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Isolation and partial characterization of forest floor and soil organic sulfur

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Abstract. The formation of organic sulfur from inorganic sulfate was investigated in hardwood forest floor and mineral horizons. All samples converted sulfate-sulfur into a non-salt extractable form which was recoverable only under conditions which release organic matter. This conversion was inhibited by azide, and depending upon the horizon, by erythromycin, candicidin, chloramphenicol and tetracycline. The form of sulfur generated in the O2 forest floor layer and in Al-horizon soil was characterized after isolation by pyrophosphate extraction at pH 8. The organosulfur extracts exhibited an average C:N:S ratio of 103:6:1. The ester sulfate content of the O2 extract was 61% by hydriodic acid (HI) reduction and 62% by hydrolysis in 3N HCl at 121 °C. However, compared to hydrolysis, reduction yielded lower estimates of ester sulfate for two of the three soil extracts analyzed. In view of the electrophoretic heterogeneity of all extracts, it is suggested that some may contain stable ester linkages that hydrolyze only after prolonged treatment and that the standard procedure for HI-reduction may provide conditions of temperature and contact time with the acid which are insufficient for the release of sulfate from these esters.

Introduction

There is an extensive literature on the *in situ* levels of ester and carbon bonded sulfur in the organic matter of agricultural soils (see e.g., Freney, 1961; Tabatabai and Bremner, 1972; Neptune et al., 1975; Bettany et al., 1980). Although interconversion may occur (Freney et al., 1975), ester sulfate predominates and, in some cases 90% of the total sulfur in these soils occurs as organic sulfur (Fitzgerald, 1976). Sulfur has also been detected as a constituent of organic matter in forest, aquatic, and peat accumulating ecosystems (Casagrande and Siefert, 1977; King and Klug, 1980; Mitchell et al., 1981; Strick et al., 1982; Landers et al., 1983). Generally, forest soils contain large quantities of sulfur as adsorbed sulfate (Fitzgerald and Johnson, 1982), and determinations for *in situ* levels of organic sulfur in a soil from an Adirondack forest indicate that much of this element is bonded directly to carbon (David et al., 1982).

The use of a ^{35}S label demonstrated that exogenous sulfate and sulfate derived from mineralization is incorporated into recently formed organic

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matter in agricultural and forest systems (Freney et al., 1971; Strickland and Fitzgerald, 1983; Fitzgerald and Andrew, 1984). A-horizon soil from forests of the Coweeta basin initially appear to incorporate most of the sulfur as ester sulfate (Fitzgerald et al., 1982). Moreover, studies of the subsequent metabolic fate of the incorporated label provide indirect evidence that sulfur in this fraction is subject to mineralization in both agricultural (Freney et al., 1975) and forest ecosystems (Strick et al., 1982; Strickland and Fitzgerald, 1983). Because methods for extraction are destructive, information relating to the composition and mineralization potential of soil organic sulfur tends to be indirect and thus somewhat equivocal. Nevertheless, these results coupled with *in situ* determinations collectively suggest that the metabolism of this form of sulfur is both complex and dynamic in soil from both systems. The composition and turnover potential of organic sulfur from forest floor and mineral soil horizons of the Coweeta basin was investigated directly in the current study using newly formed organic sulfur that had been isolated under conditions which maximize retention of all sulfur linkage groups. The use of tetrasodium pyrophosphate at pH 8 as an extractant coupled with electrophoretic analysis of the fractions obtained during isolation has made it possible to recover organic sulfur without substantial degradation. To our knowledge, this is the first report of the characterization and direct assessment of mineralization potential for this form of sulfur isolated under these conditions.

Materials and methods

Sample collection

Samples of the Al horizon and O1 and O2 forest floor layers were collected during August 1982 from Watershed 18, a mixed mature hardwood catchment, located at the Coweeta Hydrologic Laboratory near Franklin, NC. The mineral horizon comprising this watershed is in the sandy loam Ashe series and is a Typic Dystrochrept. Bulk samples of the Al horizon (approx. 350 g wet mass; 5 cm in depth) were taken at random from a ridge position, cove position and a plot adjacent to the stream along a transect of the watershed established by Swank et al. (1984). Each sampling site is characterized by contrasting vegetation and, in terms of moisture content, the ridge is more xeric compared to the stream edge. Separate samples (about 500 g wet mass) were collected at random from the O1 (2 cm) and O2 (3 cm) components of the forest floor near the stream. A detailed description of the study site is available (Swank and Douglass 1977).

Sulfur incorporation

Samples of the Al horizon (1 g wet mass, not seived) and of the O1 (finely divided) and O2 layers (both 0.5 g wet mass) were incubated at 20 °C

for 48 h without shaking after addition of a 200 µl solution containing 7.49 nmole Na_2 $^{35}SO_4$ (3.3×10^{10} Bq mmole^{-1}). Adsorbed sulfate was removed after incubation by sequential extraction three times each with Na_2SO_4, NaH_2PO_4, LiCl (all 1 M) and water (Fitzgerald et al., 1982). To determine the amount of sulfur incorporated into organic matter, the extracted residue was treated with 6 N HCl at 121 °C for 12 h, washed with water and extracted with 2 N NaOH for 12 h at room temperature. After collection of the base extract, the residue was washed with water and these fractions were combined with the acid hydrolyzate. The non salt extractable ^{35}S in this combined fraction was determined in a Beckman LS9000 Scintillation Counter using the ^{14}C-full spectrum energy window and Scinti Verse (Fisher Sci.) as the scintillation fluid. Volumes of 0.6 ml were utilized for all extractants and water washes.

Previous work (Fitzgerald et al., 1982) established: (1) that the addition of Na_2SO_4 as the first extractant effectively terminates the incorporation of ^{35}S by isotope dilution; (2) that the salt extraction sequence is essential for total recoveries of non-incorporated ^{35}S; and (3) that the entire extraction procedure recovers at least 90% of the added ^{35}S.

Isolation of organosulfur

The O2 layer adjacent to the stream and Al horizon soil from all sites were labelled separately with ^{35}S by incubating 100 g (wet mass) of each with 570 nmoles of Na_2 $^{35}SO_4$ (1.7×10^{14} Bq mmole^{-1}) for 144 h at 28 °C without shaking. Sulfate that failed to be incorporated was partially removed by extraction with 1.0 M NaH_2PO_4, pH 4.0 (500 ml with shaking for 30 min at 4 °C). No attempt at this stage was made to remove all unincorporated sulfur as preliminary work suggested that the organosulfur moiety is not completely stable at the extraction pH of 4 (see also Table 2). The residue was immediately recovered by centrifugation at 4 °C and suspended in 500 ml of 0.1 M tetrasodium pyrophosphate ($Na_4P_2O_7$) containing 0.1 M NaOH. The pH of this solution was adjusted to 8.0 with NaH_2PO_4 crystals prior to extraction. After shaking at 4 °C for 18 h, the supernatant obtained by centrifugation was retained and the residue was re-extracted as above for 30 min. The two supernatants were combined and filtered through Whatman No. 1 paper to remove floating debris and the pH of the filtrate was adjusted to 7.0 with 0.1 M NaOH. After dialysis for 36 h at 4 °C against deionized water (4ℓ, changed 5 times), the extract was concentrated to about 350 ml by rotary evaporation at 45 °C and re-dialyzed as above. A summary diagram of this isolation procedure is given in Figure 1. Extracts were held at −20 °C subsequent to analysis. Elemental analyses and determination of ester sulfate content by hydriodic acid reduction were performed on the extract from the O2 layer dialyzed as above (extract A) and after exhaustive re-dialysis (extract B). Conditions for dialysis of the latter are stated in footnote b, Table 2.

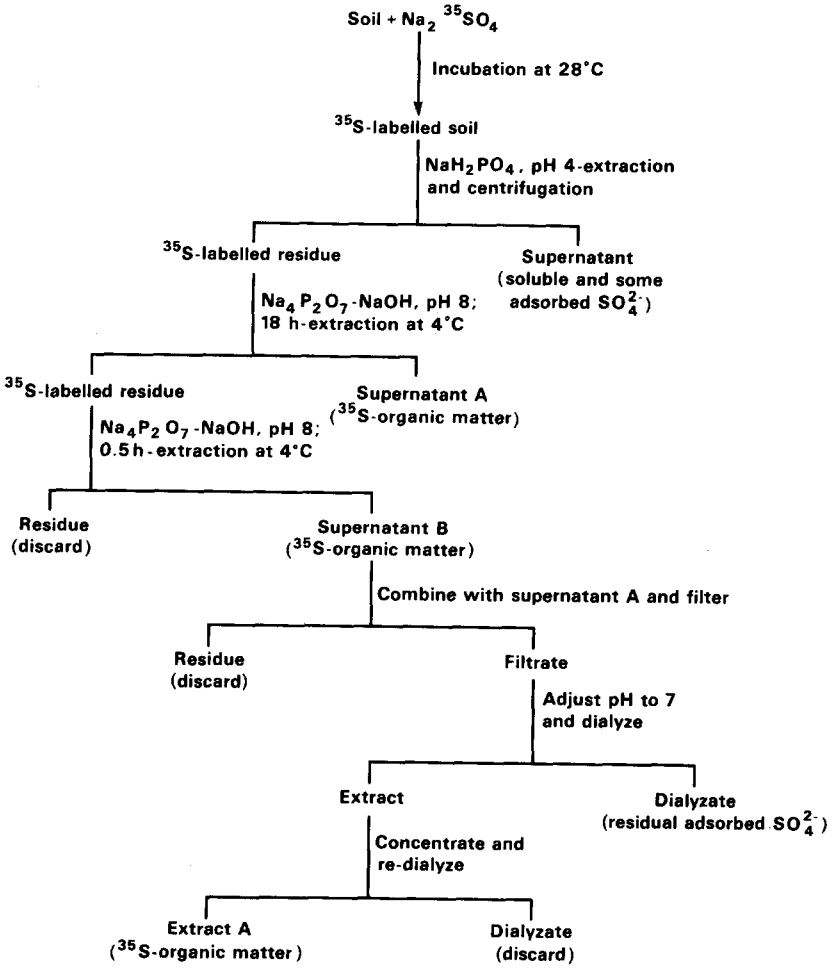

Figure 1. Procedure for the isolation of ^{35}S-labelled organosulfur from the O2 and Al horizons of watershed 18.

Determinations for total C,N,S and ester sulfate

Samples of the organosulfur extracts and each starting material were assayed for carbon and nitrogen content using a Hewlett-Packard 185 C,H,N analyzer. The samples were first dried at 50 °C for 12 h, and before analysis the soil samples were ground to a fine powder. Total sulfur was determined after oxidation of the samples in hypobromite (Tabatabai and Bremner, 1970) by reduction using the boiling hydriodic acid (HI)-mixture described by Freney (1961). Ester sulfate was determined by HI-reduction (Landers et al., 1983) and by measuring sulfate released after hydrolysis of the samples in 3 N HCl for 18 h at 30 °C followed by 1 h at 121 °C. The efficiency of the HI-reduction method was tested using choline sulfate prepared as previously described (Fitzgerald, 1973).

Mineralization of O2 layer organosulfur

Isolated organic sulfur (extract A) from the O2 layer was incubated with fresh forest floor material for 120 H at 28 °C to provide direct confirmation that this fraction was not a stable end product of sulfate metabolism. Conditions for incubation and subsequent extraction of inorganic sulfate were given previously (Strickland and Fitzgerald, 1984).

Measurement of sulfate

Inorganic sulfate present in extracts was separated from residual organosulfur and other ^{35}S-labelled components by electrophoresis. The location of each component on electrophoretograms was determined by scanning at slow speed with a Radiochromatogram Scanner and corresponding peak areas were obtained by triangulation. The accuracy of this method was confirmed by direct determinations of radioactivity on areas of the electrophoretogram that corresponded to peaks on the chart recorder paper.

Electrophoresis

Samples (80 µl) were analyzed by electrophoresis at 200 V for 2 h toward the anode on Whatman No. 1 paper in a 0.1 M $KH_2PO_4 - K_2HPO_4$ buffer, pH 7.0. Under these conditions, sulfate standards migrate about 11 cm from the origin on the electrophoretogram. The presence of sulfate in samples was confirmed by co-electrophoresis with authentic standards and by electrophoresis as above but in a 0.1 M barium acetate-acetic acid buffer, pH 4.5. In this buffer, sulfate characteristically remains at the origin of the electrophoretogram as $BaSO_4$ (Fitzgerald and Andrew, 1984).

Results and discussion

Sulfur incorporation

Sulfur from exogenous inorganic sulfate was incorporated by forest floor components and Al horizon soil into a fraction that could only be recovered by acid and base treatment of the samples. On a dry mass basis, the uppermost component of the forest floor (the O1 layer) was about 6.5 fold more active than the O2 component and this component was in turn about twice as active as the underlying soil (Table 1). Incorporation was essentially abolished in all samples by sodium azide, and levels of inhibition which were sample-dependent were noted with candicidin and erythromycin. The effect of the former antibiotic decreased with sample depth causing levels of inhibition in the O1, O2 and Al horizon of 84, 35 and 6%, respectively. A reverse effect was observed with erythromycin (27, 75 and 61% inhibition; O1, O2 and Al horizons, respectively). The broad spectrum antibacterial agents, chloramphenicol and tetracycline, inhibited the process only in the O1 and O2 layers (Table 1). The results suggest that, in these samples, candicidin-sensitive fungi (Lampen, 1969) and chloramphenicol-sensitive Gram negative bacteria are

Table 1. Incorporation of sulfur from sulfate into the non-salt extractable fraction of forest floor components and Al horizon soil[a]

Additions[b]	Incorporation (nmoles S g^{-1} dry mass)		
	O1	O2	A
None	26.3	4.0	1.8
Sodium azide	1.0	0.6	0.4
Candicidin	4.2	2.6	1.7
Erythromycin	19.2	1.0	0.7
Chloramphenicol	9.7	2.5	1.9
Tetracycline	20.5	2.1	2.6

[a]Samples were taken from watershed 18 at a position adjacent to the stream
[b]Azide (2 mmole) and the antibiotics (0.1 mmole, each) added as aqueous solutions (400 µl) with sulfate prior to incubation

primarily responsible for mediating sulfur incorporation in the O1 layer; whereas, bacteria largely mediate this process in the O2 and Al horizons. The observation that only erythromycin inhibited sulfur incorporation in the Al horizon suggests that Gram positive bacteria mediate the process in this soil.

Isolation of organosulfur

Biological mediation of the incorporation of sulfur into a fraction that is recovered during the extraction of soil organic matter (Schnitzer and Skinner, 1968) suggests that the incorporated sulfur could be covalently linked to carbon. To investigate this possibility the incorporated sulfur was isolated from the O2 layer and from three Al horizon samples by pH 8 extraction with pyrophosphate. Figure 2a shows that a component with zero electrophoretic mobility (origin component) was recovered from the O2 layer along with inorganic sulfate. The latter was removed by dialysis (Figure 2b). Our failure to detect the origin component after electrophoresis of concentrated samples of the dialyzate indicates that this component was retained during dialysis. Because compounds larger than 1.2×10^4 daltons will not pass through the tubing during dialysis, the origin component has a molecular weight $\geqslant 1.2 \times 10^4$ daltons. A similar origin component was isolated from all three soil samples (see Figure 3a for an example). Extraction with pyrophosphate at progressively increasing pH resulted in decreasing levels of this component. Moreover, extraction at pH 13, a pH commonly utilized to extract soil organic matter with pyrophosphate (see e.g. Anderson et al., 1974; Bettany et al., 1980), resulted in at least an 80% loss of the origin component. Preliminary work also suggested that the stability of this component is dependent upon the time period that is utilized for sulfur incorporation. Attempts to isolate organosulfur generated after 48 h were unsuccessful owing to pronounced instability of the origin component during both the preliminary phosphate and pH 8 pyrophosphate extraction.

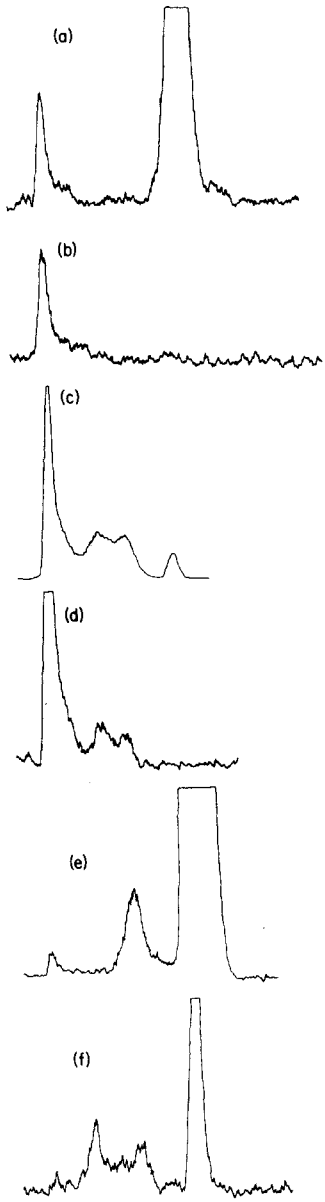

Figure 2. Electrophoretic analysis of organosulfur isolated from the O2 forest floor layer. Electrophoresis was toward the anode. The component with least anodic mobility (reading from left to right) remained at the origin and is referred to as the 'origin component'. The component with the greatest anodic mobility was identified as sulfate. Representative electrophoretograms are shown of (a), filtrate after extraction at pH 8.0; (b), filtrate after dialysis; (c), pH 7.0 filtrate after rotary evaporation; (d), after re-dialysis for 36 h; (e), salt extract after incubation at 28 °C for 120 h of the re-dialyzed preparation with O2 starting material; and (f), after hydrolysis of the re-dialyzed preparation in 3.0 N HCl for 18 h at 30 °C followed by 1 h at 121 °C.

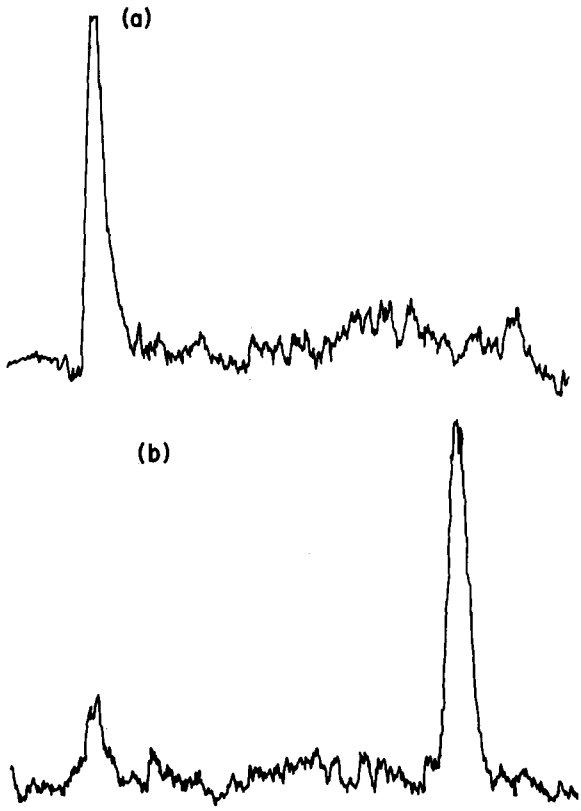

Figure 3. Electrophoretic analysis of organosulfur isolated from Al horizon soil. (a, b), Electrophoretograms of concentrated and re-dialyzed filtrate before and after hydrolysis as for Figure 2f. See legend to Figure 2 for identification of components on electrophoretograms.

Table 2. Elemental analysis of organosulfur extracts and corresponding starting material

Sample	Total (%)			
	C	N	S	C:N:S
O2 horizon, stream edge	34.83	1.32	0.18	194:7.3:1
Extract A[a]	30.62	1.48	0.32	96:4.6:1
Extract B[b]	25.61	1.50	0.21	122:7.1:1
Al horizon, ridge	4.89	0.31	0.03	163:10.3:1
Extract	18.41	1.04	0.16	115:6.5:1
Al horizon, cove	2.95	0.22	0.03	98:7.3:1
Extract	10.96	0.77	0.11	100:7.0:1
Al horizon, stream edge	6.02	0.26	0.05	120:5.2:1
Extract	15.70	0.92	0.16	98:5.8:1

[a]Organosulfur prepared according to the procedure stated in the materials and method section

[b]Organosulfur prepared as for footnote *a* except that the final extract was re-dialyzed exhaustively (4 L of water changed 20 times over a 6 day interval)

Characterization of organosulfur preparations

Elemental analysis indicated that sulfur was found together with carbon and nitrogen in each of the extracts after these had been dialyzed to remove unincorporated sulfate (Table 2). Exhaustive dialysis of one of the extracts (extract B, O2 horizon) resulted in a concomitant loss of sulfur and carbon, but nitrogen levels were retained. The decrease in sulfur content appears due primarily to a loss in carbon-bonded sulfur rather than ester sulfate (c.f. extracts A and B, Table 4). These observations again reflect the partial instability of this organosulfur preparation, but retention of about 66% of the sulfur (Table 2) even under these conditions provides assurance that sulfate was incorporated via co-valent bond formation and was not simply adsorbed to organic matter. Excepting the O2 layer in which a substantial % increase only in sulfur content was observed (Table 2), extraction resulted in substantial increases in the % of all three elements compared with levels present in each starting material.

The partial instability of organosulfur from O2 layer material is emphasized by the susceptibility of this extract to mild acid hydrolysis. Inorganic sulfate was released from this preparation by hydrolysis of ester linkages at pH 4.5. In contrast, soil organosulfur was stable under these conditions (Table 3). Although structural-dependent variation in susceptibility to acid exists, hydrolytic release of sulfate is a characteristic feature of sulfate esters which distinguishes these from substances in which sulfur is directly linked to carbon (Fitzgerald, 1976; Fitzgerald et al., 1982). The latter do not release sulfate either at low pH or elevated temperature; whereas, the more labile esters (e.g., phenolic sulfate esters) hydrolyze over a pH range of 4–6 at room temperature. Sulfate release at pH 4.5 may therefore represent an index of the more readily labile sulfate ester content of a given preparation, and Table 3 indicates that about 8% of the sulfur extracted from the O2 layer may occur in this form. This result may account for the partial instability of this preparation relative to Al horizon extracts. Collectively the acid susceptibilities of these preparations (Table 3) emphasize that, although a similar electrophoretic species was extracted in each case (Figures 2 and 3), the organic sulfur formed in the O2 layer differs, at least in susceptibility to mild acid, from that formed in the underlying A-horizon. The quantity of sulfate released with acid from the O2-organosulfur extract under conditions that ensure total hydrolysis suggests that about 62% of the sulfur occurs as ester sulfate (see, 3N HCl, 18 h at 30 °C, 1 h at 121 °C, Table 3 and Figure 2f). Similarly, in agreement with previous indirect measurements with ^{35}S-labelled soils (Fitzgerald et al., 1982), the soil organosulfur extracts also possessed high levels of ester sulfate (between 76 and 89% of the total sulfur present, Table 3 and Figure 3b). The remaining sulfur of these preparations was not altered substantially under any condition of hydrolysis (Table 3). This fraction therefore likely represents sulfur directly linked to carbon

Table 3. Acid stabilities of organosulfur extracts

Source	Treatment	% ^{35}S present after treatment as:		
		Organosulfur[a]	Sulfate	Unknown
O2 layer, extract A (stream edge)	pH 4.5,[b] 4 h at 30 °C	59.6	7.5	32.9
	3 N HCl, 4 h at 30 °C	58.7	14.9	26.4
	3 N HCl, 18 h at 30 °C	50.9	18.2	30.9
	3 N HCl, 18 h at 30 °C, 1 h at 121 °C	ND[c] (0)[d]	64.3 (60.4)	35.7 (39.6)
Al horizon, soil extract (stream edge)	pH 4.5, 4 h at 30 °C	100.0	ND	ND
	3 N HCl, 4 h at 30 °C	44.6	55.4	ND
	3 N HCl, 18 h at 30 °C	36.3	63.7	ND
	3 N HCl, 18 h at 30 °C, 1 h at 121 °C	22.0	78.0	ND
Al horizon, soil extract (ridge)	3 N HCl, 18 h at 30 °C, 1 h at 121 °C	24.1	75.9	ND
Al horizon, soil extract (cove)	3 N HCl, 18 h at 30 °C, 1 h at 121 °C	11.0	89.0	ND

[a] Origin component (Figures 2 and 3); unless otherwise stated all values were obtained by triangulation of peaks on electrophoretogram scans
[b] 0.05 M sodium acetate-acetic acid buffer, pH 4.5. All concentrations are final concentrations
[c] No discernable peak evident on electrophoretogram scans
[d] Values in parentheses determined by direct counting of sections of the electrophoretogram corresponding to peaks on the chart paper; O, no detectable level of radioactivity above background

Table 4. Analysis of organosulfur extracts by reduction with hydriodic acid

Sample	Sulfur ($\mu g\, g^{-1}$, dry mass) as:		
	Total	Ester sulfate	Carbon bonded[a]
Extract A[b]	3155.7	1928.3 (61.1)[c]	1227.4 (38.9)
Extract B	2106.7	1723.3 (81.8)	383.4 (18.2)
(O2 horizon, stream edge)			
Extract (Al horizon, ridge)	1562.6	849.6 (54.4)	713.0 (45.6)
		796.5 (51.0)[d]	766.1 (49.0)
		1148.7 (73.5)[e]	413.9 (26.5)
Extract (Al horizon, cove)	1084.2	957.0 (88.3)	127.2 (11.7)
Extract (Al horizon, stream edge)	1561.7	913.6 (58.5)	648.1 (41.5)
		836.3 (53.6)[d]	724.4 (46.5)
		855.2 (54.8)[e]	706.5 (45.2)
Choline sulfate	19.87	15.97 (80.4)	–
		19.18 (96.5)[d]	–

[a] Difference between total and ester
[b] See footnotes a, b Table 2 for explanation
[c] % of total is given in parentheses
[d] 1 h reduction with hydriodic acid increased to 3 h
[e] Reduction for 1 h with hydriodic acid of a 3 N HCl hydrolyzate of the extract (hydrolyzate obtained after 18 h at 30 °C followed by 1 h at 121 °C)

because organic sulfur in this form is resistant to degradation by acid. Both ester and carbon bonded sulfur linkages appear to be susceptible to mineralization since, as shown in Figure 2e, incubation of the dialyzed and concentrated O2-organosulfur extract with fresh O2 layer material resulted in a substantial release of sulfate. This increase in sulfate coincided with the almost complete disappearance of the origin component and the appearance of a second ^{35}S-labelled component of unknown susceptibility to mineralization (Figure 2e).

The validity of sulfate release after hydrolysis as a measurement of ester sulfate content was assessed independently by reduction with hydriodic acid. Within certain limits (Freney, 1961), sulfur which is reduced to S^{2-} in this procedure can be considered to result from ester linkages. Although no information on the mechanism of this reaction is available, it seems likely that acid hydrolysis of the ester linkage occurs releasing sulfate which is then reduced to S^{2-}. Hydriodic acid does not reduce sulfur associated with C–S linkages (which are acid stable) and, since dialyzed extracts were analyzed, the difference between total sulfur and HI-reducible sulfur represents an estimate of the carbon-bonded sulfur. Estimated values for this form of S together with determinations for ester sulfate in the various extracts are shown in Table 4. The percentage of ester sulfate determined by HI-reduction was close to that obtained by acid hydrolysis for the O2 layer extract (extract A) and for the soil extract from the cove position. However, hydriodic acid-reduction of extracts from ridge and steam-edge soils yielded values for ester sulfate that were substantially lower than those indicated by acid hydrolysis (Table 3 and 4). This discrepancy for the ridge position soil extract appears to be due to the occurrence in the extract of a number of highly stable ester linkages that fail to hydrolyze in the hydriodic acid-reducing mixture after the 1 h standard reduction time or even after 3 h (Table 4, footnote d). This possibility was confirmed by subjecting an HCl-hydrolyzate of this extract to hydriodic acid reduction. It can be seen from Table 4 (footnote e) that hydrolysis of the extract prior to HI-reduction gave a value for ester sulfate close to that obtained by HCl-hydrolysis alone (75.9% ester sulfate by HCl hydrolysis, Table 3 and 73.5% ester sulfate by HI-reduction after prior HCl-hydrolysis, Table 4). Similar treatment prior to reduction, however, failed to yield an ester sulfate percentage similar to that indicated by acid hydrolysis for the extract of the stream-edge soil. No explanation can be offered for this difference, but it should be noted (Table 4) that highly stable esters, such as choline sulfate are not completely reduced by hydriodic acid after the standard interval of 1 h. Collectively, results obtained by hydriodic acid reduction indicate that the soil organosulfur preparations exhibit some variation in composition even when the samples are taken in reasonably close proximity to each other.

The results of this study suggest that sulfur from sulfate is incorporated into organic matter primarily as ester sulfate in the Al horizon and to a

greater extent as carbon-bonded sulfur in the O2 horizon of watershed 18. The organosulfur extracted from this forest floor layer was subject to S-mineralization thus confirming indirect observations made previously (Strickland et al., 1984; Strickland and Fitzgerald, 1984). Owing to the instability of the sulfur linkages involved, it is impossible to determine if the organic sulfur isolated in the current work is representative of the total organic sulfur content of these samples. It is known that high pH is required for maximum recovery of organic matter (Schnitzer and Skinner, 1968) and Anderson et al. (1974), using pyrophosphate at pH 13, recovered about 25% more organic matter compared with the usual 0.1 M NaOH extraction procedure. However, these conditions of extraction resulted in substantial losses of organic sulfur recovered in either the O2 or Al horizon of watershed 18. Until a method is developed to stabilize the more labile sulfur linkages associated with this fraction, total extraction and direct characterization of all sulfur groups will not be possible. Earlier work by Freney (1961) demonstrated that hydriodic acid is specific for the reduction of ester sulfate and inorganic sulfur. In agricultural soils, where levels of the latter are negligible, hydriodic acid-reducible sulfur has been taken as an index of soil ester sulfate (see e.g. Fitzgerald 1976). Results of HCl hydrolysis in the current work largely confirm this assignment. However, our failure to recover by reduction levels of ester sulfate that were found by hydrolysis in two of the four samples analyzed emphasizes the need for careful interpretation of results obtained with hydriodic acid.

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