centrifuged at 2,000 *g* for 10 min and filtered through Whatman No 42 filter papers into plastic bottles (60 ml). Equivalent portions of unfumigated soils were also extracted with both of the extractants.

An additional six portions of moist soil (25.0 g) were sterilized at 120 °C for 4 h in a high-pressure sterilizer (2.5 bar). After cooling, three portions were extracted with the Bray-1 extractant (100 ml) and the remaining three portions were fumigated for 24 h prior to extraction.

Determination of P

For determining total extractable P (P_t), extracts were digested using a procedure modified from Brookes et al. (1982). Aliquots (15 ml) of extracts were pipetted into digestion tubes (75 ml). Prior to digestion, $NaHCO_3$ extracts were neutralized with 0.25 ml concentrated H_2SO_4 (added slowly) and left for 4 h with regular shaking to remove CO_2 bubbles. Tubes containing NH_4F–HCl extracts were treated with two drops of H_2SO_4 (conc.). To minimize vigorous bubbling and to protect against P loss during digestion, 1.0 g K_2SO_4 and 0.5 ml saturated $MgCl_2$ were added to each sample. Samples were then digested for 30 min at 102–105 °C with 0.2 ml H_2O_2 (30%, v/v). Where extracts remained coloured, digestion was extended for 30 min after the addition of extra H_2O_2 (0.1–0.5 ml, depending on the intensity of the colour). Then, 0.5 ml $HClO_4$ (70%, v/v) was added to each tube and the digestion continued for 1 h. After cooling, solutions were adjusted to 75 ml with distilled water and P determined spectrophotometrically as described by Olsen and Sommers (1982). Inorganic P (P_i) was determined in fresh extracts by the same spectrophotometric procedures. The amount of extractable organic P (P_o) was calculated as the difference between the measured values of P_t and P_i.

Recovery efficiency of P_i

The Olsen and Bray-1 extractants were spiked with KH_2PO_4 and ^{32}P as Na_2HPO_4 (Amersham, UK) to give a P_i concentration of 10 mg l^{-1} and a specific activity of 0.5 kBq mg^{-1} P. This concentration was selected as being representative of the range of increases

Table 1 Characteristics of the soils studied. *FYM*: farmyard manure applied at 20 t ha^{-1} per year; N, P and K fertilizers were applied at 17.5, 13 and 56 kg ha^{-1} per year to soil 3 (plot 1), and doubled for the other soils where applied

Soil no.	Site	Management	pH	Organic C[a] (%)	Total P (mg kg^{-1})	C:P ratio	Microbial biomass C (mg kg^{-1} soil)
1	East Tower Hill	Lay/arable rotation, NPK	5.7	2.4	720	34:1	670
2	Davy Houses	Lay/arable rotation, NPK	5.9	2.8	910	31:1	790
3	Palace Leas, plot 1	Grassland, FYM+NPK	5.0	5.3	2000	24:1	1880
4	Palace Leas, plot 2	Grassland, FYM	4.6	5.3	1900	28:1	1670
5	Palace Leas, plot 13	Grassland, NPK	4.1	3.7	820	45:1	1040
6	Palace Leas, plot 8	Grassland, P	4.4	4.3	1200	36:1	1770
7	Palace Leas, plot 7	Grassland, N	3.6	6.3	750	84:1	1710
8	Palace Leas, plot 6	Grassland, Nil	3.8	3.3	350	94:1	1320

[a] Values taken from Wu et al. (1994)

Table 2 Amounts of total P (P_t) and inorganic P (P_i) in unfumigated (*Unfum*) and fumigated (*Fum*) soils extracted by the Olsen extractant (0.5 M $NaHCO_3$, pH 8.5) at soil:solution ratios of 1:20 and 1:4 (w/v). The *Flush* value was calculated from the value for fumigated soil minus that for the unfumigated soil. *SDD* Standard deviation of difference, obtained by ANOVA procedures

Soil no.[a]		At 1:20				At 1:4			
		Unfum	Fum	Flush	SDD[e]	Unfum	Fum	Flush	SDD
		(mg P kg^{-1} soil)							
1	P_t	103.9	134.2	30.1	28.1	41.8	48.4	6.6	5.8
	P_i	87.3	107.8	20.4	11.1	33.8	42.6	8.8*	2.2
	P_o	16.6	26.4	9.8	32.4	18.3	21.8	3.5	2.1
2	P_t	46.7	58.4	11.7	5.2	18.3	21.8	3.5	2.1
	P_i	26.0	39.1	13.1**	2.1	11.5	16.7	5.2*	1.0
	P_o	20.7	19.3	−1.6	5.2	6.8	5.0	−1.8	1.9
3	P_t	124.1	183.0	59.0**	8.6	24.2	23.8	−0.4	0.8
	P_i	77.3	94.5	17.2**	2.1	17.4	26.5	9.1*	2.7
	P_o	46.8	88.8	41.8**	6.3	6.8	−2.7	−9.5	3.3
4	P_t	102.0	132.3	30.4	19.2	15.2	19.8	4.6*	1.6
	P_i	36.4	53.2	16.8*	4.1	7.6	12.2	4.6*	1.5
	P_o	65.6	79.1	13.5	16.4	7.6	7.6	0.0	1.1
5	P_t	98.3	114.3	16.0	9.7	36.7	40.0	3.3	4.1
	P_i	28.5	47.6	19.1**	1.6	2.9	4.1	1.2	1.9
	P_o	69.8	66.7	−3.1	10.4	33.8	35.9	2.1	5.9
6	P_t	85.7	88.9	3.2	23.5	25.7	39.1	13.4*	2.6
	P_i	4.4	5.9	1.5	0.6	6.3	12.3	6.0*	1.4
	P_o	81.3	83.0	1.7	23.9	19.4	26.8	7.4	3.8
7	P_t	69.9	92.5	22.4*	6.8	29.2	51.6	22.4**	0.4
	P_i	5.6	16.6	11.0*	1.5	1.2	3.5	2.3**	0.4
	P_o	64.3	75.9	11.4	6.9	28.0	48.1	20.1**	0.7
8	P_t	168.2	172.5	4.3	6.0	68.2	74.8	6.6**	2.0
	P_i	7.3	12.7	5.4	5.1	3.1	3.9	0.8	2.1
	P_o	160.9	159.8	−1.1	2.0	65.1	70.9	5.8**	0.8

* and ** represent significant ($P<0.05$) and highly significant ($P<0.01$), respectively
[a] See Table 1

in extractable-P obtained following fumigation of test soils (Tables 2, 3). Both extractants were used to extract soils (unfumigated) at a soil:solution ratio of 1:4 (w/v), as described above. Extracts (1 ml) were mixed with 10 ml of scintillation cocktail (Ready Safe, Beckman) in a 22-ml scintillation vial (Packard, Canberra). The vials were then counted for 5 min in a liquid scintillation counter (LS2000, Beckman Instruments). All counts were automatically corrected using a quench curve for ^{32}P stored in the scintillation counter. The recovery of added P_i was calculated from ^{32}P activities in the extracts expressed as a percentage of that counted in an equivalent volume of the extractants.

Recovery of ^{32}P added as cultured 'bacterial' and 'fungal' biomass

^{32}P-labelled soil bacterial and fungal biomass were grown separately in the laboratory, as described by McLaughlin et al. (1986). To grow bacterial biomass labelled with ^{32}P, soil suspensions (1 ml at 10^{-4} dilution) were inoculated into 750 ml TSB medium (0.3%) containing 100 mg cycloheximide and 75 MBq ^{32}P (in Na_2HPO_4) in a 2.5-l flask. After incubation at 20 °C for 5 days in the dark without shaking, the biomass was harvested by centrifugation (10,000 *g*) for 20 min at 4 °C and repeatedly washed by resuspending in 0.85% (w/v) NaCl in a 250 ml tube and centrifuging to remove any unincorporated ^{32}P.

To produce ^{32}P-labelled fungal biomass, a medium containing (l^{-1}) 660 mg $NaNO_3$, 330 mg KH_2PO_4, 165 mg KCl, 165 mg $MgSO_4$.7H2O, 6.6 mg $FeSO_4$, 165 mg yeast extract, 10 g sucrose, 100 mg streptomycin sulphate, 5 mg tetracycline hydrochloride, and 100 MBq ^{32}P was prepared. Fungal inoculum was provided by adding a soil suspension (1 ml at 10^{-1} dilution) to 750 ml of the medium in a 2.5 l flask. The flask was incubated at 20 °C for 5 days without shaking. The 'fungal mass' produced was collected by vacuum filtration (nitrocellulose membrane), washed thoroughly with 0.85% NaCl, blotted dry, and cut into small pieces before use.

Aliquots of the 'bacterial' suspension (1 ml, containing approximately 1 kBq ^{32}P) and the portions of 'fungal' mass (70 mg, containing approximately 1 kBq ^{32}P) were thoroughly mixed with 12 portions of moist soil (5.0 g). Six were extracted with the Olsen or Bray-1 extractants (each 20 ml) without fumigation. The remaining portions were fumigated before extraction. The procedures for fumigation, extraction and the determination of ^{32}P were as described above, except that 5 ml of extract was used for counting ^{32}P. ^{32}P activity in the 'bacterial' and 'fungal' biomass was counted in separate portions which had not been mixed with soil. The recovery of biomass ^{32}P was calculated from the difference between ^{32}P recovered with and without fumigation expressed as a percentage of ^{32}P in the added biomass.

Soil properties and statistics

For the measurement of soil biomass C, soils were fumigated and extracted according to Vance et al. (1987). Extracts were analysed as described by Wu et al. (1990). Total soil P was measured spectrophotometrically following Na_2CO_3-fusion (Olsen and Sommers 1982). Soil pH was determined in 1 M KCl at a soil:solution ratio of 1:2.5 (w/v). All measurements were done in triplicate. All data were analysed by ANOVA and the means tested for significant differences using the *t*-test routine provided in the STATISTICA 4.0 software package. In these analyses, the statistical significance of the P_t, P_i and P_o flushes, calculated from the mean values for the fumigated soil minus those for the unfumigated soil, was equivalent to that of the difference between the means.

Table 3 Amounts of total P (P_t) and inorganic P (P_i) in the soils extracted by the Bray-1 extractant (0.03 M NH_4F–0.025 M HCl) at soil:solution ratios of 1:20 and 1:4 (w/v)

Soil no.[a]		At 1:20				At 1:4			
		Unfum[a]	Fum	Flush	SDD	Unfum	Fum	Flush	SDD
		(mg P kg^{-1} soil)							
1	P_t	162.7	175.5	12.8	24.6	47.6	57.9	10.4*	3.8
	P_i	149.1	154.8	5.7	5.8	40.3	50.0	9.7*	2.6
	P_o	13.6	20.7	7.1	16.5	7.3	8.0	0.7	7.7
2	P_t	112.3	133.7	21.4	8.9	15.6	20.9	5.3*	1.5
	P_i	110.1	123.5	13.4*	4.2	13.9	23.1	9.2**	0.5
	P_o	2.1	10.0	7.9	9.1	1.8	−2.2	−4.0	3.1
3	P_t	233.6	282.1	48.5**	7.0	59.0	91.1	32.1**	0.8
	P_i	186.0	212.3	26.3*	4.9	56.6	82.7	26.1**	0.1
	P_o	47.6	69.8	22.2	11.7	2.4	8.4	6.0*	0.9
4	P_t	106.6	153.0	46.3**	8.4	22.8	49.6	26.8**	3.8
	P_i	83.4	121.0	37.6**	4.1	19.8	42.2	22.4**	1.2
	P_o	23.2	32.0	8.8	11.8	3.0	7.3	4.3	3.5
5	P_t	46.0	68.5	22.5*	6.1	7.6	18.5	10.9**	1.3
	P_i	35.9	53.2	17.3*	4.7	5.4	17.6	12.2**	0.8
	P_o	10.1	15.3	5.2	4.2	2.2	0.9	−1.3	1.8
6	P_t	59.0	91.4	32.4**	5.3	10.3	25.1	14.8**	0.5
	P_i	48.7	78.6	29.9**	2.3	8.8	27.0	18.2**	0.5
	P_o	10.3	12.8	2.5	3.2	1.5	−1.9	−3.4	1.1
7	P_t	10.6	26.9	16.3**	2.4	1.7	3.5	1.8*	0.7
	P_i	4.3	18.6	14.3**	0.3	0.5	8.1	7.6**	0.3
	P_o	6.3	8.3	2.0	2.6	1.2	−4.6	−5.8	0.4
8	P_t	8.3	11.7	3.4	2.0	3.7	6.0	2.2*	0.5
	P_i	8.2	18.1	9.9**	0.8	1.4	7.5	6.1**	0.5
	P_o	0.1	−6.6	−6.7	2.2	2.3	−1.5	−3.9	0.9

[a] See Table 2 for description of abbreviations

Results and discussion

Effectiveness of the Olsen extractant in extracting P released by fumigation in acid soil

Increased dissolution of humic materials (as judged by the darkening of the extracts) from the acid soils with the Olsen extractant, made complete digestion of the extracts very difficult and resulted in large variations in the values of P_t in the extracts (Table 2). Thus, the value of the P_t flush (difference between total extractable P in the fumigated soil and that in the unfumigated soil) determined by the Olsen extractant at 1:20 (w/v) was not statistically significant ($P>0.05$) in the majority (75%) of the soils used. When extracted at 1:4, a significant P_t flush was found in 50% of the soils used. At both ratios, the coefficient of variation (CV) of the P_t flush $[CV=(SDD/\sqrt{2})/(\text{the flush value})\times 100\%]$ was generally larger than 20%. As a result, the value of the P_o flushes calculated from P_t flushes minus P_i flushes varied widely (from –3.1 to 41.8 mg P kg^{-1} soil at 1:20 and −9.5 to 22.4 mg P kg^{-1} soil at 1:4), and in most of the soils were not statistically significant.

The performance of the Olsen extractant was better when the P_i flush (difference between extractable inorganic P in the fumigated soil and that in the unfumigated soil) was measured. This is shown by the fact that for 50% of the soils extracted at 1:4 and for 75% at 1:20, the values for the P_i flush were statistically significant or very significant ($P<0.05$ or <0.01, respectively) and the CV values were generally less than 20% (Table 2). However, the values of the P_i flushes for soils 1 and 2 determined at both ratios (1:20 and 1:4), were quite different (by 1.6- to 1.7-fold, Table 2), although both soils had been maintained under the same management and had developed similar pH, C:P ratio, and microbial biomass values (Table 1). In soil 6, which had received P fertilizer continuously since 1896, the value for the P_i flush determined at 1:20 was small (1.5 mg P kg^{-1} soil). In contrast, for soils 7 and 8, which had not received P fertilizer since 1896, the magnitude of the P_i flush was 3.6–7.3 times greater than in soil 6. Furthermore, the value of the P_i flush, determined at 1:20, was 12.7 times larger in soil 5 than in soil 6, whereas at 1:4 the value was five times larger in soil 6. These results clearly do not reflect the effects of fertilizer inputs and the size of the microbial biomass on the amount of microbial biomass P in these soils.

As shown in Table 2, neither the P_t flush nor the P_i flush determined using the Olsen extractant at both soil:solution ratios (1:20 and 1:4) provided good indicators of microbial biomass P in the acid soils tested. This is in agreement with previous work that also questioned the reliability of the Olsen extraction procedure for biomass P in acid soils (Potter et al. 1991; Oberson et al. 1997).

Table 4 Amounts of total P (P_t), inorganic P (P_i), and organic P (P_o) in unfumigated and fumigated sterilized soil extracted with the Bray-1 extractant (0.03 M NH_4F–0.025 M HCl) at 1:4 (w/v). Data presented are means and standard errors

Soil no.	Unfumigated			Fumigated		
	P_t	P_i	P_o	P_t	P_i	P_o
	(mg P kg^{-1} soil)					
1	72.5 ± 4.4	71.4 ± 1.1	1.1	63.9 ± 4.5	65.7 ± 0.6	−1.8
2	43.3 ± 1.7	43.2 ± 0.6	0.1	44.1 ± 1.8	43.2 ± 0.4	0.9
3	104.6 ± 3.3	88.0 ± 1.5	16.6	86.6 ± 2.6	78.5 ± 0.3	8.1
4	61.3 ± 2.5	33.9 ± 1.5	27.4	55.4 ± 1.2	29.0 ± 0.1	26.4
5	39.0 ± 1.0	21.2 ± 0.2	17.8	31.5 ± 1.7	18.7 ± 1.0	12.8
6	19.4 ± 1.0	17.3 ± 0.2	2.1	20.7 ± 0.7	19.6 ± 0.1	1.1
7	16.0 ± 0.4	5.7 ± 0.1	10.3	12.5 ± 0.8	5.3 ± 1.2	7.1
8	14.9 ± 1.3	12.9 ± 1.0	2.0	16.5 ± 1.6	14.7 ± 1.0	1.8

Effectiveness of the Bray-1 extractant in extracting P released by fumigation in acid soil

In most of the test soils, the values of the P_i and P_t flush, measured by Bray-1 extractant at a soil:solution ratio of 1:20, were significant or very significant ($P>0.05$ and 0.01, respectively; Table 3). The CV ranged between 10% and 136% for the P_t flush and between 1.5% and 72% for the P_i flush. With the exception of soil 8, the P_o flush (2.0–22.2 mg P kg^{-1} soil) accounted for a substantial proportion (7.7–55.5%) of the P_t flush but was not statistically significant. In soil 8, the P_o flush appeared as a negative value (–6.7 mg P kg^{-1} soil). As with the Olsen extractant, the use of the P_o flush data is unreliable in interpreting the P_t flush obtained using the Bray-1 extractant at 1:20.

Of the methods used in this study, the Bray-1 extractant at 1:4 gave the best performance. At this ratio, the P_t flush was significant (four soils) or very significant (also four soils) for the soils tested (Table 3). The CV of the P_t flush varied from 1.8% to 27.5%. The P_i flush determined at this ratio was highly reproducible in all of the soils ($P<0.01$, except soil 1 with $P<0.05$; Table 3). In addition, the standard deviation of the P_i flush was generally below 1 mg P kg^{-1} soil and the calculated CV was small (0.3–19%). It is interesting to note that the P_o flush in the Bray-1 extracts at 1:4 was very different from that at 1:20 or that found with the Olsen extracts at both of the soil:solution ratios tested. For most of the soils, the P_o flush in the Bray-1 extracts at 1:4 was negative (from −1.0 to −6.0 mg P kg^{-1} soil). In the remaining soils (1, 3 and 4), where a positive P_o flush was obtained, its size was relatively small (0.7–6.0 mg P kg^{-1} soil, equivalent to 7–23% of P_i). This provides further evidence that the P_o flush is of little importance in estimating biomass P in acid soils but only adds to the variance in the P_t flushes.

The investigation was extended to test whether $CHCl_3$-fumigation increased the solubility of non-biomass P in acid soils. Sterilization at high pressure (2.5 bar) at 120 °C could kill microorganisms in soil and release P from the biomass into non-biomass forms. Thus, any increase in extractable P by fumigating sterilized soils can be considered as an effect of fumigation. The data presented in Table 4 suggest that this was not the case. After the sterilized soils were fumigated, the amounts of P_t, P_o and P_i either decreased (in soils 1 and 3–5) or remained constant (in soils 2 and 6–8; Table 4). The decrease was most likely due to the resorption of biomass P released by sterilization during the 24-h fumigation period. Nevertheless, the data indicate that $CHCl_3$-fumigation followed by extraction with the Bray-1 extractant does not increase the extractability of non-biomass P in acid soils. This is in agreement with the findings of previous studies conducted with Bray-1 (Oberson et al. 1997) and Olsen extractions (Brookes et al. 1982; Hedley and Stewart 1982).

Recovery of added P_i and microbial biomass P

When extracted in the presence of soil, the recovery of ^{32}P-labelled P_i spiked in both of the Olsen and Bray-1 extractants was influenced by soil pH (Fig. 1). General-

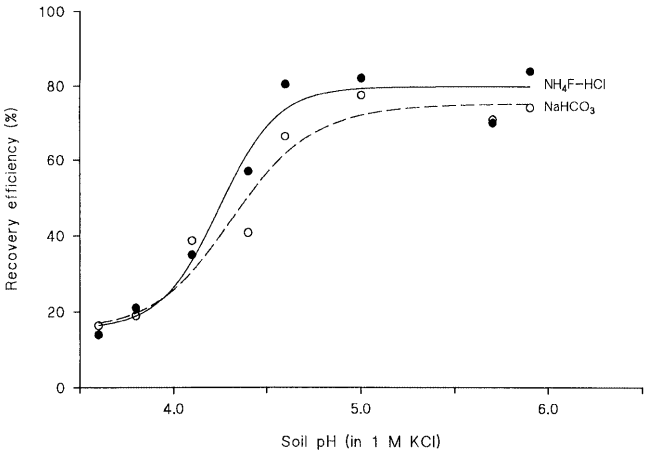

Fig. 1 Relationship between the recovery using the Olsen and Bray-1 extractants of ^{32}P-labelled inorganic orthophosphate P added to soils and soil pH, determined at a soil:solution ratio of 1:4

ly, the recovery of ^{32}P-labelled P_i was 10% higher in the Bray-1 extractant than in the Olsen extractant, indicating a lower resorption capacity of P_i in the Bray-1 extractant. In soils with pH values of 4.6–5.9, the recovery of added P_i in both extractants remained relatively constant and close to the maximum value predicted from the curves (76 ± 7% for the Olsen extractant and 86 ± 6% for the Bray-1 extractant; Fig. 1). Over this pH range, the recovery of added P_i in the Olsen extractant was comparable to that found previously in neutral and calcareous soils (Brookes et al. 1982, 1984; McLaughlin et al. 1986). However, as soil pH decreased below 4.6, the recovery of added P_i in both extractants decreased sharply until at pH 3.6 the recovery became very low (13–16%; Fig. 1).

As shown in Table 5, the percentage recovery of 'bacterial P' and 'fungal P' added to the soils and extracted by the Olsen extractant (1:4, w/v) ranged from 1.7% to 29.1% (average 19.6%) and 3% to 16.5% (average 10.3%), respectively. These values were much smaller than those (33–57%) measured previously in neutral and calcareous soils (Brookes et al. 1982; Hedley and Stewart 1982; McLaughlin et al. 1986). When extracted with the Bray-1 extractant, the recovery of the added 'bacterial P' and 'fungal P' ranged from 11.6% to 36.7% (average of 27.9%) and from 9.1% to 25.7% (average of 18.9%), respectively (Table 5). The average recovery values were 1.4–1.8 times larger than those for the Bray-1 extractant. These results indicate clearly that the Olsen extractant is not as effective as the Bray-1 extractant for measuring microbial biomass P in acid soils.

In the methods proposed by Hedley and Stewart (1982) and later by McLaughlin et al. (1986), the P_t flush was not corrected for the recovery of added P_i, as proposed by Brookes et al. (1982). The former workers suggested that no simple relationship exists between the recovery of biomass P determined as P_t and that of added P_i. Data presented in Table 5 indicate that the recovery of both 'bacterial P' and 'fungal P' (labelled with ^{32}P) added to the acid soils and extracted in either the Olsen or the Bray-1 extractant was relatively constant (24.1–36.7% and 15.7–25.7%, respectively), with the exception of soil 7, and showed no relationship with soil pH (Table 1). A possible hypothesis is that the biomass P released by fumigation is not completely hydrolysed to P_i during the 30 min extraction period (a reaction possible during the subsequent analysis procedure due to the presence of H_2SO_4). As such the sorption pattern of P derived from the killed biomass in acid soils would be quite different from that of added P_i, because the recovery of added P_i largely depended upon soil pH (Fig. 1). This questions the validity of using added P_i for establishing the resorption of released biomass P during the 30-min extraction period. The data in Table 3 show that with the Bray-1 extractant, particularly at a soil:solution ratio of 1:4, the difference between the P_t and P_i flush in all of the soils was not statistically significant. Thus, correcting either the P_t or the P_i flush for the recovery of added P_i is inappropriate.

Table 5 Recovery of ^{32}P-labelled bacterial P and fungal P added to the soils extracted by the Olsen extractant (0.5 M $NaHCO_3$, pH 8.5) and the Bray-1 extractant (0.03 M NH_4F–0.025 M HCl) at 1:4 (w/v). Values for the control (extracted without fumigation) have been subtracted. *SEM* Standard error of the mean

Soil no.[a]	In the Olsen extractant		In the Bray-1 extractant	
	Bacterial P (% of addition)	Fungal P (% of addition)	Bacterial P	Fungal P
1	21.6 ± 0.3	13.9 ± 0.7	28.1 ± 0.7	25.7 ± 0.8
2	29.1 ± 0.4	12.7 ± 0.4	24.1 ± 1.4	15.7 ± 0.3
3	20.1 ± 0.4	6.0 ± 0.4	31.1 ± 1.6	16.2 ± 0.9
4	22.8 ± 1.2	7.4 ± 0.7	29.1 ± 0.5	21.5 ± 2.1
5	21.2 ± 1.1	10.2 ± 0.4	36.7 ± 1.2	22.6 ± 1.4
6	25.9 ± 0.3	16.5 ± 0.9	35.8 ± 0.9	21.6 ± 0.3
7	1.7 ± 0.2	3.0 ± 0.1	11.6 ± 0.5	9.1 ± 1.4
8	14.4 ± 1.1	13.0 ± 0.3	26.5 ± 0.2	19.1 ± 0.5
Mean ± SEM	19.6 ± 3.0	10.3 ± 1.6	27.9 ± 2.8	18.9 ± 1.8

[a] See Table 1

Estimating biomass P in the acid soils

As identified above, the P_i flushes obtained using the Bray-1 extractant (at 1:20 and 1:4) provide better information for estimating biomass P in the test soils than data obtained by any of the other approaches investigated (the P_t and P_o flushes obtained by the Olsen extractant or the P_t flushes by the Bray-1 extractant). Thus, data from the former were used to calculate biomass P in the soils. It has been suggested that the value of k_P is best determined separately for each soil type, because of differences in P-sorbing ability and microbial community structure (Hedley and Stewart 1982; McLaughlin et al. 1986). However, previous workers have generally used a k_P value of 0.4 to calculate the amount of biomass P, despite disagreement over the need to correct for the recovery of biomass P using added P_i (Brookes et al. 1982; Hedley and Stewart 1982). In order to keep our calculation procedure comparable with previous work, this value (k_p 0.4) was also adopted. The procedure for correcting the P_i flush using the recovery of added P_i, as proposed by Brookes et al. (1982), was not used here because the recovery of added P_i did not represent the recovery of microbial biomass P in these acid soils (see above). Furthermore, previous studies have established that the amount of microbial biomass P in soil is related to cultivation, fertilizer inputs and the size of the microbial biomass (Brookes et al. 1984; He et al. 1997). In this study, the soils used were carefully selected as pairs (i.e. nos 1 and 2, nos 3 and 4, and nos 7 and 8). Each pair of soils was similar in terms of cultivation type and P inputs, and had comparable pH values, C:P ratios, and size of the microbial biomass (Table 1). The exceptions were soils

Table 6 Quantity (calculated from the P_i flush divided by a k_P value of 0.4) of microbial biomass P in the soils determined using the Bray-1 extractant (0.03 M NH_4F–0.025 M HCl) at 1:20 and 1:4 (w/v) and the relationship with biomass C (C:P) and total P (see data in Table 1)

Soil no.	At 1:20			At 1:4		
	Biomass P (mg kg^{-1} soil)	C:P ratio	Biomass P Total P (%)	Biomass P (mg kg^{-1} soil)	C:P ratio	Biomass P Total P (%)
1	14.3 ± 14.5	47:1	2.0	24.3 ± 6.5	28:1	3.5
2	33.5 ± 10.5	24:1	3.8	23.0 ± 1.3	34:1	2.6
3	65.7 ± 12.3	29:1	3.3	65.5 ± 0.3	29:1	3.0
4	94.0 ± 10.3	18:1	4.9	56.0 ± 3.0	30:1	3.0
5	43.3 ± 11.8	24:1	5.4	30.5 ± 2.0	34:1	3.7
6	74.8 ± 5.8	24:1	6.2	45.5 ± 1.3	39:1	3.8
7	35.8 ± 0.8	48:1	5.1	19.0 ± 0.7	90:1	2.5
8	24.8 ± 2.0	53:1	8.3	15.3 ± 1.3	86:1	5.1

5 and 6, which cannot be considered as a pair, because of distinct differences in the size of the microbial biomass.

When calculated from the value of the P_i flush obtained with the Bray-1 extractant at 1:20, the amount of biomass P in the soils ranged from 14 to 94 mg P kg^{-1} soil, and the C:P ratio of the biomass ranged between 18:1 and 53:1 (Table 6). However, the amounts of biomass P and the C:P ratios of the biomass in the two soils within the same pairs (see above) were quite different, generally by a factor greater than 1.4. If these values are accepted then management, particularly P inputs and soil P content, is not related to C:P ratios in these soils (Table 1).

The amounts of biomass P calculated from the value of the P_i flush determined at 1:4 ranged from 15 to 65 mg P kg^{-1} soil (Table 6). This represents 2.5–5.1% of soil total P. The amounts of biomass P and the C:P ratios of the biomass were comparable for soils 1 and 2, soils 3 and 4, and also for soils 7 and 8. For soil 5, the amount of biomass P was 67% of that in soil 6, reflecting the difference in the size of the microbial biomass. However, the C:P ratios of the biomass in these soils were also close to each other (34:1 and 39:1), showing good agreement with soil P status (Table 1). In soils 1–6, the C:P ratios of the biomass (28–39:1) were within the ranges published previously (generally 15–35:1; Brookes et al. 1982, 1984; Sarathchandra et al. 1984; Srivastava and Singh 1988; Srivastava et al. 1989). In soils 7 and 8, where the low P status did not prevent the establishment of a large microbial biomass (1,380–1,760 mg biomass C kg^{-1} soil, Table 1), the C:P ratio of the biomass was, surprisingly, not much wider (86–90:1, Table 6). Thus, in all of the soils used, the amounts of biomass P determined using the Bray-1 extractant at 1:4 (w/v) reflect both the known P status of the soils and the size of the microbial biomass.

In conclusion, extraction with the Bray-1 extractant following $CHCl_3$-fumigation provides a better estimate of microbial biomass P in acid soils (pH 3.6–5.9) than does the Olsen extractant. Extraction at a soil:solution ratio of 1:4 is recommended because the results are considered more reliable and consistent with other measures of P status and biomass C in the test soils. The data also show that the resorption of biomass P released by $CHCl_3$–fumigation and recovered in the Bray-1 extractant is different from that of added P_i, making it inadvisable to correct for the recovery of biomass P using added P_i.

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