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SHORT COMMUNICATION

DETERMINATION OF PHOSPHORUS RELEASED BY
CHLOROFORM FUMIGATION FROM MICROBIAL
BIOMASS IN HIGH P SORBING TROPICAL SOILSA. OBERSON,^{1,2*} D. K. FRIESEN,^{1,3} C. MOREL⁴ and H. TIESSEN⁵

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Soil microbial biomass is known to be an important reservoir of phosphorus in soils (Brookes *et al.*, 1984; Srivastava and Singh, 1988; Morel *et al.*, 1997). Microbial P (P_{mic}) is usually determined by fumigating samples of soil with chloroform ($CHCl_3$) and measuring the inorganic P (P_i) released by lysis of microbial biomass (P_{chl}). P_{mic} is calculated from P_{chl} by applying a correction factor (k_p) to correct for the incomplete release of P from microbial cells during fumigation (Brookes *et al.*, 1982; Hedley and Stewart, 1982). In this method of Brookes *et al.* (1982), it is assumed that the reactions of P_i with soil constituents during 24 h of fumigation are negligible, and only sorption reactions occurring during extraction are accounted for. However, P_i added to soil solution initially reacts rapidly, and then more slowly with soil mineral surfaces with rates and amounts depending on soil properties (Barrow and Shaw, 1975; Morel *et al.*, 1994). Morel *et al.* (1997) proposed a fumigation–sorption method to account for these sorption reactions. In this procedure, a short fumigation period (75 min) relative to the subsequent equilibration period (40 h) is used so that P_{chl} is released as a spike of P_i into soil solution. A parallel set of unfumigated samples are equilibrated in a range of aqueous P_i solutions whose concentrations bracket the expected P_{chl} of the fumigated soils. P_{chl} prior to sorption is calculated from the P_i sorption isotherm constructed on the unfumigated samples. Morel *et al.* (1997) found that P_{chl} determined by fumigation–extraction (Brookes *et al.*, 1982) was 18–53% of the P_{chl} determined by the fumigation–sorption method on a range of temper-

ate zone soils differing in pedogenesis and cropping history. The greatest underestimation was observed with the soil having the highest sorption capacity (Morel *et al.*, 1997).

The fumigation–sorption method was developed with soils having medium to low P sorption capacity (Morel *et al.*, 1997). Our attempts to apply the method to highly weathered tropical soils (Oxisols and Ultisols) with medium to high P sorption capacity were frustrated by our inability to detect variations in P_i in solution following equilibration with P additions in the expected range of the P_{chl} spike. We therefore describe the adaptations that are required to apply the fumigation–sorption method of Morel *et al.* (1997) to estimate P_{chl} in Oxisols and Ultisols. They mainly concern the choice of a suitable extractant.

Soil samples were taken from two long-term field experiments located on Oxisols (tropheptic haplustox isohyperthermic) on the Eastern Plains of Colombia. Both soils have medium to high P sorption capacity, according to the classification of Juo and Fox (1977). The fractions of clay, silt and sand of the 0–10-cm layer were, respectively, 39, 42 and 19% at the Carimagua site and approximately 40, 30 and 30% at the Matazul site. Treatments included native savanna, improved pastures and monoculture rice receiving increasing P fertilizer inputs (Table 1). Samples were cooled immediately in the field and brought back to the laboratory in ice-chests where they were stored at 4°C in plastic bags prior to analysis within 4 wk of sampling. Within this period, no effect of storage on P_{chl} could be detected. Morel *et al.* (1997) report reproducible values for storage periods of 8 wk.

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Table 1. Description of the soils used

Soil no.	Site and cropping history	Total P input (kg ha ⁻¹)	pH	Total P ^c	Chemical soil characteristics ^b		Total N	Organic C (mg kg ⁻¹)
					Organic P ^d	Bray II-P ^e (mg kg ⁻¹)		
1	Carimagua; native savanna	0	4.8 ± 0.03	185 ± 3	63.8 ± 2.6	1.3 ± 0.1	1454 ± 28	23.7 ± 0.7
2	Carimagua; grass-only pasture, since 1978	103	4.9 ± 0.03	193 ± 6	68.4 ± 3.6	1.4 ± 0.2	1555 ± 24	22.6 ± 0.5
3	Carimagua; grass-legume pasture, since 1978	106	5.0 ± 0.03	227 ± 5	77.1 ± 3.8	2.2 ± 0.3	1670 ± 41	24.9 ± 0.5
4	Matazul; continuous rice monocrop, since early 1989; low fertilization level	168	4.3 ± 0.05	274 ± 5	ND	15.1 ± 1.1	1476 ± 37	24.0 ± 0.1
5	Matazul; continous rice monocrop, since early 1989; moderate fertilization level	250	4.8 ± 0.04	307 ± 14	107 ± 0.7	20.2 ± 1.6	1330 ± 14	19.8 ± 1.0

^aTotal P input from start of the field experiment until date of soil sampling.

^bMean and standard error of mean of eight or six samples per treatment for the Carimagua and Matazul site, respectively.

^cTotal P determined by perchloric acid digestion.

^dSum of organic P fractions extracted using the method of Tiessen and Moir (1993).

^e100 mM HCl, 30 mM NH₄F.

Estimation of P_{chl} was based on the fumigation-sorption method of Morel *et al.* (1997) using field-moist samples equivalent to 10 g of oven-dry soil in which the extractants 10 mM CaCl₂, 500 mM NaHCO₃ (Bic) and dilute acid-fluoride (30 mM NH₄F, 25 mM HCl) (Bray) replaced the water used by Morel *et al.* (1997). Four subsamples were fumigated for 75 min using chloroform liquid and vapor. At the end of the fumigation, a standard series of P_i solutions (0–50 mg P kg⁻¹) were added to duplicate unfumigated samples. Afterwards, deionized water (or, in the case of CaCl₂ extracts, 10 mM CaCl₂) was added to all subsamples to raise the soil-to-solution ratio to 1:1. All subsamples were then stored at 4°C for 1 d. For CaCl₂ extracts, the soil-to-solution ratio was further raised the next day to 1:10 using CaCl₂, and the subsamples were again stored at 4°C for an additional overnight period before analysis. This procedure corresponds to that of Morel *et al.* (1997) where CaCl₂ is substituted for water as extractant and the second overnight equilibration represents the extraction step. In the experiments with Bic or Bray, extraction was after the first 1-d equilibration period using soil-to-solution ratios of 1:20 and 1:10 and extraction times of 30 min and 1 min, respectively, as indicated in Olsen and Sommers (1982) for these standard methods.

In all cases, solutions were separated from soils by centrifugation (8 min, 5000 rev min⁻¹), and supernatants were decanted and vacuum filtered

through 0.45-µm filter disks. P_i in the extracts was determined by the method of Murphy and Riley (1962), as described by Tiessen and Moir (1993).

P adsorption during equilibration is described according to equation (1), adapted by Morel *et al.* (1997) from Barrow and Shaw (1975) for a fixed equilibration period at soil solution ratio of 1:1:

$$Ext_P = Ext_0 + b_1 P_{ad}^{b_2}, \quad (1)$$

where: Ext_P = P_i concentration (mg l⁻¹) extracted after equilibration with different amounts of P added; Ext_0 = P_i concentration extracted without P

Fig. 1. Comparison of 10 mM CaCl₂, 500 mM NaHCO₃ and dilute acid-fluoride as extractants to assess modifications in P concentrations induced by P additions in an Oxisol sample taken in a plot planted with grass-legume pasture.

Table 2. Determination of chloroform-released P corrected for sorption using 500 mM NaHCO₃ (Bic) or dilute acid-fluoride (30 mM NH₄F, 25 mM HCl) (Bray) as extractant

Extractant soil	Concentration		Regression coefficients			P _{Chl} (mg P kg ⁻¹)
	Ext ₀ (mg P l ⁻¹)	Ext _{Chl}	b ₁	b ₂	R ²	
Bic^a						
1	0.062	0.192	0.031	0.752	0.991	6.7
			0.017	—	0.924	7.6
2	0.100	0.283	0.012	1.030	0.997	14.1
			0.013	—	0.996	14.1
3	0.120	0.296	0.037	0.607	0.927	13.1
			0.014	—	0.817	12.6
4	0.289	0.398	0.006	1.234	0.735	10.5
			0.010	—	0.725	10.9
5	0.422	0.554	0.046	0.799	0.950	3.7
			0.029	—	0.930	4.6
Bray^a						
1	0.092	0.285	0.051	1.001	0.991	3.8
			0.051	—	0.991	3.8
2	0.120	0.401	0.033	1.139	0.997	6.6
			0.046	—	0.992	6.1
3	0.126	0.456	0.039	1.057	0.999	7.6
			0.045	—	0.998	7.3
4	0.860	1.206	0.064	0.937	0.991	6.1
			0.055	—	0.989	6.3
5	1.205	1.504	0.061	0.991	0.987	5.0
			0.060	—	0.987	5.0

^aThe first line of each row shows the regression coefficients, R² and P_{Chl} obtained using equations (1) and (2), respectively. In the second line, the linear regression coefficient and R² from the equation Ext_P = Ext₀ + b₁P_{ad}, and P_{Chl} = [(Ext_{Chl} - Ext₀)/b₁], are indicated.

addition; b₁, b₂ = coefficients estimated by non-linear regression of the mean value of Ext_P against P_{ad} for the unfumigated samples; and P_{ad} = amount of P added (0–50 mg P kg⁻¹).

Chloroform released P corresponds to a P addition and is calculated from a rearrangement of equation (1) as follows:

$$P_{\text{Chl}} = [(Ext_{\text{Chl}} - Ext_0)/b_1]^{1/b_2}, \quad (2)$$

where P_{Chl} = chloroform released P (mg kg⁻¹), and Ext_{Chl} = P_i concentration in extracts of fumigated samples.

Total P (P_t) in the extracts of fumigated samples and unamended, unfumigated samples was

measured after digestion with K₂S₂O₈ (Bowman, 1989). Organic P (P_o) was calculated as the difference between P_t and P_i.

The relationships between extracted inorganic P (Ext_P) and added P (P_{ad}) using either 10 M CaCl₂, Bic, or Bray are shown in Fig. 1 for one soil sample. Similar relationships were found for the other soil samples. In 10 M CaCl₂, detectable variations in Ext_P only occurred for P additions higher than 100 mg P kg⁻¹. Both Bic and Bray extractants showed detectable variations in the range of 0–20 mg P kg⁻¹ of added P. P_i released by fumigation (P_{Chl}) was not detectable in CaCl₂ extracts but ranged between 3 and 14 mg P kg⁻¹ soil in Bic and

Table 3. Inorganic and organic P contents in unfumigated and chloroform fumigated samples extracted with 5 mM NaHCO₃ (Bic) and dilute acid-fluoride (30 mM NH₄F, 25 mM HCl) (Bray)

Soil	Extractant	Unfumigated ^a		P _i /P _t	P _t	Fumigated P _i (mg kg ⁻¹)	P _i /P _t
		P _i	P _i (mg kg ⁻¹)				
1	Bic	1.2 (0.7) ^b	5.4 (0.5)	4.5	3.8 (0.2)	6.2 (0.2)	1.8
	Bray	0.9 (0.2)	0.4 (0.1)	0.4	2.9 (0.2)	0.5 (0.1)	0.2
2	Bic	2.0 (0.5)	7.2 (0.2)	3.6	5.7 (0.2)	8.3 (0.2)	1.5
	Bray	1.2 (0.1)	0.4 (0.1)	0.3	4.0 (0.1)	0.7 (0.2)	0.2
3	Bic	2.4 (0.6)	5.9 (0.5)	2.5	5.9 (0.7)	7.2 (0.6)	1.2
	Bray	1.3 (0.0)	0.3 (0.1)	0.2	4.6 (0.1)	0.4 (0.1)	0.1
4	Bic	5.8 (1.1)	7.2 (0.2)	1.2	8.0 (0.7)	8.7 (0.4)	1.1
	Bray	8.6 (0.3)	0.4 (0.3)	0.1	12.1 (1.1)	0.7 (0.2)	0.1
5	Bic	8.9 (0.9)	4.9 (0.9)	0.6	11.6 (0.6)	7.1 (0.1)	0.6
	Bray	12.1 (0.2)	ND ^c	ND	15.0 (0.5)	ND	ND

^aThe unfumigated sample was not P-amended.

^bSDs are given in parentheses.

^cND, not determined.

Bray extracts (Table 2). Thus, determination of P_{Chl} in these soils was only possible with the latter extractants. Concentration increases as a function of P additions were higher in Bray extracts than in Bic extracts (Fig. 1 and Table 2), indicating greater sensitivity to small differences in P_{Chl} in the Bray extractant. Furthermore, P extracted by Bray increased linearly with rate of P addition, and linear estimates are more precise for the Bray than for the Bic extractant (Table 2).

The method of Brookes *et al.* (1982) is based on the difference in Bic-extractable P_i between fumigated and unfumigated samples, and not on total P (which includes P_o), since they found a high and reasonably constant percentage of P_i in the CHCl_3 released total P. Limitation of the measurement to P_i requires furthermore that equal amounts of P_o are extracted in fumigated and unfumigated subsamples. To test whether this requirement was valid for our soils and extractants, and because P_o hydrolysis can cause errors during photometric P_i determination (Gerke and Jungk, 1991), the amount of P_o extracted from fumigated and unfumigated samples was compared for both the Bic and Bray extractants (Table 3). In unfumigated and unamended samples, P_o contents in Bic extracts were 0.6–4.5 times higher than P_i . With Bray, P_o contents were 0.1–0.4 times higher than the P_i content.

The P_o -to- P_i ratios in fumigated samples were lower than those observed in unfumigated samples (Table 3), primarily because fumigation increased the P_i more than the P_o contents of the extracts. Within the same soil, Bray-extracted P_o amounts were similar in fumigated and unfumigated subsamples, whereas for Bic, differences were 0.8–2.2 mg P_o kg^{-1} soil.

Bic extracted 12–20 times more P_o than Bray (Table 3). This value is probably an underestimation since a fraction of the P_o soluble in alkaline extracts may be hydrolyzed when determining P_i by the usual photometric methods (Gerke and Jungk, 1991). The yellow–brownish color of the Bic extracts caused by iron oxides and organic matter also increased uncertainty in the results, even though individual blanks were laboriously read for each aliquot. Blank readings were 50–70% of the sample readings for the Carimagua soils, and 10–20% for the Matazul soils with higher extractable P_i contents.

The effect of chloroform on non-microbial P_i extractable with Bray was tested using soil samples that had been sterilized by autoclaving for 4 h. P concentration in Bray extracts was the same for fumigated autoclaved samples as for unfumigated autoclaved samples without P addition (data not shown). Consequently, we assume that chloroform had no effect on Bray extractable non-microbial P in these residue poor soils, in agreement with the results of Brookes *et al.* (1982), Hedley and Stewart

(1982) and Morel *et al.* (1997), using Bic or water as extractants.

We conclude that the Bray extractant is more suitable than Bic for assessing the amounts of P released during CHCl_3 fumigation of low P acid soils with medium to strong P sorption capacity. The Bray extractant overcame problems of low P concentrations and weak slopes of P addition curves, which hindered the other extractants tested. It was sensitive to variations in concentration caused by P additions of less than 5 mg P kg^{-1} soil and to the low P_{Chl} values found in the soils used to elaborate the method. Furthermore, Bray extracted much less P_o than Bic and was thus less susceptible to errors caused by P_o hydrolysis during photometric P_i determination.

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