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ORIGINAL PAPER

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Determination of soil microbial biomass phosphorus in acid red soils from southern China

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Abstract A $CHCl_3$ fumigation and 0.03 M NH_4F-0.025 M HCl extraction procedure was used to measure microbial biomass P (P_{mic}) in 11 acid red soils (pH <6.0) from southern China and the results compared to those obtained by the commonly-used $CHCl_3$ fumigation and 0.5 M $NaHCO_3$ extraction method. Extraction with NH_4F-HCl was found to be more effective and accurate than $NaHCO_3$ extraction for detecting the increase of P from microbial biomass P following chloroform fumigation due to its higher efficiency in extracting both native labile phosphate and added phosphate (^{32}P) in the soils. This was confirmed by the recovery of ^{32}P from in situ ^{32}P-labeled soil microbial biomass following fumigation and extraction by the NH_4F-HCl solution. Soil microbial biomass P, measured by the NH_4F-HCl extraction method, was more comparable with soil microbial biomass C (with a more narrow C:P ratio range of 4.3 to 22.3 and a mean of 15.6 in the microbial biomass), than that obtained by $NaHCO_3$ solution (with a mean C:P ratio of 30.7 and a wide range of 14.9 to 48.9). K_p, the fraction of soil microbial biomass P extracted after $CHCl_3$ fumigation, by the NH_4F-HCl solution was 0.34. The amount of microbial biomass P determined (using K_p =0.34) was 3–400% (mean 131%) higher than that obtained by the $NaHCO_3$ extraction (using K_p =0.40) for the 11 red soils studied. The results suggest that the $CHCl_3$ fumigation and NH_4F-HCl extraction method is more reliable for measuring microbial biomass P than the $NaHCO_3$ extraction method in acid red soils.

Keywords Fumigation-extraction method · Microbial biomass phosphorus · Acid red soils

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Introduction

Soil organisms are the driving force behind C turnover and plant nutrient transformations and play an important role in soil fertility and ecosystem functioning (Smith and Paul 1990). Both microbial biomass and activity can be used as an indicator of nutrient availability and the effect of agricultural practices and environmental impact on soil sustainability for agriculture.

Over the last two decades, great progress has been made in measuring microbial biomass C (C_{mic}), N (N_{mic}), P (P_{mic}) and S (S_{mic}) with the development of a rapid fumigation-extraction technique in soil (Saggar et al. 1981; Brookes et al. 1982, 1985; Hedley and Stewart 1982; Wu et al. 1990, 1994). Although microbial biomass P has been reported to make a large contribution to the plant-available P pool in soil (Brookes et al. 1984; Sparling et al. 1987; Srivastava and Singh 1988; Perrott and Sarathchandra 1989; Smith and Paul 1990; Chen et al. 2000), it is not always a good indicator of plant-available P (Srivastava et al. 1989). For measuring P_{mic}, 0.5 M $NaHCO_3$ is usually used as the extractant after chloroform fumigation. However, this method was developed and tested mainly with neutral and mildly alkaline soils (pH 6.0–7.9; Brookes et al. 1982; Hedley and Stewart 1982; McLaughlin and Alston 1986) and requires validation for different types of soils, particularly those which are moderately or strongly acidic (pH below 6.0). In principle, a reliable extractant for P_{mic} measurement should be effective in removing labile P released by soil fumigation without being much affected by soil properties such as P-sorption capacity. As far as extraction efficiency is concerned, 0.5 M $NaHCO_3$ is less effective than some acidic chemical reagents for extracting labile P from

acid soils or soils with a large amount of labile P (Holford and Cullis 1985; He et al. 1988, 1991; Shuman et al. 1988) although it has been widely used as an index of available P in neutral to alkaline soils (Olsen and Summers 1982). In addition, being an alkaline solution (pH 8.5), the $NaHCO_3$ extracts are usually coloured due to dissolved organic matter and this often makes the determination of P concentration difficult. In recent years, it has been reported that $NaHCO_3$ as an extractant is not suitable for measuring P_{mic} in some acid soils. Wu et al. (2000) found that 0.03 M NH_4F-0.025 M HCl as an extractant improve reproducibility of the P flush following fumigation, contributing to a better estimate of P_{mic}, compared with 0.5 M $NaHCO_3$ in eight acid soils from the United Kingdom. Oberson et al. (1997) recommended the use of the NH_4F-HCl extractant to measure P_{mic} from tropical soils with a high P-sorption ability. However more work is needed to validate whether or not NH_4F-HCl is indeed a suitable extractant for measuring P_{mic} in a large range of acid soils, including the red soils with strong P-sorption capacity widespread in southern China as well as in many tropical and subtropical regions.

In this study, the two extractants were compared for their ability to extract labile P as well as PO_4^{3-}-^{32}P added to soils. The recovery of native soil microbial biomass ^{32}P was further investigated, and the C:P ratio of soil microbial biomass by the two extractants was also examined to clarify which extractant was more appropriate for measuring P_{mic}.

Materials and methods

Soils

The 11 red soils used in this study were collected from Lanxi county of Zhejiang Province, southern China. Soil samples were taken from the 0- to 20-cm soil layer. Selected soil properties are shown in Table 1. Soil organic matter was determined by a dichromate oxidation method (Nelson and Sommers 1982). Soil pH (water) was measured using a Beckman 120 pH meter (Beckman, Calif.) at a soil:solution ratio of 1:1. Total P was measured by the $HClO_4$-H_2SO_4 digestion method. Soil extractable P was determined by the 0.03 M NH_4F-0.025 M HCl extraction method (Bray1 P) or by the 0.5 M $NaHCO_3$ extraction method, respectively (Fig. 1). Phosphorus concentration in the digest or extract was determined by ascorbic acid reduction colorimetry (Olsen and Sommers 1982).

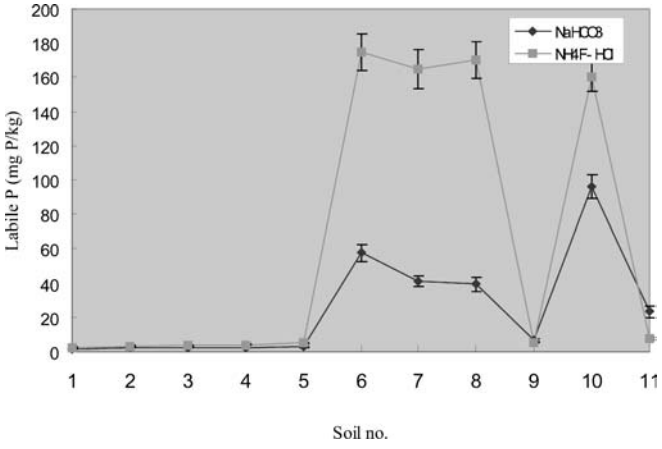

Fig. 1 Comparison of soil labile P extracted by 0.5 M $NaHCO_3$ and 0.03 M NH_4F-0.025 M HCl

Samples were adjusted to 40% of field water-holding capacity (WHC), sieved (<2 mm) and stored at 4°C. Before use, the soils were incubated at 100% humidity and 25°C for 10 days.

Measurement of C_{mic} and P_{mic}

The basic procedure for measuring C_{mic} and P_{mic} was as described by Jenkinson and Powlson (1976), Brookes et al. (1982) and Wu et al. (1990). Briefly, fresh moist soil samples were exposed to alcohol-free $CHCl_3$ vapour in a vacuum desiccator at room temperature for 24 h. The fumigated soils were then placed in a clean empty desiccator and residual $CHCl_3$ removed from the fumigated soil by repeated evacuation. Microbial biomass C was measured by extracting the fumigated soil immediately following $CHCl_3$ removal by shaking for 30 min with 0.5 M K_2SO_4 at a solution:soil ratio of 4:1. After filtration through a Whatman no. 42 filter paper, the filtrate was analyzed for organic C using an automated TOC analyzer (500 model; Shimadzu, Japan). Microbial biomass C was calculated as follows: $C_{mic} = E_c/K_c$, where E_c is the increase in extractable C in the fumigated soil over that in the control (without fumigation), and F_c is the fraction of C_{mic} extracted following fumigation. Here a value of K_c =0.45 was used (Wu et al. 1990). Microbial biomass P was measured as described by Wu et al. (2000) by extracting both the fumigated and non-fumigated soil samples with 0.03 M NH_4F 0.025 M HCl or 0.5 M $NaHCO_3$ for 30 min at solution:soil ratio of 4:1. Suspensions were centrifuged (3,000× *g* for 10 min) and filtered through Whatman no. 42 filter paper. Phosphorus concentration in the filtrate was determined colorimetrically (Olsen and Sommers 1982). Microbial biomass P was calculated from the following formula: $P_{mic} = E_p/K_p$, where E_p

Table 1 Some basic properties of the tested soils

Soil no.	Land use	pH		Organic C (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})
		H_2O	KCl			
1	Eroded fallow	4.5	3.8	1.87	0.46	0.40
2	Woodland (China fir)	4.5	3.9	7.42	0.63	0.38
3	Woodland (maple)	4.4	3.7	5.80	0.52	0.22
4	Woodland (metasequoia)	4.5	3.6	10.61	0.76	0.40
5	Bamboo land	4.9	3.8	5.92	0.52	0.49
6	Citrus grove	5.0	4.0	6.90	0.64	1.25
7	Fallow grassland	5.9	5.0	4.00	0.55	1.12
8	Upland	6.0	5.5	5.57	0.70	1.13
9	Tea garden	4.2	3.5	6.84	0.50	0.43
10	Vegetable field	5.6	4.9	12.30	0.81	1.30
11	Paddy field	5.0	3.9	11.66	0.84	1.23

is the increase in extractable P in the fumigated soil over that in the control, and K_p is the fraction of P_{mic} extracted after fumigation. Correction of microbial biomass P was made using recovery of added phosphate as described below (Brookes et al. 1982).

Recovery of added phosphate (^{32}P)

Twenty millilitres 0.5 M $NaHCO_3$ (pH 8.5) or 0.03 M NH_4F-0.025 M HCl containing a specific P concentration (as KH_2PO_4) and 5 kBq ^{32}P (as $NaH_2{}^{32}PO_4$, carrier-free) were added to a 5.0-g soil sample (oven-dry basis) in a 50-ml plastic centrifuge tube. A specific P concentration similar to the amount of P released from soil microbial biomass was chosen, based on a preliminary experiment. The soil suspensions were shaken on an end-over-end shaker at 400 rpm for 30 min and then centrifuged (3,000× *g* for 10 min) and filtered through Whatman no. 42 filter paper. ^{32}P activity in the extract was determined using a liquid scintillation counter (Wallace Winspectry, Finland) with automatic correction of ^{32}P natural decay. The recovery of added P was calculated from the ratio of the activity in the extract over that of the added ^{32}P.

Recovery of soil microbial biomass ^{32}P

^{32}P-labelled tryptic soy broth (TSB) was prepared in suspension by mixing TSB, N (in the form of NH_4NO_3) and P (^{32}P and ^{31}P, in the form of NaH_2PO_4) fertilizers with different amounts of deionized water containing glucose. The suspension was added to the field-moist soils (5.0 g oven-dry basis) in 50-ml polypropylene centrifuge tubes at a rate which supplied 2,000 μg C g^{-1} oven-dry soil, 225 μg N g^{-1} oven-dry soil and 60 μg P, 5 kBq ^{32}P g^{-1} oven-dry soil. Sufficient water was added to bring the moisture content of the soils to 40% WHC. The samples were then placed in jars with 100% humidity and soda lime to absorb CO_2 and incubated at 20°C. When microbial activity reached a peak (after approximately 3 days), the soils were fumigated, and both the fumigated and the nonfumigated samples were extracted with 20 ml 0.03 M NH_4F-0.025 M HCl or 0.5 M $NaHCO_3$ for 30 min. The conditions of the extraction, centrifugation, and filtration of the soil suspensions, as well as the measurement of ^{32}P activity in the extract, were exactly as described earlier. The recovery of ^{32}P in both the control (NF) and fumigated (F) soil was calculated as follows:

$$\%\text{recovery} = {}^{32}\text{P activity recovered}/{}^{32}\text{P activity added}$$

Any increase in the recovery of ^{32}P from the fumigated treatments over the control was attributed solely to lysis and extraction of P from soil micro-organisms in situ. The K_p value from the flush of soil microbial phosphorus was calculated from the following formula:

$$K_p = (F/r - NF/r)/\left[{}^{32}\text{P added} - (NF/r)\right]$$

where F is the ^{32}P activity recovered from the fumigated sample, NF is the ^{32}P activity recovered from the control, and r is the recovery of added P without incubation (as obtained from the above experiment). Therefore, (F/r – NF/r) or (F – NF)/r is the amount of ^{32}P released from the microbial biomass after the soil has been fumigated, and [^{32}P added – (NF/r)] is the amount of ^{32}P which had been incorporated into the soil microbial biomass after a 3-day incubation. All analyses were conducted in triplicate.

Results and discussion

Effectiveness of P extraction and the determination of extracted P

The ability of the two reagents to extract labile P (usually called available P) varied substantially between the soils, and was affected by soil P status (Fig. 1). For soils with a high labile P content (soils 6, 7 and 8), 0.03 M NH_4F-0.025 M HCl was much more effective than 0.5 M $NaHCO_3$. The amount of P extracted by NH_4F-HCl was 1.7 to 4.3 times larger than that extracted by $NaHCO_3$ (Fig. 1). For soils with very low extractable P contents (soils 1–5, 9 and 11), however, NH_4F-HCl and $NaHCO_3$ were almost equally effective. For soils 1–5, the amount of P extracted by NH_4F-HCl was slightly higher (1.4–1.8 times) than that extracted by $NaHCO_3$, whereas, for soils 9 and 11, $NaHCO_3$ extracted more P (1.2–3.1 times) than NH_4F-HCl (Fig. 1). In addition, the dark colour of the $NaHCO_3$ extracts presented problems with P determination. For some soils (soils 4 and 11), repeated treatment with activated charcoal was required to remove the dissolved organic material from the $NaHCO_3$ extract and this probably decreased the accuracy of P determination for the soils with low extractable P contents. In contrast, little organic matter was dissolved by the dilute acid fluoride reagent (NH_4F-HCl) during extraction and thus there was no problem with the determination of the extracted P.

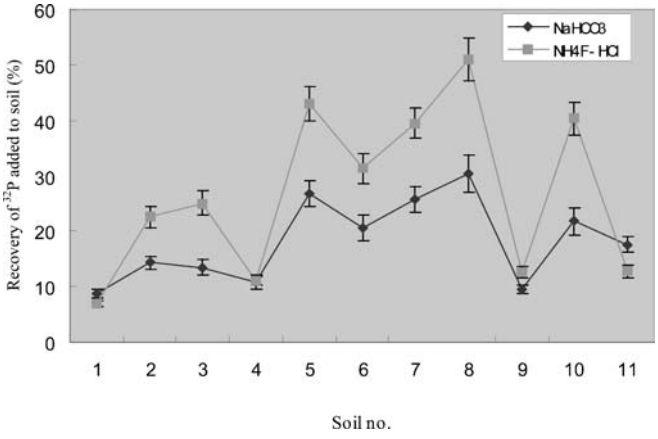

Fig. 2 Recovery of inorganic ^{32}P added to soil by 0.5 M $NaHCO_3$ or 0.03 M NH_4F-0.025 M HCl

Figure 2 shows the recovery of added P ($H_2{}^{32}PO_4^-$) by the two extractants. The NH_4F-HCl extractant recovered a larger amount of the added P (^{32}P) than did the $NaHCO_3$ extractant from most of the test soils, particularly from those with a high labile P content or those soils with a relatively high P recovery (soils 2, 3, 5–8 and 10). Exceptions were soils 1, 4, 9 and 11, for which the recovery of added ^{32}P by NH_4F-HCl was slightly higher or lower than that by $NaHCO_3$. Furthermore, it can be seen that the recovery rate of P by the two extractants, which ranged from 6.9% to 51.1%, was low for these soils, suggesting that the red soils have a strong P-sorbing ability.

Table 2 Recovery of soil microbial biomass ^{32}P by 0.5°M $NaHCO_3$ or 0.03 M NH_4F-0.025 M HCl

Soil no.	$NaHCO_3$				HCl-NH_4F			
	Unfumigated	Fumigated	Flush	K_P	Unfumigated	Fumigated	Flush	K_P
	%				%			
1	6.9±0.4	8.1±0.3	1.2	0.67±0.10	4.1±0.2	5.5±0.3	1.4	0.50±0.07
2	8.4±0.2	10.2±0.3	1.8	0.30±0.02	14.5±0.5	17.7±0.7	3.2	0.39±0.02
3	7.6±0.1	8.3±0.2	0.7	0.12±0.02	16.9±0.3	18.9±0.2	2.0	0.24±0.01
4	6.2±0.2	7.2±0.1	1.0	0.22±0.03	7.4±0.2	9.0±0.4	1.6	0.42±0.03
5	15.8±0.7	17.9±0.8	2.1	0.19±0.01	21.3±1.1	28.2±0.9	6.9	0.32±0.02
6	8.4±0.6	10.0±0.3	1.6	0.13±0.02	16.8±0.8	20.8±0.8	4.0	0.27±0.01
7	10.3±0.1	13.4±0.5	3.1	0.20±0.03	20.2±1.0	27.4±1.1	7.2	0.37±0.03
8	17.7±0.9	21.7±0.3	4.0	0.31±0.01	27.6±0.6	35.7±1.0	8.1	0.34±0.02
9	4.8±0.2	5.9±0.1	1.1	0.23±0.03	5.2±0.2	7.1±0.3	1.9	0.25±0.02
10	11.4±0.3	12.4±0.2	1.0	0.09±0.01	21.5±0.9	25.6±0.8	4.1	0.22±0.01
11	10.1±0.4	14.3±0.6	4.2	0.56±0.08	5.5±0.3	8.9±0.5	3.4	0.46±0.08
Mean			2.0	0.27			4.0	0.34

Recovery of P_{mic}

The measurement of soil microbial biomass P requires an extractant that can detect the P flush with accuracy after the soil has been fumigated. An extractant with a high recovery efficiency for soil labile P or for added P may not necessarily be able to detect the flush of P following fumigation (Brookes et al. 1982; McLaughlin and Alston 1986). To test the effectiveness of these two extractants for the detection of the P flush from microbial biomass after biocidal treatments, soils were labelled with ^{32}P-$H_2PO_4^-$ in situ prior to extraction of microbial ^{32}P with either 0.03 M NH_4F-0.025 M HCl or 0.5 M $NaHCO_3$ following $CHCl_3$ fumigation. The ^{32}P flush was attributed entirely to microbial ^{32}P on the assumption that (1) the labelled organisms represent the whole microbial population in the soil and (2) the $CHCl_3$ fumigation has little effect on the extractability of non-microbial ^{32}P (Brookes et al.1982; Hedley and Stewart 1982; McLaughlin and Alston 1986). The results obtained (Table 2) indicated that a larger ^{32}P flush was detected with NH_4F-HCl than with $NaHCO_3$, with only one exception (soil 11). The difference in the ^{32}P recovery between the fumigated soils and the controls, measured using NH_4F-HCl extractant, was 1.2 to 4.1 times larger than that obtained using $NaHCO_3$ extractant for soils 1–10, whereas the P flush by NH_4F-HCl extraction was slightly lower than that by $NaHCO_3$ extraction for soil 11. These results are in good agreement with those found for the extraction of labile P or added P (^{32}P) from these soils, suggesting that $NaHCO_3$ extraction may underestimate P_{mic} of these acidic red soils.

K_p, the fraction of soil biomass P released and extracted following fumigation (Brookes et al. 1982), is usually determined by adding cultured micro-organisms to the soil (Brookes et al. 1982; Hedley and Stewart 1982; McLaughlin and Alston 1986). Brookes et al. (1982) proposed a K_p value of 0.4, using 0.5 M $NaHCO_3$ as the extractant, based on their results using four bacteria and four fungal species added to three soils. This value of K_p is similar to that reported by Hedley and Stewart (0.37; 1982) using two different bacteria and two different fungi added to one soil. McLaughlin and Alston (1986) found K_p values of 0.33, 0.40 and 0.57, respectively, for the three soils using a mixed population of soil micro-organisms, fumigation by hexanol and 0.5 M $NaHCO_3$ as the extractant. The K_p values are best determined for indigenous micro-organisms with different soil types (Wu et al. 2000) and an alternative way to determine K_p is to measure the recovery of ^{32}P-labeled native soil microbial biomass P. This way is arguably more reliable due to the fact that it is labelling native soil micro-organisms and as such is superior to adding cultured micro-organisms to soils.

In the present study, K_p was measured using 11 red soils with microbial populations developed in situ by amending the soil with glucose and $H_2PO_4^-$-^{32}P-labelled tryptic soy broth. In estimating K_p in this way, it was assumed that the amount of added P-^{32}P transformed to soil organic matter-^{32}P and not extractable by $NaHCO_3$ or NH_4F-HCl during the 3-day period of incubation following amendment, was minimal. Furthermore, the recovery of ^{32}P in the $NaHCO_3$ or NH_4F-HCl-extractable fraction of the non-biomass organic-^{32}P was assumed to be comparable to that of the untransformed PO_4^{3-}-^{32}P remaining in the soil. Therefore, the total non-biomass-^{32}P (non-biomass organic-^{32}P + the remaining PO_4^{3-}-^{32}P) in soil can be calculated from the total extractable ^{32}P in the non-fumigated soil corrected using the recovery efficiency of PO_4^{3-}-^{32}P. The microbial biomass-^{32}P (i.e. the added PO_4^{3-}-^{32}P immobilized by the biomass during the 3-day incubation) is the difference between the added PO_4^{3-}-^{32}P and the total soil non-biomass-^{32}P. Thus, K_p can be calculated from the flush of the extractable-^{32}P measured in the fumigated soil divided by the microbial biomass-^{32}P developed in the soil. The results (Table 2) indicated that, for the 11 soils, there was considerable variation in the K_p values (0.09 to 0.67) measured using $NaHCO_3$ extraction with the average value of K_p (0.27) substantially lower than the value (0.40) reported by Brookes et al. (1982). This suggests that the $NaHCO_3$ extraction probably failed to detect the full flush of P from the

Table 3 Comparison of soil microbial biomass P measured using the 0.5 M $NaHCO_3$ or 0.03 M NH_4F-0.025 M HCl extraction method

Soil no.	C_{mic}	C_{mic}/C_{org} [a]	$NaHCO_3$		HCl-NH_4F	
			P_{mic} [b]	C_{mic}/P_{mic}	P_{mic}	C_{mic}/P_{mic}
	(mg kg^{-1})	(%)	(mg kg^{-1})		(mg kg^{-1})	
1	68.3±2.6	3.7	2.0±0.16	34.2	5.6±0.3	12.2
2	165.6±10.1	2.2	6.6±0.51	25.1	7.7±0.6	21.5
3	93.6±5.7	1.6	2.8±0.18	33.5	4.5±0.2	20.8
4	186.3±14.5	1.8	5.7±0.42	32.6	9.7±0.8	19.2
5	149.4±11.2	2.5	6.9±0.33	21.7	6.7±0.5	22.3
6	141.5±10.8	2.1	5.3±0.40	26.7	12.2±0.9	11.6
7	132.6±9.7	3.3	3.5±0.24	38.0	10.2±0.6	13.0
8	167.3±12.4	3.0	5.0±0.36	33.5	7.5±0.2	22.3
9	117.6±8.7	1.7	2.4±0.19	48.9	12.0±0.5	9.8
10	205.6±15.3	1.7	7.2±0.62	28.7	13.8±0.6	14.9
11	224.9±17.9	1.9	15.1±1.21	14.9	52.3±2.1	4.3
Mean				30.7		15.6

[a] C_{mic} Microbial biomass C, C_{org} total organic C
[b] P_{mic} Microbial biomass P

microbial biomass following fumigation, as indicated by the very small K_p values (0.09 to 0.13) for some of the tested soils (soils 3, 6 and 10). In contrast, K_p values (from 0.22 to 0.50) measured with NH_4F-HCl extraction varied much less between the tested soils (Table 2), and the average K_p value (0.34) was higher than that obtained by $NaHCO_3$ but similar to other reported values (0.33 to 0.57; Hedley and Stewart 1982; McLaughlin and Alston 1986; Wu et al. 2000). The NH_4F-HCl extraction procedure seems superior to the $NaHCO_3$ procedure for the determination of K_p and the estimation of soil microbial biomass P in these acid soils based on the recovery of soil microbial biomass ^{32}P.

P_{mic} measured by NH_4F-HCl extraction

To evaluate further the NH_4F-HCl method, C_{mic} of the tested soils was also determined. The results are shown in Table 3. The C_{mic} in the soils ranged from 68.3 to 225 mg C kg^{-1}, accounting for 1.6 to 3.7% (mean 2.3%) of the total organic C. These data are in good agreement with previous results (Anderson and Domsch 1980; Hasebe et al. 1985). P_{mic} in the present study measured by the NH_4F-HCL method, ranged from 4.5 to 52.3 mg P kg^{-1} compared to 2.0 to 15.1 mg P kg^{-1} using the $NaHCO_3$ extraction. On average, P_{mic} increased by 131% using NH_4F-HCL compared to that obtained using $NaHCO_3$. The mean ratio of C:P in soil microbial biomass was 15.6, ranging from 4.3 to 22.3 using NH_4F-HCL extraction, whereas the corresponding value with $NaHCO_3$ was 30.7, ranging from 14.9 to 48.9. There is considerable variation in the reported C:P ratios of soil microbial biomass. Based on C_{mic} measured by 0.5 mol/l K_2SO_4 and P_{mic} by 0.5 mol/l $NaHCO_3$, Brookes et al. (1984) found the mean soil microbial biomass C:P ratio was 14, ranging from 11 to 36 in 15 soils from both arable and grassland. This was much lower than the mean C:P ratio reported by other authors; e.g., a mean value of 27 (ranging from 15 to 63) for 21 New Zealand pasture soils (Perrott and Sarathchandra 1982) and a value 29 for five tropical, coal mine spoils (Srivastava et al. 1989) but higher than the value of 9 reported by Patra et al. (1990) for a grassland soil. The present C:P ratio values ranging from 14.9 to 48.9, mean value of 30.7, based on the same method (i.e., C_{mic} by 0.5 mol/l K_2SO_4 and P_{mic} by 0.5 mol/l $NaHCO_3$) in the 11 soils were very similar to the values of 27 to 29 given by Perrott and Sarathchandra (1982) and Srivastava et al. (1989), but substantially higher than the value of 14 reported by Brookes et al. (1984) and Patra et al. (1990). It is interesting to note that most of the soils used by Perrott and Sarathchandra (1982) and Srivastava et al. (1989) were acid soils (pH <6.0) compared to the mostly neutral soils (pH 6.6–7.5) studied by Brookes et al. (1984) and Patra et al. (1990). The C:P ratio in the present study for the acid red soils using NH_4F-HCl extraction were similar to those reported by Brookes et al. (1984) and Patra et al. (1990) for neutral soils with 0.5 M $NaHCO_3$ extraction, but markedly lower than those determined using the $NaHCO_3$ method in low pH soils (Perrott and Sarathchandra 1982; Srivastava et al. 1989). Therefore, the C:P ratio also supports the idea that the $NaHCO_3$ extractant is less effective than the NH_4F-HCl extractant in detecting the flush of P from fumigated biomass in low pH soils.

In conclusion, for acid soils (pH <6.0), the 0.03 M NH_4F-0.025 M HCl extraction is a more reliable method for measuring soil microbial biomass P compared to the 0.5 M $NaHCO_3$ extraction because it gives higher recovery of both PO_4^{3-}-^{32}P added to soils and soil microbial biomass ^{32}P by isotope tracing. Furthermore, the K_p values and C:P ratio for microbial biomass determined using the NH_4F-HCl extraction procedure are more comparable to the commonly reported values, provided one accepts that the 0.5 M $NaHCO_3$ extraction gives a reliable estimate of soil microbial biomass P for neutral soils. The 0.5 M $NaHCO_3$ extraction would therefore seem unsuitable for measuring microbial biomass P in acid red soils, particularly where the extractable P content is high, as it fails to detect the full flush of P

from the microbial biomass following $CHCl_3$ fumigation as evidenced by the low K_p value for these soils.

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