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SHORT COMMUNICATION

MINERALIZATION OF ORGANIC SULPHUR IN THE O2 HORIZON OF A HARDWOOD FOREST: INVOLVEMENT OF SULPHATASE ENZYMES

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The incorporation of sulphate-S (SO_4^{2-}-S) into organic matter in soil of deciduous forests of North Carolina has been suggested as a mechanism contributing to S accumulation in these ecosystems (Swank *et al.*, 1984). This process is microbially mediated and a substantial amount of the SO_4^{2-}-S was incorporated as phenolic sulphate-ester linkages (Fitzgerald *et al.*, 1983, 1985). The ester sulphate content of O1, O2 and A1 horizons was 13, 27 and 40% of total S, respectively (Fitzgerald *et al.*, 1987) indicating a standing stock of ester-sulphate in these combined surface horizons of 104 kg S ha^{-1}.

Once organic-S is formed, it can become mineralized (Strickland *et al.*, 1984, 1986). The release of SO_4^{2-} from organic matter may proceed by one of two mechanisms: oxidation of the carbon skeleton to yield energy and carbon for biosynthesis with SO_4^{2-} being released as a by-product, or directly after hydrolysis of ester-sulphate linkages comprising the organic-S (Dodgson *et al.*, 1982). Work with forest soil and litter (Strickland and Fitzgerald, 1984) suggests that depolymerization of organic-S coupled to hydrolysis is the preferred pathway. Sulphate-ester hydrolysis is catalyzed by sulphatase enzymes of differing substrate specificity and competitive inhibition of both arylsulphatase and alkylsulphatase activity by orthophosphate has been reported (Dodgson *et al.*, 1982; Al-khafaji and Tabatabai, 1979). As for the analogous phosphatase enzymes present in soil (Speir and Ross, 1978), some indirect evidence (Strickland and Fitzgerald, 1984) suggests that SO_4^{2-} release from organic-S is mediated by preformed sulphatase enzymes. In order to obtain direct evidence for the involvement of these enzymes, S mineralization occurring in organic matter extracts was assayed in the presence and absence of PO_4^{3-} and sodium azide. The latter agent inhibits microbial growth (and thus new sulphatase synthesis and product assimilation) but not sulphatase activity. Orthophosphate should inhibit the action of this enzyme. The use of extracts as opposed to parent material (Strickland and Fitzgerald, 1985) minimizes problems of interpretation associated with partial adsorption of SO_4^{2-} or added inhibitors.

The O2 horizon from a hardwood forest (watershed 18 of the Coweeta Hydrologic Laboratory, near Franklin, N.C.) was sampled in August 1984. Following the procedure of Strickland *et al.* (1986), organic matter was extracted from the sample with pyrophosphate at pH 8, the extract dialyzed to remove SO_4^{2-}, incubated for 24 h with glucose to stimulate microbial growth and finally incubated for 24 h with $^{35}SO_4^{2-}$ to permit formation of labelled organic-S. To determine if PO_4^{3-} inhibited S mineralization, the pyrophosphate extract containing the organic ^{35}S was dialyzed again for 24 h at 10°C against either 50 mM glycyl glycine-NaOH buffer or 50 mM KH_2PO_4-K_2HPO_4 buffer, both pH 8. The buffer/extract ratio was 20:1 and the buffer was changed 6 times. Each dialyzed extract was made 0.1 M with sodium azide to inhibit microbial growth and thereby prevent the re-incorporation into organic matter of $^{35}SO_4^{2-}$ released during mineralization (Strickland and Fitzgerald, 1984). The supplemented extracts were then held at 30°C with shaking and sub-samples (80 μl) were subjected to electrophoresis (Strickland and Fitzgerald, 1985) to determine the amount of $^{35}SO_4^{2-}$ released.

Strickland *et al.* (1986) showed that organic-S from the O2 horizon consists of two components both containing sulphate esters which can be distinguished by their electrophoretic mobility relative to that of SO_4^{2-} (taken as unity). One component remains at the origin and is believed to represent microbial biomass (origin component, $RSO_4^{2-} = 0$) whereas the other component migrates toward the anode with an $RSO_4^{2-} = 0.75$. Incubation after dialysis in the presence and absence of PO_4^{3-} as above resulted in a substantial decrease in the origin component which was accompanied by a corresponding increase in the mobile (RSO_4^{2-}, 0.75) component (Table 1). Because components with electrophoretic mobility are, by necessity, always smaller in size than those that remain at the origin, it is

Table 1. Influence of dialysis buffer and incubation time on the electrophoretic composition of organic ^{35}S[a]

Buffer (pH 8) and time (h)	^{35}S (% of total) of components having RSO_4^{2-} values of [b] 0	0.75	1.0
Glycine glycine			
0	51	49	ND[c]
96	17	75	8
288	14	71	15
Phosphate			
0	55	45	ND
96	36	64	ND
288	27	73	ND

[a]Dialyzed extracts containing organic ^{35}S were incubated with 0.1 M sodium azide at 30°C.
[b]RSO_4^{2-}, relative electrophoretic mobility compared to that of SO_4^{2-} taken as unity.
[c]Not Detected, see Fig. 1.

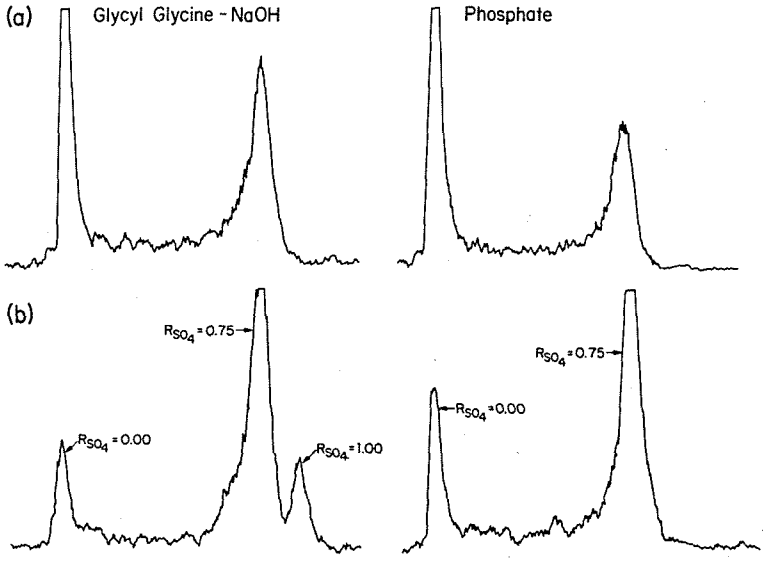

Fig. 1. Electrophoretograms of organic ^{35}S after dialysis against glycyl glycine-NaOH or phosphate buffer, (a) starting material and (b) after incubation with 0.1 M azide at 30°C for 288 h. Components were separated by electrophoresis on paper strips and after drying, the strips were scanned with a radiochromatogram scanner to produce the profiles shown above.

tempting to suggest that this conversion represents the depolymerization of insoluble organic-S which is believed to preceed S-mineralization (Houghton and Rose, 1976; Strickland *et al.*, 1984). In addition to the origin and mobile component, a third electrophoretic component (Fig. 1) was detected after incubation only of the extract dialyzed in glycyl glycine. The mobility of this component was identical to authentic SO_4^{2-} and it was undetectable, even in trace amounts, in the starting material or in the PO_4^{3-} dialyzed extract (Fig. 1). This latter result serves as a control for the non-enzymic release of SO_4^{2-}. Such a control is desirable particularly in view of the instability of some ester linkages in organic-S (Fitzgerald *et al.*, 1985). Inorganic $^{35}SO_4^{2-}$ represented 15% of the total radioactivity (Table 1) and failure to detect this anion in the extract containing PO_4^{3-} suggests the involvement of sulphatase enzymes in S mineralization. Because SO_4^{2-} was released in the presence of azide (glycyl glycine, Fig. 1), these enzymes must have been present in the extract before incubation. Further, microbial growth was not required to sustain enzyme activity suggesting that S mineralization is mediated by preformed, possibly extracellular, enzymes.

Sulphur mineralization was also assessed under conditions suitable for microbial growth. In this case, the organic ^{35}S in the initial pyrophosphate extract was added as the source of C and S (5% by vol, unsterilized) to a basal salts medium (Fitzgerald, 1973) lacking added SO_4^{2-}. The mixture was held at 30°C with shaking and samples were periodically taken for electrophoresis. A steady increase in the release of SO_4^{2-} was associated with decreasing amounts of both the origin as well as the mobile organic ^{35}S (Fig. 2). Sterilization was not possible because the organic-S occurs as a fine colloid thus precluding membrane filtration. Autoclaving was also ruled out because this treatment ruptures labile ester-sulphate linkages in the extract (Fitzgerald *et al.*, 1985). Nevertheless, several Gram-negative bacteria have been isolated from the O2 horizon of this forest and, when used as an inoculum for this basal salts medium, each isolate produced essentially the same pattern of release of SO_4^{2-} from the organic ^{35}S as that shown in Fig. 2.

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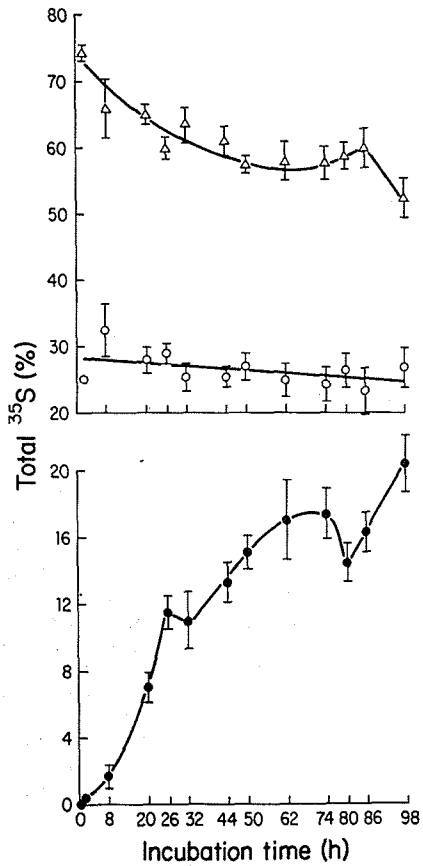

Fig. 2. Changes in electrophoretic composition of organic ^{35}S after incubation at 30°C under conditions favourable for microbial growth. After electrophoresis and scanning of electrophoretograms, ^{35}S present in each component was expressed as a % of the total radioactivity ($\pm$ one Standard Error, $N = 4$) (△), component with $RSO_4^{2-} = 0$; (○), component with $RSO_4^{2-} = 0.75$; (●), SO_4^{2-}.

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