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Factors involved in potential sulfur accumulation in litter and soil from a coastal pine forest

M.E. WATWOOD, J.W. FITZGERALD[1,*], W.T. SWANK[2] & E.R. BLOOD[3]
[1]*Dept. of Microbiology and Institute of Ecology, University of Georgia, Athens, GA 30602; (*requests for offprints);*
[2]*Southeastern Forest Experiment Station, Coweeta Hydrologic Laboratory, Otto, NC 28763;*
[3]*Belle W. Baruch Institute, Georgetown, SC 29208, USA*

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Abstract. Samples of 01/02, A1, E2 and Bh horizons collected from a coastal pine forest were assayed for the potential capacity to adsorb and to form organic sulfur from added sulfate. The subsequent mobilization (mineralization) of organic S was also assayed to determine potential capacities of the samples for organic S accumulation. Organic and inorganic forms of S were quantified, as were total carbon and nitrogen levels. Relationships between these parameters and the above S processing capacities for each sample were examined, and fluctuations were statistically analyzed. Of the organic S present, sulfonate-S (non-Raney Ni-reducible S) was found to be the major component irrespective of horizon. Adsorbed and soluble S were found to be low, suggesting that loss by leaching may be an important fate of incoming sulfate at this site. Results from this study were compared with those obtained for two additional sites, which have been examined in detail and documented previously to be S accumulating. Soils from the pine forest site adsorbed substantially less sulfate than those from the other two sites. The organic S accumulation capacity was relatively low for the pine site, averaging less than one third of the potential established for the other two sites.

Introduction

A thorough understanding of sulfur cycling in forest litter and soil is mandated by the increasing concern over possible effects of acidic deposition on forest ecosystems. Sulfate, which is a major anion entering forests in precipitation (Likens & Bormann 1974), is subject to multiple fates upon contact with the forest floor and soil. If the anion remains soluble, it can be leached by water moving through the soil profile and thus removed from the forest by streamflow (Murphy 1980; Huete & McColl 1984). The loss of various nutrient cations has been shown to be facilitated by ionic association with mobile sulfate (Johnson 1980; Johnson & Cole 1977; Singh et al. 1980), and this phenomenon is a major concern where increased levels of incoming sulfate associated with acidic precipitation have been correlated with nutrient loss (David et al. 1984; Johnson et al. 1981). Alternatively, if the proper physical conditions exist, then the anion can become adsorbed either specifically or non-specifically, and may remain so for various periods of time, depending on changes in the physico-chemical environment which influence desorption (Johnson & Henderson 1979). Although there is controversy concerning the role of soil formation versus anthropogenic impact with respect to soil acidification (Krug & Frink 1983), sulfate adsorption has been shown to reduce cation leaching in several acidic soil systems (Johnson 1980; Wiklander 1980; Rechcigl & Sparks 1985; Richter et al. 1983).

Another fate of sulfate-S is incorporation into organic matter via the formation of covalent linkages. This process, which is referred to as organic S formation, is the result of microbial metabolism in forest soil and litter (Fitzgerald et al. 1983; Strickland & Fitzgerald 1984; Fuller et al. 1986). Organic S represents a possible storage form of S within the environment (Fitzgerald et al. 1982, 1985; Strick et al. 1982). However, yet another process has been documented which may temper organic S formation as an S retention mechanism. The mobilization of recently formed organic S, which may involve depolymerization of the organic matrix to yield less complex, more soluble moieties, followed by mineralization to yield sulfate, occurs to varying extents depending upon the system under investigation (Freney et al. 1975; Strickland & Fitzgerald 1984). This process is believed to result from the action of preformed enzymes, depolymerases and sulfohydrolases, existing in the soil (Strickland et al. 1984; 1986b). The amount of added sulfate-S retained as organic S is thus dependent, not only on the microbially mediated formation process, but also on the subsequent rate of mobilization of this fraction (McLaren et al. 1985; Schindler et al. 1986; Fuller et al. 1986). Potential levels of each process must be known before estimates of organic S accumulation within a forest system can be obtained.

The current study was undertaken to determine potentials for sulfate adsorption, organic S formation and mobilization in forest floor and soil profile samples taken from a coastal pine stand in South Carolina. In addition, amounts of sulfate and various forms of organic S were quantified for these samples to further characterize the study site and to provide verification for the laboratory-derived process level data. This forest is located within the North Inlet, Long Term Ecological Research (LTER) site. In an effort to foster inter-site comparison among LTER and non-LTER sites, mean S processing potentials for this pine forest are compared with those derived previously for a hardwood forest (LTER site; Fitzgerald et al. 1988) and several forests of differing vegetation located along an environmental gradient (Non-LTER site; Watwood et al. 1986).

Materials and methods

Site description and sampling

The study area is located in the Coastal Plain region, 1.6 km north of Georgetown, South Carolina. The specific site is adjacent to the North Inlet estuary about 1.2 km from the Atlantic Ocean. The 20-year-old loblolly pine (*Pinus taeda*) stand selected is typical of natural regeneration found in the Coastal Plain. The stand was established on abandoned agricultural land after 1872. A pine seed tree cut was conducted in 1956 followed by a second cut in 1965. In 1985 the stand contained 2200 stems ha^{-1} with a basal area of 25.5 m^{-2} ha^{-1} and a mean tree dbh of 14.5 cm. Sparse hardwood vegetation is comprised primarily of wax myrtle (*Myrica cerifera*) in the understory and occasional *Quercus* and *Acer* species in the overstory. Annual precipitation in the study area averages 115 cm. The climate is characteristically maritime with temperatures ranging from ·4 °C to 36 °C. Annual precipitation patterns are highly variable due to the episodic occurrence of tropical storms and hurricanes. The pine forest is exposed to atmospheric sulfate inputs from oceanic aerosols and from a paper mill located within five miles of the site. Soils are Spodosols composed of Aeric Haplaquods (Leon classification) on the drier portions and Typic Haplaquods (Lynn Haven classification) on the wetter areas. The forest floor averages about 5 cm in depth and horizon thickness for the A1, E2, and Bh averages 5.2, 9.8, and 35 cm, respectively.

Samples of litter (01/02), A1, E2, and Bh soil horizons were taken from nine plots within the study site in May and August 1983, July 1984, and February 1985. All samples were maintained in sealed bags at 5 °C. Root material was removed by hand, and analyses were performed within one week of each sampling date.

Sulfate adsorption and organic-S formation

Field moist samples (1 g wet weight, not sieved) were incubated at 20 °C for 48 h with 7.5 nmoles $Na_2^{35}SO_4$ (3.3×10^{10} Bq $mmole^{-1}$, Amersham) and then extracted according to the method of Fitzgerald et al. (1983) to yield soil water, salt, acid and base extracts. Preliminary work demonstrated that

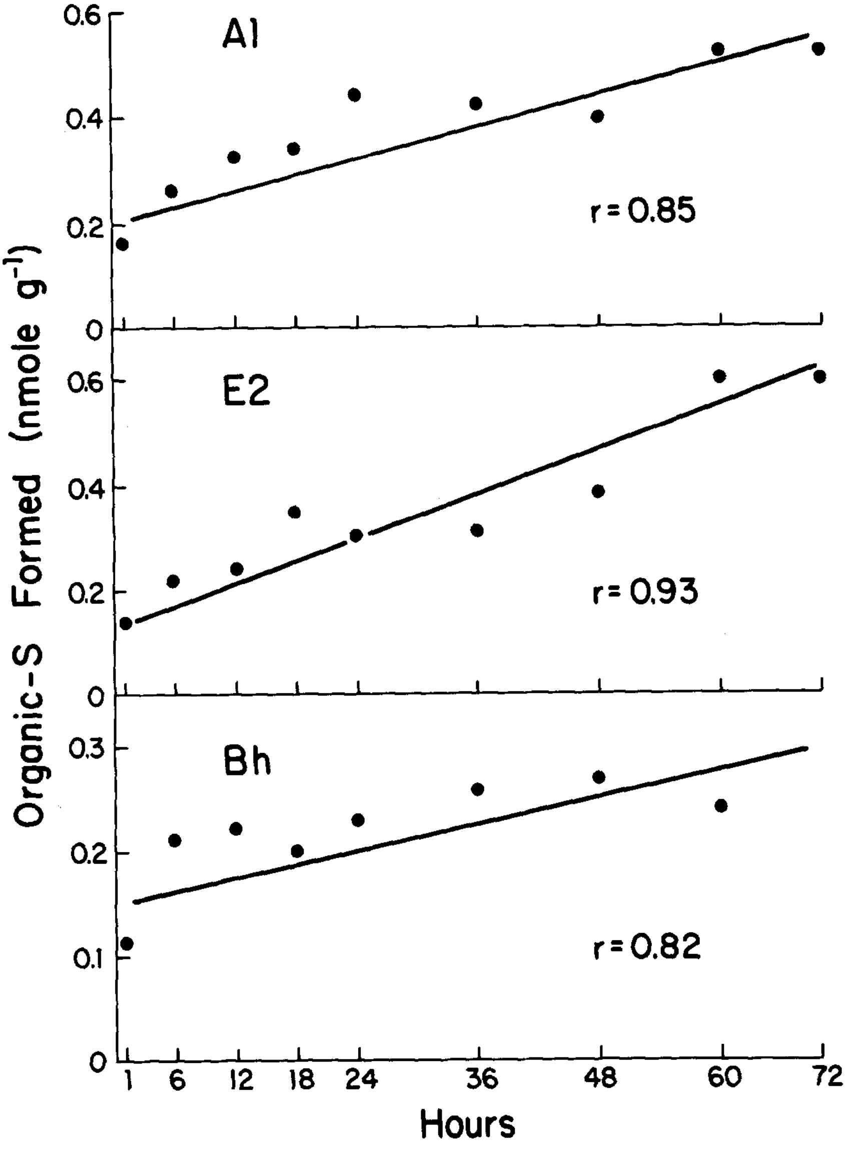

Fig. 1. Relationship between organic S formation and incubation time for various mineral horizons of a coastal pine forest. Composites of samples collected from all nine plots during February, 1985 were assayed. The correlation coefficient (*r*) is given for each horizon.

salt extraction (successive washes with 1 *M* Na_2SO_4, NaH_2PO_4, and LiCl) completely recoverd adsorbed ^{35}S-sulfate, and that the subsequent acid and base extraction removed ^{35}S incorporated into organic matter. Organic S, which is obtained under these conditions, has also been isolated with minimal destruction of sulfur linkage groups (Fitzgerald et al. 1985). Incubations for the assay were conducted for 48 h because time course experiments showed this interval to be well within the range of constant linearity for the formation process (Fig. 1). With respect to the adsorption process (assayed as salt-extractable ^{35}S), r values for each mineral horizon indicated a lack of correlation with incubation time ($r = -0.21, 0.26$, and -0.30 for A1, E2, and Bh horizons, respectively). Moreover, the predicted slope of each line was not significantly different from zero, indicating that sulfate adsorption was independent of incubation time. Prior to analysis, composite samples of litter and A1 horizon soil collected in July were subjected to either autoclaving (120 °C) for 3 h or treatment with sodium azide ($0.128\,g\,200\,\mu L^{-1}$). Effects of each treatment on the processes of adsorption and formation were determined.

Mobilization of recently formed organic S

This assay was conducted according to the method of Strickland et al. (1984). Briefly, samples (1 g wet weight, not sieved) were incubated for 24 h at 20 °C with 7.5 nmoles $^{35}SO_4$ to permit the formation of organic S. The samples were then extracted with phosphate to remove sulfate which had not been incorporated into organic matter. At this point any ^{35}S remaining in the samples was present as covalently linked organic S. Following centrifugation, the supernatant from a water slurry of each sample (subsample:water = 1:5) was added to reconstitute microflora removed during salt extraction. Samples were then incubated at 20 °C for a second 24 h period, and extracted successively with Na_2SO_4, NaH_2PO_4, and LiCl to recover soluble organic S and/or sulfate released due to the mobilization process. Following this extraction, samples were treated with 6 *M* HCl and then with 2 *M* NaOH to release the remaining organic ^{35}S which had not been mobilized. Mobilized S was expressed as a percentage of the ^{35}S incorporated into organic matter during the initial incubation. Although the amount of mobilized organic S is determined indirectly, the validity of this method has been confirmed by the direct addition of isolated organic S to samples in laboratory and field incubations (Strickland & Fitzgerald 1984; Strickland et al. 1986b).

Aliquots of the salt wash following the second incubation were routinely subjected to paper electropheresis for 2 h at 200 V in sodium acetate-acetic acid and barium acetate-acetic acid buffers. The paper strips were then scanned for radioactivity with a Packard radiochromatograph scanner to distinguish inorganic and organic ^{35}S components generated by the mobilization process. Inorganic sulfate remains at the origin during electropheresis in barium acetate and migrates about 13 cm when electropheresis is carried out in sodium acetate (Fitzgerald & Andrew 1984). Insoluble organic S remains at the origin whereas soluble forms exhibit mobilities less than that of sulfate when electrophoresis is carried out in the sodium acetate buffer or in buffers lacking Ba^{2+} (Fitzgerald et al. 1985).

S analysis

Intrinsic S forms, present naturally in samples which have received no laboratory S supplements, were quantified for samples collected in May and February. Total S was determined by hydriodic acid (HI) reduction following sample oxidation in hypobromite (Tabatabai & Bremner 1970), whereas HI reducible-S (ester linked-S and inorganic sulfate) was determined by direct sample reduction with HI (Frenery 1961). Total carbon-bonded S was calculated as the difference between total S and the amount of HI reducible-S. Amino acid-S was quantified for the February samples by reduction with Raney nickel (Freney et al. 1970), thus providing further differentiation of carbon-bonded S into amino acid and non amino acid-S (possibly, sulfonate-S). Analysis in this laboratory of a wide variety of different sulfonates support the generally accepted premise that the sulfonate linkage is the only naturally occurring organic S linkage which is both resistant to reduction with HI as well as to reduction with the nickel catalyst (Fitzgerald 1976).

To obtain soluble S, 4 g (wet weight) of each sample was mixed with 20 ml water, shaken for 15 min and centrifuged. The supernatant which contained the soluble S was retained. Adsorbed S was obtained by shaking the residue from the water wash with 20 ml of 0.02 M Na_2HPO_4 for 30 min. The mixture was centrifuged and the supernatant was retained. An additional 20 ml of NaH_2PO_4 was added to the pellet and the extraction was repeated. The two supernatants were combined to yield the phosphate extract. The S content of each fraction was determined by HI reduction and amounts of inorganic sulfate (February samples only) were determined for each of the extracts by anion chromatography (Dick & Tabatabai 1979). Ester sulfate present in the soluble and phosphate extracts was calculated as the difference between the amount of S detected by direct HI reduction and the amount of inorganic sulfate determined chromatographically.

Statistical analysis

Data derived from sulfur processing studies and intrinsic sulfur determinations were analyzed for differences between and within horizons using analysis of variance and Duncan's multiple range test at $\alpha = 0.05$.

Table 1. Elemental analysis of litter and mineral soil horizons collected from a pine forest site.[a]

Horizon	Total (%)			C:N:S
	C	N	S	
01/02	28.81	0.81	0.11	272: 7.6:1
A1	4.81	0.20	0.02	273:10.6:1
E2	2.11	0.16	0.01	211:15.8:1
Bh	1.44	0.13	0.01	165:17.8:1

[a] Samples were collected in May 1983; mean values are reported with n = 4.

Results

Elemental analysis of the samples revealed that while C, N, and S levels showed a steady decline with increasing sample depth, the C:S and N:S ratios throughout the profile varied substantially (Table 1). C:S ratios ranged from about 273:1 in the litter (01/02) layer of A1 horizon, to 165:1 within the Bh horizon, whereas ratios of N:S increased consistently with increasing soil depth, from about 8:1 in the litter layer to 18:1 in the Bh soil horizon.

Litter (01/02) samples were very active with respect to S retention measured as salt-extractable ^{35}S. While the salt-extractable fraction is considered to be adsorbed S in mineral horizons, the exact nature of this fraction in litter is uncertain. Regardless, within litter salt-extractable ^{35}S recovered following incubation included S which did not remain water soluble and was not incorporated into organic matter through covalent linkages by microorganisms. Therefore even though the mechanism is unclear, the phenomenon clearly reflects some type of potential S retention. Litter samples collected in May, August, and February were more active, on a dry weight basis, than those of the mineral horizons (ANOVA, Duncan's Multiple Range Test $\alpha = 0.05$) with respect to salt-extractable S, organic S formation, organic S mobilization (with the exception of February), and potential organic S accumulation (Table 2). When values for all collection dates were combined, litter samples accumulated an average of 1.1 nmole of organic S over a 24 h period. Differences in the levels of the S processing potentials with respect to the soil horizons showed no distinct patterns, and the horizons examined did not differ significantly with respect to the accumulation of organic S, with a combined average potential of 0.24 nmole being accumulated over a 24 h period. Assays of each horizon demonstrated that a substantial portion of the anion was retained in non-soluble forms (Table 2). Adsorption (salt-extractable S), rather than organic S formation, was responsible for

Table 2. Sulfur processing potentials of litter and soil collected from a pine forest site.

Sampling date	Horizon	Mean (± one standard error), n = 9			
		Sulfate adsorbed[a]	Organic sulfur formed	Organic sulfur mobilized	Organic sulfur accumulated[b]
		(nmole S g^{-1} dry wt 48 h^{-1})		(% 24 h^{-1})	(nmole S g^{-1} 24 h^{-1})
May 1983	01/02	4.3(0.5)	3.2(0.6)	80.8(2.3)	0.6(0.1)
	A1	1.4(0.1)	0.8(0.1)	66.9(4.9)	0.3(0.03)
	E2	1.7(0.2)	0.7(0.2)	59.6(4.8)	0.3(0.1)
	Bh	2.4(0.4)	0.6(0.1)	59.4(5.4)	0.2(0.03)
August 1983	01/02	7.4(0.7)	2.2(0.2)	71.5(2.6)	0.6(0.1)
	A1	1.2(0.1)	0.3(0.1)	51.4(2.9)	0.2(0.03)
	E2	1.6(0.1)	0.4(0.03)	49.4(1.5)	0.2(0.02)
	Bh	2.8(0.4)	0.4(0.1)	50.9(2.4)	0.2(0.02)
February 1985	01/02	3.7(0.5)	3.5(0.3)	44.1(2.7)	2.0(0.2)
	A1	0.9(0.1)	0.8(0.1)	47.5(3.4)	0.4(0.04)
	E2	0.7(0.04)	0.5(0.02)	45.3(2.8)	0.3(0.02)
	Bh	0.7(0.1)	0.6(0.1)	56.8(1.4)	0.3(0.03

[a] Salt-extractable S. [b]Values were calculated by substracting (% mobilized × nmole formed) from the number of nmole formed.

significantly more of this retention in August litter samples, soil horizons from May and August, and in the February E2 horizon. For other samples both processes contributed equally to the retention of added sulfate (Table 2).

The initial rate (0–1 h) of organic S formation (Fig. 1), was very high, indicating that pre-formed enzymes within the soil profile contributed initially to the process. Similar findings were obtained with soil from other forests (Watwood et al. 1986). Of the organic S which was formed within the litter (01/02) layer, between 44 and 81% was mobilized within 24 h. Unlike results obtained with hardwood forests (Strickland & Fitzgerald 1984), in the mineral horizons of the pine forest, much more (45 to 67%) of the organic S was mobilized during the same time period, and electrophoresis demonstrated that inorganic sulfate (as opposed to a mixture of soluble organic S and sulfate) was the sole end product of the mobilization process. These mobilization rates contributed to estimates of potential organic S accumulation in the pine forest which were (with some exceptions) less than 50% of the amount of organic S orginally formed (Table 2).

Seasonal variation with respect to salt-extractable S involved the litter layer (Table 2) in which samples collected in August exhibited higher values (7.4 nmole) than other months, and the E2 and Bh horizons, where February samples (0.69 and 0.73 nmole, respectively) were significantly lower than values for those collected in other months. Organic S formation potentials were constant for all three months except in the A1 horizon where August samples were low (0.32 nmole) compared with other months (0.84 and 0.79 nmole for samples taken in May and February, respectively). With respect to the mobilization process, the litter layer was most active in May (80.8%) and least active in February (44.1%), whereas the A1 and E2 horizons were also most active in May (66.9 and 59.6%, respectively), but exhibited similar levels in August (51.4 and 49.4%) and February (47.5 and 45.3%). For the litter layer and A1 horizon, potential accumulation of organic S was highest in February (2.0 and 0.41 nmole, respectively). The E2 and Bh horizons showed no such seasonal effects with respect to this estimated potential (Table 2).

Treatment of samples with sodium azide (Table 3) resulted in 20 and 80% decreases in levels of salt-extractable S and 43 and 55% decreases in organic S formation rates for the 01/02 and A1 horizon, respectively. While autoclaving appeared to increase levels of salt-extractable S (litter layer) or had no effet (A1 horizon), the treatment effectively stopped the process of organic S formation in both horizons.

Quantitation of the various forms of organic and inorganic S present in samples collected in May and February (Table 4) revealed that, on a dry weight basis, all forms of S were highest in the litter layer and, with a few exceptions (adsorbed S in May, soluble S in February), the A1 horizon exhibited the next highest levels (ANOVA, Duncan's multiple range test, =0.05). Analysis of soluble and adsorbed S in the February samples revealed that all of the S in each horizon was present as sulfate. Differences in sulfur fractions within horizons and with respect to sampling dates are

Table 3. Influence of respiratory inhibitor and sterilization on sulfate adsorption and organic S formation.[a]

Treatment	Horizon	Sulfate adsorbed[b]	Organic S formed
		(nmole S g^{-1} dry weight 48 h^{-1})	
None	01/02	2.0	2.0
	A1	1.5	1.2
Sodium azide[c]	01/02	1.6	1.2
	A1	0.3	0.5
Autoclaving[d]	01/02	2.5	0.0
	A1	1.5	0.0

[a] Samples collected from each of nine plots in July 1984 were pooled and composites were treated. [b]Salt-extractable S. [c]Added (0.128 g 200 μL^{-1}) together with $^{35}SO_4^{2-}$ to each sample prior to incubation. [d]121 °C for 3 h with cooling prior to addition of $^{35}SO_4^{2-}$ and incubation.

Table 4. Intrinsic sulfur fractions of litter and mineral soil horizons from a pine forest site.[a]

Sampling date	Horizon	Mean, n = 3					
		Total S	HI-reducible S	Carbon-bonded S	Insoluble S^{b}	Soluble S^{c}	Adsorbed S
		($\mu g\ g^{1}$ dry wt)	(percent of total S)				
May 1983	01/02	1044	19^{a1}	81^{b1}	15^{a1}	2^{c1}	2^{c1}
	A1	225	39^{a1}	61^{b1}	28^{a1}	8^{c1}	3^{c1}
	E2	74	54^{a1}	46^{a1}	33^{a1}	10^{b1}	11^{b1}
	Bh	48	52^{a1}	48^{a1}	23^{b1}	20^{b1}	9^{c1}
February 1985	01/02	1373	19^{a1}	81^{b1}	13^{a1}	6^{c1}	1^{c1}
	A1	394	18^{a2}	82^{b2}	12^{a1}	1^{c2}	5^{c2}
	E2	88	24^{a2}	76^{b2}	22^{a1}	1^{c2}	0^{c2}
	Bh	149	15^{a2}	86^{b2}	12^{a2}	3^{c2}	0^{c2}

[a] Differences within horizon are denoted by different letters; seasonal differences, by numbers (Duncan's multiple range test at $\alpha = 0.05$). [b] Non-phosphate extractable ester sulfate. [c] All soluble and adsorbed S was present as SO_4^{2-} in samples collected in February. May samples were not analyzed for SO_4^{2-} and the values given are based on determinations for HI-reducible S.

Table 5. Composition of carbon-bonded sulfur fraction.

Horizon	Amino Acid-S[a]	Sulfonate-S[b]	Amino Acid-S	Sulfonate-S	Amino Acid-S	Sulfonate-S
	($\mu g\ g^{-1}$ dry wt)		(percent of total S)		(percent of C-bonded S)	
01/02	144[a]	967[a]	11	70	13	87
A1	26[b]	303[b]	9	73	11	89
E2	7[c]	61[c]	7	69	10	90
Bh	5[c]	130[d]	2	83	2	98

[a] Horizn differences are denoted by different letters (ANOVA, Duncan's multiple range test at $\alpha = 0.05$). Samples were collected in February, 1985. [b]Non-Raney Ni-reducible S.

Table 6. Comparison of sulfur processing potentials of litter and soil collected from three forest sites.

Site	Vegetation	Sample	Sulfate adsorbed[a]	Organic sulfur formed	Organic sulfur mobilized	Organic sulfur accumulated
			($nmole\ S\ g^{-1}$ dry wt $48\ h^{-1}$)		(% $24\ h^{-1}$)	($nmole\ S\ g^{-1}\ 24\ h^{-1}$)
North inlet[b]	Pine	01/02	4.9(0.6)	3.0(0.4)	65.8(2.7)	1.0
		A1	1.2(0.1)	0.7(0.1)	55.6(3.7)	0.3
Coweeta[c]	Hardwood	01 and 02	6.3(0.4)	11.0(0.7)	33.6(2.3)	7.3
		A1	6.0(0.2)	1.7(0.04)	41.3(2.0)	1.0
Santa Fe	Mixed[d]	02	3.1(0.3)	4.8(0.6)	29.9(4.2)	3.4
National Forest		A1	5.0(0.4)	3.0(0.2)	31.6(2.6)	2.1

[a] Salt-extractable S. [b]This study, n = 27. ± one standard error is given in parentheses. [c]Coweeta Hydrologic Laboratory, watershed 18, n = 120 (Fitzgerald et al. 1988). [d]Spruce fir, Aspen and Pine, n = 15 (Watwood et al. 1986).

noted in Table 4. With respect to the February samples, further differentiation of intrinsic carbon-bonded S revealed horizon differences in absolute quantities of amino acid and sulfonate-S (non-Raney Ni-reducible S), but no such differences existed when values were expressed as percentages of total S (Table 5). For each horizon, sulfonate-S was found to be the predominant form of carbon-bonded S.

When compared with processing capacities determined for two other forests (Table 6), the A1 horizon soil from the pine forest exhibited much lower capacities for sulfate adsorption and organic S formation, and a higher capacity for organic S mobilization. The potential for accumulation of organic S was therefore substantially less than potentials derived for the other systems. The same comparative results were obtained for litter samples with respect to organic S accumulation, whereas an intermediate value for retention as salt-extractable S was determined for the pine forest samples.

Discussion

The accumulation of incoming S in litter and soil has been documented for several forest ecosystems including those located at Coweeta (Swank et al. 1984; Swank & Douglas 1977; Shriner & Henderson 1978). Two soil processes account for S accumulation, and these processes are influenced by several factors. The process of sulfate adsorption is controlled by pH (Freney 1979; Freney & Williams 1983), organic matter content (Johnson & Todd 1983), iron and aluminum oxides (Johnson & Todd 1983; Parfitt & Smart 1978), and clay content and type of clay (Freney 1979; Johnson 1980). The low levels of sulfate adsorption observed for the pine forest soils, as compared with those from Coweeta and the Santa Fe forests (Table 6), may be partially due to differences in sesquioxides which provide exposed sites for ion adsorption. Analyses of pine soils at North Inlet for amorphous and crystalline forms of iron oxides using the methods of Johnson & Todd (1983) showed that concentrations are nearly two orders of magnitude lower than iron oxide concentrations found for Coweeta soils (Johnson & Todd 1983). In addition C:S ratios indicate a relative abundance of organic matter in the A1 and E2 horizons of the pine forest as compared with a mean ratio of 200:1, which has been documented for a variety of forest soils from different regions of the world (Freney & Williams 1983). Because organic matter has been shown to have a negative influence on sulfate adsorption in other forest soils due to blockage of exposed sites (Johnson & Todd 1983), organic matter content may also contribute to the low levels of adsorption observed with samples collected from the pine forest site. Based on this established physico-chemical nature of sulfate adsorption, it was not unexpected that autoclaving had no effect on the process in the A1 horizon. However, the reduction in adsorption noted after treatment with sodium azide may suggest a previously undocumented role of aerobic populations in this type of S retention. Results involving the corresponding salt extractable S fraction for the litter layer, which decreased somewhat following sodium azide treatment, also suggest an aerobic microbial component in this retention process. However, autoclaving, which kills aerobic and anaerobic cells and denatures enzymes, caused an increase in salt extractable S levels for litter. This is clearly an unexpected result, and further elucidation of the mechanism by which salt extractable S is retained in litter is necessary for interpretation. Regardless, low intrinsic levels of adsorbed sulfate in the mineral horizons compare favorably with the low adsorption potentials observed using added sulfate in the laboratory incubations.

The other process contributing to sulfate retention is organic S formation which involves the formation of hydriodic acid (HI) reducible C-O-S (ester) linkages and non-HI reducible C-S linkages. This process has been shown to be microbially mediated in many forest ecosystems (Freney 1979; Strickland & Fitzgerald 1984), and this appears to be the case with the pine forest site based upon the observation that sterilization by autoclaving effectively eliminated the process in both soil and litter samples. Samples treated with excess amounts of sodium azide exhibited reduced organic S formation potentials but no total elimination, as was achieved by autoclaving. A possible explanation for this result is that azide causes the lysis only of aerobic microorganisms. This respiratory inhibitor has no effect on anaerobic populations which may carry out substantial amounts of organic S formation at the study site, especially since it is saturated with water occasionally during the year. Moreover, autoclaving causes the inactivation of pre-formed enzymes, whereas treatment with azide may not. The rapid onset of organic S formation observed in time course experiments suggests that pre-formed enzymes, which are not subject to inactivation by azide, contribute substantially to this process.

Organic S formation potentials were found to be relatively low in both litter and soil from the pine forest site compared with the other selected sites (Table 6). This may be due to low levels of microbial activity for which there could be numerous explanations. C:S and N:S ratios may be related to the formation process. It has been hypothesized (Stotzky & Norman 1961) that C:S ratios of less than 900:1 provide adequate S for maximum microbial activity, so that the active formation of organic S by microorganisms is not imperative. C:S ratios of both litter and soil at the pine forest site were much lower than 900:1. Although lower and more variable limits on this ratio with respect to organic S formation have been cited by others (Barrow 1961), the ratios determined for this site are still low. In addition, a large portion (at least 70%) of the total S present in litter and soil is present as organic S. Thus, the formation of organic S from added sulfate by microbial populations may not be favored under these conditions. While N:S ratios have not been strongly correlated with organic S formation, it is interesting to note that in the pine forest soils the ratios were higher than the 10:1 ratio documented previously for a variety of forest soils (Freney & Williams 1983).

Another explanation for the low organic S formation potentials concerns the mobilization process. This process was observed to occur at relatively high rates in both litter and soil, and it could be occurring concomitant with organic S formation during incubations (McLaren et al. 1985; Schindler et al. 1986). If so, this would have the effect of lowering apparent levels of organic S formation. Thus, although formation potentials appear to be relatively low for the pine forest site, the process could indeed be occuring at higher rates which are masked by the rapid mobilization of the organic S to yield sulfate. Over half of the organic S formed in 48 h within litter and soil was mobilized within a subsequent 24 h period. At each of the other two sites (Table 6) less than 42% of recently formed organic S was mobilized during similar incubations. The end result is that less sulfur is retained through adsorption and organic S formation than in other systems examined in detail to date. Accordingly, estimations of organic S accumulation for the pine forest site indicate a potential of less than one third of that for the other sites in both soil and litter.

Of the organic S present, from 46 to 86% existed as carbon-bonded S within the soil horizons. Similar observations were made by David et al. (1982) for a hardwood forest in the Adirondack Mountains of New York. It is interesting to note that in May, carbon-bonded S represented less of the total S within the E2 and Bh horizons of the pine forest site than that present in samples collected in other months. One possible explanation for this result is the higher mobilization rates noted for this month. Mobilization of organic S may involve direct oxidation of C-S linkages or conversion of C-S linkages to ester sulfate linkages with subsequent release of sulfate by hydrolysis. This explanation is supported by the observation that elevated levels of soluble S were noted for the May samples. This soluble fraction may be totally (as in the case of the February samples) or partially composed of sulfate, the sole end product of the mobilization process. Analysis of the February samples revealed that sulfonate-S (non-Raney Ni-reducible S) acounted for over 87% of the carbon-bonded S and between 69 and 83% of the total S in each horizon. Amounts of carbon-bonded S in hardwood forests of North Carolina (Strickland et al. 1986a, b) are generally much higher (by almost 2-fold) in the litter (01/02) layers than in the A1 horizon. Moreover, levels of amino acid S comprising the fraction are usually much higher relative to levels of sulfonate-S. These results are in direct contrast to those obtained with February samples from the pine forest suggesting that sulfonate-S is an important storage form of sulfur in this forest.

In conclusion, it appears that litter and soil of this pine forest currently have a relatively low capacity for retaining incoming sulfate, either as adsorbed S or as organic S. The mobilization process occurs at such high rates as to insure that most of the anion remains either in a soluble form, subject to leaching loss (as indicated by low intrinsic levels of soluble S), or is converted to organic forms of S which are in turn rapidly remineralized. Overall, the potential for accumulation of sulfate as organic S is low, and sulfate adsorption capacity may also be too low to buffer cation leaching.

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References

Barrow, N.J. (1961) Studies on mineralization of sulfur from soil organic matter. Australian Journal of Agricultural Research 12: 306–319.

David, M.B., M.J. Mitchell & J.P. Nakas (1982) Organic and inorganic sulfur constituents of a forest soil and their relationship to microbial activity. Soil Science Society of America Journal 46: 847–852.

David, M.B., M.J. Mitchell & S.C. Schindler (1984) Dynamics of organic and inorganic sulfur constituents in hardwood forest soils. In: E.L. Stone (Ed) Forest Soils and Treatment Impacts pp. 221–245. Proceedings of Sixth North American Forest Soils Conference, Knoxville, Tennessee.

Dick, W.A. & M.A. Tabatabai (1979) Ion chromatrographic determinations of sulfate and nitrate in soils. Soil Science Society of America Journal 43: 899–904.

Fitzgerald, J.W. (1976) Sulfate ester formation and hydrolysis: a potentially important yet often ignored aspect of the sulfur cycle of aerobic soils. Bacteriological Reviews 40: 698–721.

Fitzgerald, J.W., T.C. Strickland & W.T. Swank (1982) Metabolic fate of inorganic sulphate in soil samples from undisturbed and managed forest ecosystems. Soil Biology and Biochemistry 14: 529–536.

Fitzgerald, J.W., J.T. Ash, T.C. Strickland & W.T. Swank (1983) Formation of organic sulfur in forest soils: a biologically mediated process. Canadian Journal of Forest Research 13: 1077–1082.

Fitzgerald, J.W. & T.L. Andrew (1984) Mineralization of methionine sulphur in soils and forest floor layers. Soil Biology and Biochemistry 16: 565–570.

Fitzgerald, J.W., T.C. Strickland & J.T. Ash (1985) Isolation and partial characterization of forest floor and soil organic sulfur. Biogeochemistry 1:155–167.

Fitzgerald, J.W., W.T. Swank, T.C. Strickland, J.T. Ash, D.D. Hale, T.L. Andrew & M. Watwood (1988) Sulfur pools and transformation in litter and surface soil of a hardwood forest. In: W.T. Swank & D.A. Crossley (Eds) Forest Hydrology and Ecology at Coweeta, Chap. 18, (pp. 245–253). Springer-Verlag, New York.

Freney, J.R. (1961) Some observations on the nature of organic sulphur compounds in soil. Australian Journal of Agricultural Research 12: 424–432.

Freney, J.R. (1979) Sulfur transformations. In: R.W. Fairbridge & C.W. Finkl (Eds) The Encyclopedia of Soil Science. Part I. Physics, Chemistry, Biology, Fertility and Technology (pp. 536–544). Dowden, Hutchinson and Ross, Incorp. Stroudsburg, Pennsylvania.

Freney, J.R., G.E. Melville & C.H. Williams (1970) The determination of carbon bonded sulfur in soil. Soil Science 109: 310–318.

Freney, J.R., G.E. Melville & C.H. Williams (1975) Soil organic matter fractions as sources of plant available sulphur. Soil Biology and Biochemistry 7: 217–221.

Freney, J.R. & C.H. Williams (1983) The sulphur cycle in soil. In: M.V. Ivanov and J.R. Freney (Eds) The Global Biogeochemical Sulphur Cycle (pp. 129–201). John Wiley and Sons, New York.

Fuller, R.D., C.T. Driscoll, S.C. Schindler & M.J. Mitchell (1986) Modelling of sulfur transformations in forested spodosols. Biogeochemistry 2: 313–328.

Huete, A.R. & J.G. McColl (1984) Soil cation leaching by 'Acid rain' with varying nitrate to sulfate ratios. Journal of Environmental Quality 13: 366–371.

Johnson, D.W. (1980) Site susceptibility to leaching by H_2SO_4 in acid rainfall. In: T.C. Hutchinson & M. Havas (Eds) Effects of Acid Precipitation on Terrestrial Ecosystems (pp. 525–535). Plenum Press, New York.

Johnson, D.W. & D.W. Cole (1977) Sulfate mobility in an outwash soil in western Washingtón. Water, Air and Soil Pollution 7: 489–495.

Johnson, D.W. & G.S. Henderson (1979) Sulfate adsorption and sulfur fractions in a highly weathered soil under a mixed deciduous forest. Soil Science 128: 34–40.

Johnson, D.W., G.S. Henderson & D.E. Todd (1981) Evidence of modern accumulations of adsorbed sulfate in an east Tennessee forested ultisol. Soil Science 132: 422–426.

Johnson, D.W. & D.E. Todd (1983) Relationships among iron, aluminum, carbon, and sulfate in variety of forest soils. Soil Science Society of America Journal 47: 792–800.

Krug, E.C. & C.R. Frink (1983) Acid rain on acid soil: a new perspective. Science 221: 520–525.

Likens, G.E. & F.H. Bormann (1974) Acid rain: a serious regional environmental problem. Science 184: 1176–1179.

McLaren, R.G., J.I. Keer & R.S. Swift (1985) Sulphur transformations in soils using sulphur-35 labelling. Soil Biology and Biochemistry 17: 73—79.

Murphy, M.D. (1980) Essential micronutrients III: sulphur. In: B.E. Davies (Ed) Applied Soil Trace Elements (pp 235–258). John Wiley and Sons, New York.

Parfitt, R.L. & R.S.C. Smart (1978) The mechanism of sulfate adsorption on iron oxides. Soil Science Society of America Journal 42: 48–50.

Rechcigl, J.E. & D.L. Sparks (1985) Effect of acid rain on the soil environment: a review. Communications in Soil Science and Plant Analysis 16: 653–680.

Richter, D.D., D.W. Johnson & D.E. Todd (1983) Atmospheric sulfur deposition, neutralization, and ion leaching in two deciduous forest ecosystems. Journal of Environmental Quality 12: 263–270.

Schindler, S.C., M.J. Mitchell, T.J. Scott, R.D. Fuller & C.T. Driscoll (1986) Incorporation of ^{35}S-sulfate into inorganic and organic constituents of two forest soils. Soil Science Society of America Journal 50: 457–462.

Shriner, D.S. & G.S. Henderson (1978) Sulfur distribution and cycling in a deciduous forest watershed. Journal of Environmental Quality 7: 392–397.

Singh, B.R., G. Abrahamsen & A.O. Stvanes (1980) Effect of simulated acid rain on sulfate movement in acid forest soils. Soil Science Society of America Journal 44: 75–80.

Stotzky, G. & A.G. Norman (1961) Factors limiting microbial activities in soil. II. The effect of sulfur. Archives of Microbiology 40: 370–382.

Strick, J.E., S.C. Schindler, M.B. David, M.J. Mitchell & J.P. Nakas (1982) Importance of organic sulfur constituents and microbial activity to sulfur transformations in an Adirondak forest soil. Northeastern Environmental Science 1: 161–169.

Strickland, T.C. & J.W. Fitzgerald (1984) Formation and mineralization of organic sulfur in forest soils. Biogeochemistry 1: 79–95.

Strickland, T.C., J.W. Fitzgerald & W.T. Swank (1984) Mobilization of recently formed forest soil organic sulfur. Canadian Journal of Forest Research 14: 63–67.

Strickland, T.C., J.W. Fitzgerald & W.T. Swank (1986a) In situ measurements of sulfate incorporation into forest floor and soil organic matter. Canadian Journal of Forest Research 16: 549–553.

Strickland, T.C., J.W. Fitzgerald & W.T. Swank (1986b) In situ mobilization of ^{35}S-labelled organic sulphur in litter and soil from a hardwood forest. Soil Biology and Biochemistry 18: 463–468.

Swank, W.T. & J.E. Douglass (1977) Nutrient budgets for undisturbed and manipulated hardwood forest ecosystems in the mountains of North Carolina. In: D.L. Correll (Ed) Watershed Research in Eastern North America: A Workshop to Compare Results. Vol. 1, (pp. 343–363). Smithsonian Institute, Edgewater, Maryland.

Swank, W.T., J.W. Fitzgerald & J.T. Ash (1984) Microbial transformations of sulfate in forest soils. Science 223: 182–184.

Tabatabai, M.A. & J.M. Bremner (1970) An alkaline oxidation method for determination of total sulfur in soils. Soil Science Society of America Journal 34: 62–65.

Watwood, M.E., J.W. Fitzgerald & J.R. Gosz (1986) Sulfur processing in forest soil and litter along an elevational and vegetative gradient. Canadian Journal of Forest Research 16: 689–695.

Wiklander, L. (1980) Interaction between cations and anions influencing adsorption and leaching. In: T.C. Hutchinson & M. Havas (Eds) Effects of Acid Precipitation on Terrestrial Ecosystems (pp. 239–254). Plenum Press, New York.