Reprinted from the *Soil Science Society of America Journal*
Volume 52, no. 5, September–October 1988
677 South Segoe Rd., Madison, WI 53711 USA

Sulfur Transformations in Forest Litter and Soil: Results of Laboratory and Field Incubations

M. E. WATWOOD AND J. W. FITZGERALD

Sulfur Transformations in Forest Litter and Soil: Results of Laboratory and Field Incubations

M. E. WATWOOD* AND J. W. FITZGERALD

ABSTRACT

Field incubations of litter (01 and 02 layers) and A horizon soil utilizing ^{35}S-labeled inorganic sulfate were conducted during July 1985 in an eastern white pine (*Pinus strobus* L.) and a hardwood forest. Samples were assayed for the capacity to form ^{35}S-labeled organic S, and in most cases these capacities were very similar to those determined in corresponding laboratory incubations. The A horizon soils from both watersheds formed approximately 3.0 nmol of organic S g^{-1} dry weight of sample during 2-d field or laboratory incubations. No substantial increase in this amount was observed after 7-d field incubations. Intrinsic S fractions of samples were quantified prior to and following field incubations, and organic S represented the majority of the total S in all cases. Total S ranged from 260 to 1180 and from 150 to 270 mg kg^{-1} in the litter layers and the A horizon, respectively. Sulfonate S (13–74% of total S) and nonphosphate extractable ester sulfate (16–82% of total S) were the largest organic S pools. Water soluble and phosphate extractable S pools were comprised of both inorganic sulfate and ester sulfate, and the latter was found to represent up to 8.1% of total S. The distribution of ^{35}S following field incubations was determined, and organic ^{35}S fractions in a variety of linkage groups were again found to predominate. Total C, moisture content, and throughfall sulfate concentrations during the field incubations were also determined.

THE RETENTION of sulfate derived from atmospheric deposition has been documented for litter and soil horizons of many forests (Swank and Douglass, 1977; Shriner and Henderson, 1978; Johnson et al., 1982). Sulfate adsorption has been implicated as a mechanism of S retention, and much research has focused on the role of this process in various forest systems (Johnson and Henderson, 1979; Johnson et al., 1980; Johnson et al., 1982). Laboratory studies indicate that the incorporation of sulfate-S into organic matter through the microbially mediated formation of covalent linkages may also contribute to observed S retention (Fitzgerald et al., 1982; Strick et al., 1982, Strickland and Fitzgerald, 1984; Swank et al., 1984; McLaren et al., 1985). With few exceptions, however, relatively little detailed work has been conducted to document the importance of organic S formation as a retention mechanism under field conditions (Freney et al., 1971; Schindler et al., 1986; Strickland et al., 1986a). Field incubations were therefore conducted with litter and surface soil of two adjacent forested watersheds to determine amounts of sulfate adsorption and organic S formation, and these data are compared with those derived from similarly designed laboratory experiments. Quantification of intrinsic S forms has been used in conjunction with laboratory determined S retention potentials to characterize S cycling within various forest systems (McLaren et al., 1985; Fitzgerald et al., 1987; Schindler et al., 1986; Watwood et al., 1986). In the current study this approach is used to trace the fate of ^{35}S-labeled sulfate within litter and soil under field conditions.

Practical constraints prohibited the replication of field results required for rigorous statistical comparison with laboratory data. Results of this study, however, are appropriate as a preliminary numerical comparison to indicate whether laboratory derived S retention potentials are similar under field conditions.

M.E. Watwood, Biology Dep., Univ. of New Mexico, Albuquerque, NM 87131; and J.W. Fitzgerald, Microbiology Dep., Univ. of Georgia, Athens, GA 30602. Research conducted as part of the Integrated Forest Study. Received 20 July 1987. *Corresponding author.

Published in Soil Sci. Soc. Am. J. 52:1478–1483 (1988).

MATERIALS AND METHODS

Site Description

Field incubations were conducted in July 1985 on two watersheds within the Coweeta basin located near Otto, NC. Watershed 1, a 30-yr-old eastern white pine plantation, and watershed 2, an undisturbed mixed mature hardwood forest are adjacent catchments having south facing slopes. Soils on watershed 1 are in the fine-loamy Fannin series of the Typic Hapludults, and soils on watershed 2 are in the sandy loam Chandler series of the Typic Dystrochrepts. In general, Coweeta surface soils are moderately acid (pH~4.74) with a relatively low cation exchange capacity (~11 $cmol_c$ kg^{-1}) and percent base saturation of approximately 77.2% (Swank and Crossley, 1988).

Field Incubations

An intact section (about 8 kg, wet weight) of the A horizon (approximately 15 cm, in depth), covered with intact 01 (01 = Oi) and 02 (02 = mixture of Oe and Oa) litter layers, was removed from the study site on each watershed at mid-elevation. The section was placed into a 25 by 25 by 25-cm plexiglass incubation chamber through a slider opening in one side of the chamber. This chamber, with an open top and a bottom panel interspersed with small drainage holes, was then placed upon 5-cm supports inside of a larger, watertight, drainage container. The entire double container was placed in the cavity left in the hillslope by the removal of the soil section. A solution of aqueous ^{35}S-labeled sulfate (1.1×10^{-4} TBq $mmol^{-1}$, 7.8 nmol, Amersham Corp., Arlington Heights, IL) was pipetted in successive 10-mL aliquots slowly and evenly over the surface of the 01 litter layer. The application of each aliquot of label was followed by the addition of 10 mL of deionized, distilled water to facilitate even distribution of the label. The total volume of liquid added was 200 mL. For each watershed, separate field incubations of 2- and 7-d duration were carried out. There was a 2.8 cm of rainfall during the initial 2-d incubation and 6.3 cm was recorded between Days 2 and 7. Mean sulfate concentrations in throughfall (determined by ion chromatography) were 2.29 and 1.56 mg L^{-1} for watersheds 1 and 2, respectively.

Following incubation, the entire double container was removed from the watershed and transported to the laboratory, where the volume of water present in each drainage container was measured. The amount and composition of ^{35}S present in this water was determined by electrophoresis on Whatman no. 1 paper in KH_2PO_4–K_2HPO_4 buffer, pH 8.0 (2 h at 200 V), followed by scanning of the dried paper strips with a Hewlett-Packard radiochromatogram scanner (Hewlett-Packard Co., Palo Alto, CA). The 01 and 02 litter layers and the A horizon were separated, and each horizon was sieved (<1 cm) and mixed well. All samples were stored

at 5 °C in sealed bags, and analyses were performed within 4 d following the field incubations. Six subsamples (1 g, wet weight) of each homogenized soil sample were extracted (Fitzgerald et al., 1983) to recover ^{35}S present in water soluble, salt extractable (successive washes of 1 M Na_2SO_4, NaH_2PO_4, and LiCl), acid extractable (6M HCl, 121 °C, 12 h) and base extractable (2M NaOH, 25 °C, 12 h) fractions. The amount of ^{35}S present in each extract was determined by liquid scintillation counting. Amounts of ^{35}S present in the acid and base extractable fractions were added together to represent the total amount of organic ^{35}S, since previous work (Fitzgerald et al., 1982) has shown that salt extraction completely removes any adsorbed ^{35}S, and the ^{35}S that is extractable only by strong acid and base is therefore present as covalently bonded organic S. Routinely, 90 to 98% recoveries of added label were achieved by the complete extraction procedure.

Immediately prior to and following field incubations, intrinsic components in each horizon (sieved and mixed subsamples) were quantitated as follows. Total S was determined by the hypobromite oxidation method of Tabatabai and Bremner (1970). Organically bound ester sulfate and inorganic S were determined by direct sample reduction with hydriodic acid (HI) (Johnson and Nishita, 1952). The carbon-bonded S component of organic S was calculated as the difference between total S and HI-reducible S. Amino acid S present in organic matter was quantified by reduction with Raney nickel (Freney et al., 1970), and sulfonate S (a form of carbon-bonded S) was estimated as the difference between total carbon-bonded S and amino acid S. Results of preliminary work in this laboratory utilizing a wide range of sulfonates of varying carbon chain length (Fitzgerald and Franklin, 1982) demonstrated that this sulfur linkage is not reduced by either HI or Raney nickel. Thus carbon-bonded S, which is not reducible by this catalyst, is considered to represent sulfonate linked S. Water soluble S was obtained by shaking samples (1:5, sample solution) for 15 min with deionized distilled water, followed by centrifugation. Phosphate extractable S was subsequently recovered by shaking the water washed residue with 0.02 M Na_2HPO_4 for 30 min, followed by centrifugation. This extraction was repeated, and the two supernatants were combined. Following determinations for HI-reducible S, each extract was analyzed for inorganic sulfate by ion chromatography. Because HI reduces both inorganic S as well as ester sulfate (Freney, 1961), the ester sulfate content of these extracts was calculated as the difference between the amounts of HI-reducible S and sulfate-S. Nonphosphate extractable (insoluble) ester sulfate was determined as the difference between total HI-reducible S and the HI-reducible S present in both water and phosphate extracts.

The final step in each intrinsic S assay (except ion chromatographic determinations) involved colorimetric determination of the S^{2-} generated by each specific reducing agent. Subsamples of solutions used for the determination of intrinsic S following field incubations were also assayed for radioactivity using liquid scintillation counting to measure the amount of ^{35}S incorporated into various forms of S.

Laboratory Studies

Laboratory incubations were conducted with 01 and 02 litter and A horizon soil samples collected from six randomly selected locations near each study site at the beginning of each field incubation. Following collection, samples were stored at 5 °C in sealed bags, and all analyses were conducted within a week of sampling. Samples (1 g, wet weight) were incubated with ^{35}S-labeled sulfate (3.3×10^{-2} TBq $mmol^{-1}$, 7.5 nmol, Amersham) in scintered glass filtersticks for 2 d at 20 °C, and then extracted as described previously to determine the amount of label present in water soluble, salt extractable, and organic (acid and base extractable) fractions.

Total C was determined in triplicate for six randomly collected samples of each horizon using a Hewlett-Packard C analyzer, and moisture content (after drying at 50 °C for 2 d) of each horizon was determined for subsamples within each chamber prior to and following field incubations.

Table 1. Total C and moisture content before and after field incubations [mean (SE)].

Watershed and horizon	Total C content†	Moisture content‡		
		Day 0	Day 2	Day 7
	g kg^{-1}			
WS1 O1	460 (4)	321 (10)	2640 (55)	1901 (30)
O2	428 (10)	1480 (87)	1806 (54)	1470 (41)
A	40 (3)	189 (4)	402 (1)	454 (2)
WS2 O1	452 (4)	125 (2)	1956 (83)	1900 (77)
O2	357 (14)	655 (56)	1873 (36)	1535 (25)
A	44 (3)	207 (4)	452 (3)	517 (5)

† n = 6 random samples. ‡ n = 6 chamber subsamples.

RESULTS AND DISCUSSION

Total C content of the 01 and 02 litter layers and the A horizon soil, determined prior to the field incubations, was similar for both watersheds, ranging from about 40 g kg^{-1} in the A horizon to over 450 g kg^{-1} in the 01 litter layer (Table 1). Although total C is not the most precise indicator of organic matter and energy, it is a useful reference for comparison among sites (Johnson and Todd, 1983; Watwood et al., 1988b). In this study, the similarity of total C content in litter and soil of the two forested stands may indicate that similar amounts of organic matter and energy are present in these systems. Since the retention of S via sulfate adsorption and organic S formation has been shown to be related to these variables (Johnson and Todd, 1983; Strickland and Fitzgerald, 1984), the two systems might be expected to exhibit similar S retention capacities, despite the differences in vegetative cover.

Moisture content (Table 1), which has also been shown to have an effect on organic S formation (Watwood et al., 1988a), was also very similar for the A horizon of both watersheds, although there was considerable variation in moisture content of the heterogeneous litter layers. Within each watershed, moisture content in each horizon increased considerably during the 2-d incubation but after 7 d was roughly equal to or lower than that after 2 d. The A horizon moisture content increased slightly during the longer incubation period. These postincubation values reflect the large amounts of rainfall recorded at the study sites. Moisture contents of samples used in laboratory incubations were very similar to those determined within the incubation chambers prior to field incubations.

Table 2 shows results of the field and laboratory ^{35}S incubations. With respect to 2 day laboratory incubation of the 01 litter layer for watersheds 1 and 2, respectively, 5.3 and 5.8 nmol ^{35}S g^{-1} dry weight was recovered as water soluble S, 2.8 and 2.5 nmol as salt extractable, and 16.7 and 15.4 nmol was recovered only by treatment with strong acid and base. Similar results were obtained for the 02 layer, although less of

Table 2. Water soluble, salt extractable, and acid and base extractable ^{35}S fractions recovered following laboratory and field incubations with ^{35}S-labeled sulfate [mean (SE)].

Incubation type and duration	Watershed and horizon	Water soluble	Salt extractable	Acid and base extractable
		μmol S kg^{-1} dry weight		
Lab, 2 d†	WS1 O1	5.3 (1.0)	2.8 (0.2)	16.7 (2.2)
	O2	5.4 (1.3)	2.6 (0.5)	8.4 (1.4)
	A	1.9 (0.2)	7.5 (0.5)	3.1 (0.3)
	WS2 O1	5.8 (1.8)	2.5 (0.5)	15.4 (3.1)
	O2	2.4 (1.0)	2.2 (0.6)	8.2 (1.4)
	A	2.2 (0.4)	8.2 (0.5)	2.6 (0.3)
Field, 2 d‡	WS1 O1	4.0 (0.2)	4.1 (0.2)	19.2 (0.2)
	O2	5.7 (0.2)	5.3 (0.1)	10.2 (0.3)
	A	1.5 (0.1)	6.7 (0.1)	2.4 (0.1)
	WS2 O1	2.4 (0.1)	3.6 (0.2)	16.2 (0.1)
	O2	3.8 (0.1)	6.4 (0.1)	11.4 (0.2)
	A	1.0 (0.1)	7.1 (0.1)	2.9 (0.1)
Field, 7 d‡	WS1 O1	2.2 (0.2)	3.0 (0.3)	16.6 (0.4)
	O2	3.4 (0.4)	5.3 (0.4)	9.9 (0.6)
	A	1.0 (0.1)	7.0 (0.2)	2.9 (0.2)
	WS2 O1	3.0 (0.3)	2.5 (0.3)	16.3 (0.6)
	O2	2.5 (0.3)	4.2 (0.3)	12.3 (0.5)
	A	1.3 (0.2)	6.5 (0.2)	3.6 (0.1)

† n = 6 random samples. ‡ n = 6 chamber subsamples.

the labeled sulfate S had become incorporated into acid and base extractable S during the incubation (8.4 and 8.2 nmol for watersheds 1 and 2, respectively). More ^{35}S was recovered as salt extractable S, and less as water soluble or acid and base extractable S for each A horizon. This result was not unexpected since the salt extractable fraction is considered to represent ^{35}S, which has become adsorbed during incubation, and more adsorption sites are present in soil as opposed to litter. Also, since S retention capacities are expressed on a dry weight basis, the organic S formation capacity observed with the A horizon was much greater than that of the litter samples because of the greater bulk density of the former horizon. For example, the application of conversion factors derived for a Coweeta control hardwood forest (Swank et al., 1984) to the current laboratory derived data for watershed 2 yields estimates of annual potential S fluxes in the 01, 02, and A horizons, respectively, of 0.10, 0.10, and 56.27 kg ha^{-1}yr^{-1} for adsorption and 0.54, 0.28 and 17.84 kg ha^{-1}yr^{-1} for microbial incorporation.

With a few exceptions, results of the 2-d field incubation (Table 2) were similar to those obtained with laboratory incubations. With respect to the litter layers of both watersheds, more salt extractable ^{35}S was recovered following field incubation than following laboratory incubation. The A horizon of watershed 1 incorporated slightly less of the labeled sulfate into acid and base extractable S (3.1 and 2.4 nmol g^{-1} dry weight, laboratory and field, respectively), but for watershed 2, the values for laboratory and field incubations were very similar.

Results of the 7-d field incubation were similar to those of the 2-d laboratory and field incubations, with the majority of the ^{35}S being recovered as acid and base extractable S (organic S) for both litter layers, and as salt extractable S (adsorbed S) for the A horizons. Slightly more ^{35}S was recovered as acid and base extractable S in the A horizon of each watershed after 7 d than after 2 d of field incubation. These values (2.9 and 3.6 nmol g^{-1} dry weight, watershed 1 and 2 respectively) did not greatly exceed those obtained in the 2-d laboratory incubations. Although organic S formation rates may have increased during the longer 7-d incubation, the concomitant process of organic S mineralization, which has been documented with forest soils under laboratory conditions (David et al., 1983; Strickland and Fitzgerald, 1984) and field conditions (Strickland et al., 1986b) may have masked net increases in organic S formation during this period.

The amount of ^{35}S present in water collected in each drainage container was extremely low, and in no case comprised more than 2% of the total ^{35}S recovered. Electrophoresis of samples revealed that the sole component in the water was ^{35}S-labeled sulfate in all cases. Examination of the S status of the samples prior to and following field incubations reveals considerable variation in total S content in the litter layers (Table 3). This result was not surprising considering the heterogeneous nature of this material, and the large increases in total S noted after the 2-d incubation may be due to sample variation instead of an actual increase in S content. However, total S also increased somewhat in the A horizons of each watershed, indicating that perhaps elevated inputs of S in the litter

Table 3. Forms and distribution of intrinsic S fractions prior to and following field incubations [mean (SE), n = 3 chamber subsamples].

Duration of incubation, d	Watershed and horizon	Total S	Amino acid†	Sulfonate	Water soluble	Phosphate extractable	Nonphosphate extractable water
		mg kg^{-1} dry weight					
0	WS1 O1	262.5 (9.7)	24.2 (7.9)	110.8 (7.9)	11.8 (4.2)	52.2 (8.4)	64.1 (7.1)
	O2	484.5 (63.5)	11.1 (1.9)	264.1 (1.9)	3.9 (2.4)	34.4 (4.4)	171.0 (4.8)
	A	150.1 (6.1)	20.4 (5.1)	28.8 (5.1)	3.9 (0.5)	9.6 (1.2)	87.4 (0.5)
	WS2 O1	481.4 (91.5)	6.7 (0.9)	355.8 (1.0)	8.7 (0.5)	30.8 (1.0)	79.4 (1.0)
	O2	283.9 (54.4)	38.3 (7.7)	38.9 (7.7)	6.2 (1.4)	32.1 (1.7)	168.1 (1.4)
	A	226.2 (7.7)	55.9 (7.2)	88.7 (7.2)	9.0 (2.0)	7.7 (2.0)	64.9 (0.5)
2	WS1 O1	1182.6 (92.6)	33.1 (1.2)	655.2 (1.2)	34.3 (9.5)	54.5 (4.7)	408.0 (5.9)
	O2	833.8 (57.0)	45.0 (2.5)	428.6 (2.5)	20.8 (4.2)	24.2 (1.7)	314.3 (3.3)
	A	216.0 (82.8)	0.9 (0.2)	43.2 (1.1)	4.8 (0.4)	20.5 (3.5)	146.2 (0.6)
	WS2 O1	801.5 (31.8)	34.5 (2.4)	307.8 (2.4)	38.5 (4.8)	109.0 (7.2)	313.4 (3.2)
	O2	775.6 (175.7)	37.2 (3.9)	170.6 (3.9)	25.6 (0.8)	27.1 (3.9)	515.8 (3.1)
	A	269.8 (4.6)	7.6 (0.3)	82.6 (0.3)	14.3 (0.3)	24.0 (1.9)	141.9 (1.1)
7	WS1 O1	1071.6 (127.4)	27.9 (5.4)	637.6 (5.4)	12.9 (1.1)	55.7 (3.2)	338.6 (2.1)
	O2	503.1 (42.)2	41.8 (4.0)	16.1 (4.0)	17.6 (2.0)	17.1 (1.5)	411.0 (2.5)
	A	229.6 (22.9)	15.2 (0.7)	33.3 (0.7)	9.4 (0.5)	27.6 (1.2)	144.4 (0.7)
	WS2 O1	736.0 (10.2)	45.6 (2.9)	265.0 (2.9)	32.4 (4.4)	55.9 (4.4)	337.1 (3.7)
	O2	637.2 (77.2)	63.1 (5.1)	377.2 (0.6)	15.9 (1.9)	18.5 (1.9)	162.5 (1.3)
	A	226.6 (61.3)	3.6 (3.2)	72.5 (1.4)	10.7 (0.2)	15.4 (1.6)	124.6 (1.8)

† Raney Ni reducible S.

layers did take place during this time, and that some of the additional S leached into the A horizon, resulting in the observed increases. In all cases the majority of the total S was comprised of organic S, which is in agreement with findings of Mitchell et al. (1986) for soils from a Rocky Mountain forested watershed.

In the present study, organic S was characterized further on the basis of S linkage groups. With the exception of the 02 horizon of watershed 1 (7-d incubation), sulfonate S was the major form of carbon-bonded S present, ranging from 14 to 60% of total S. Amino acid S accounted for <25% of the total S in all cases, and a pronounced decrease in this component was noted for the A horizon of both watersheds during the initial 2-d incubation. Amounts of amino acid S in the surface soil subsequently increased during the 7-d incubation for watershed 1, and remained constant for watershed 2. Nonextractable (insoluble) ester sulfate represented 25 to 81% of the total S, and in watershed 1 this component remained fairly constant throughout the duration of the incubations, with the exception of the 02 litter layer, in which ester sulfate increased substantially after 7 d. This fraction showed much more variation for watershed 2. With a few exceptions (Table 3), amounts of water soluble and phosphate extractable S in each horizon were higher in watershed 2 than in watershed 1. Both of these fractions were present in low quantities, but when considered together, they accounted for over 10% of the total S in several cases.

Further differentiation of water soluble and phosphate extractable S into organic and inorganic components indicated that ester sulfate was often detected in both pools (Table 4). In some cases the amounts of ester sulfate were actually higher than those of inorganic sulfate, especially in the phosphate extractable fraction of litter layers. The findings that organic S can be adsorbed and can occur in this form at levels higher than inorganic sulfate confirm the suggestions of Strickland and Fitzgerald (1984), and Mitchell et al. (1986) that adsorbed S (phosphate extractable S) may be a very dynamic fraction in forest systems. The observation that organic S is present in water soluble forms (Table 4) confirms results obtained previously from samples collected from other watersheds in the Coweeta basin (Fitzgerald et al., 1982).

The distribution of ^{35}S following field incubations indicated that the majority of the added label was incorporated into organic matter (Table 5); a result in agreement with the predominance of intrinsic unlabeled organic S in these horizons (Table 3). Moreover, amounts of amino acid S in samples from the study sites prior to incubation were generally low, and correspondingly <10% of the ^{35}S was incorporated into this fraction during field incubations (Table 5). These results may be due to the high capacity of these soils to mineralize amino acid S (Fitzgerald and Andrew, 1984). In most instances, the majority of the organic ^{35}S was recovered as sulfonate ^{35}S. This result is unexpected in view of the findings of Schindler et al. (1986), who found a more rapid incorporation of added ^{35}S-sulfate into the ester sulfate fraction of organic S in soils from a northern hardwood forest. However, the enhanced lability of the ester sulfate linkage to either enzymatic or nonenzymatic hydrolysis, documented for soils of the Coweeta basin (Fitzgerald et al., 1985), may have rendered this fraction more susceptible than sulfonate S to mineralization during these incubations. This would mean that a higher net percentage of ^{35}S would be detected as the more stable sulfonate linkage following incubation.

With respect to the distribution of soluble and phosphate extractable ^{35}S, intrinsic amounts of each were

Table 4. Composition of intrinsic water soluble and phosphate extractable S fractions assayed prior to and following field incubations [mean (SE), n = 3 chamber subsamples].

Duration of incubation, d	Watershed and horizon	Water soluble		Phosphate extractable	
		Sulfate S	Ester S	Sulfate S	Ester S
		mg kg^{-1} dry weight			
0	WS1 O1	5.4 (0.3)	6.3 (0.3)	5.0 (0.3)	47.2 (0.3)
	O2	3.7 (0.4)	ND†	13.5 (3.3)	20.8 (3.3)
	A	2.4 (0.2)	1.4 (0.2)	9.6 (0.1)	ND
	WS2 O1	4.2 (0.4)	4.3 (0.4)	0.9 (0.9)	29.9 (0.9)
	O2	6.3 (0.8)	ND	6.7 (0.7)	25.4 (0.7)
	A	9.0 (0.2)	ND	7.7 (0.9)	ND
2	WS1 O1	34.8 (6.9)	ND	39.0 (9.7)	15.2 (7.6)
	O2	12.1 (2.3)	9.0 (2.3)	ND	24.4 (0.1)
	A	4.7 (0.5)	ND	20.6 (0.3)	ND
	WS2 O1	30.1 (0.4)	8.7 (0.4)	97.6 (18.4)	11.7 (8.1)
	O2	25.0 (1.0)	0.6 (0.3)	7.6 (0.8)	19.3 (0.8)
	A	14.0 (0.2)	0.2 (0.1)	23.6 (0.5)	0.4 (0.2)
7	WS1 O1	12.5 (0.9)	ND	ND	55.4 (0.2)
	O2	7.1 (0.1)	10.5 (0.1)	ND	17.0 (0.4)
	A	9.3 (0.1)	ND	27.5 (1.8)	ND
	WS2 O1	13.9 (0.5)	18.6 (0.5)	ND	56.1 (0.2)
	O2	8.2 (0.4)	7.6 (0.4)	ND	18.6 (0.4)
	A	6.1 (1.9)	4.5 (1.9)	13.8 (0.2)	1.5 (0.3)

† ND = not detected.

Table 5. Forms and distribution of ^{35}S following field incubations [mean (SE), n = 3 chamber subsamples].

Duration of incubation, d	Watershed and horizon	Amino acid†	Sulfonate	Water soluble	Phosphate extractable	Nonphosphate extractable ester
		% of total ^{35}S recovered				
2	WS1 O1	5.4 (2.4)	50.7 (1.1)	7.9 (0.5)	7.1 (0.5)	28.9 (2.4)
	O2	9.2 (3.7)	35.8 (6.8)	15.0 (2.9)	11.4 (2.2)	28.7 (10.6)
	A	5.6 (0.6)	35.4 (11.8)	14.2 (1.2)	15.8 (2.4)	29.1 (12.5)
	WS2 O1	5.2 (1.3)	52.2 (6.8)	19.6 (0.1)	10.5 (0.1)	12.5 (3.2)
	O2	6.8 (2.8)	21.8 (3.0)	13.8 (2.0)	9.5 (1.4)	48.2 (3.1)
	A	5.6 (2.9)	66.9 (5.7)	9.7 (0.3)	12.0 (0.3)	5.9 (2.4)
7	WS1 O1	5.6 (0.8)	43.2 (7.5)	2.8 (0.3)	5.0 (0.5)	43.4 (7.3)
	O2	9.4 (1.4)	24.0 (13.9)	10.3 (1.9)	11.9 (2.2)	44.6 (9.1)
	A	6.4 (0.4)	36.3 (5.9)	8.8 (0.3)	11.3 (0.4)	37.2 (6.2)
	WS2 O1	9.6 (3.5)	41.9 (15.0)	5.8 (2.1)	7.8 (2.9)	35.7 (7.2)
	O2	9.2 (3.8)	30.6 (11.0)	19.0 (3.2)	5.7 (1.0)	35.7 (7.2)
	A	9.6 (1.1)	32.0 (7.4)	21.6 (2.7)	25.3 (3.1)	11.6 (2.3)

† Raney Ni redicible ^{35}S.

generally low (Table 3), but following field incubation, up to 47% of the added ^{35}S was recovered in these combined fractions (Table 5). In most cases, amounts of water soluble ^{35}S decreased during the longer 7-d incubation, although a net increase during this period was observed for the 02 and A horizons of watershed 2. Net amounts of phosphate extractable ^{35}S exhibited little variation between the two incubation periods in the litter layers. With respect to the A horizon, a slight decrease in this fraction occurred after 7 d in watershed 1 (15.8 and 11.3%, 2 and 7 d, respectively), whereas amounts of this fraction for watershed 2 increased (12.0 and 25.3%, 2 and 7 d, respectively). These results, considered together with findings suggesting the presence of ester sulfate in intrinsic soluble and phosphate extractable pools, indicate that interconversion of inorganic and organic S components comprising these pools may occur frequently. Furthermore, increases in nonphosphate extractable ester ^{35}S-sulfate after the 7-d incubation may reflect a transfer of S from water soluble or phosphate extractable pools to nonphosphate extractable (insoluble) ester sulfate, as previously suggested by Strickland and Fitzgerald (1984).

SUMMARY AND CONCLUSIONS

Overall, these initial results suggest that laboratory determined S retention potentials for litter and surface soil in these forests accurately reflect capacities existing in the field at least during the summer. Extensively replicated field studies will be required to statistically verify this preliminary study, but such an endeavor will obviously be very cost and labor intensive. With respect to S status, this study verifies that organic forms dominate the total S pool in the litter layers and A horizon soil in the watersheds examined. Sulfonate S and insoluble ester sulfate appear to be important organic fractions, as is the ester sulfate component in the soluble and phosphate extractable fractions. The dynamic role of S present in these fractions is further substantiated by the elevated recoveries of ^{35}S in these fractions following incubation compared with preexisting pool sizes. It is noteworthy that the majority of the ^{35}S was recovered in organic forms, predominantly as sulfonate ^{35}S. The process of organic S mineralization may be relevant with respect to regulation of organic S levels, and transfers between the soluble, extractable, and insoluble organic S pools are clearly dynamic. The similarity of S retention potentials between the pine and hardwood watersheds indicates that S cycling may be regulated by factors such as available C or energy, irrespective of vegetation or past management practices.

ACKNOWLEDGMENT

The authors wish to thank W.T. Swank for aid in study site selection at Coweeta, F.G. Lehmann for expert field assistance, and S. Sommer for pertinent editorial comments. We also thank three anonymous reviewers for their valuable suggestions with respect to revision of the manuscript.

REFERENCES

David, M.B., S.C. Schindler, M.J. Mitchell, and J.E. Strick. 1983. Importance of organic and inorganic sulfur to mineralization processes in a forest soil. Soil Biol. Biochem. 15:671–677.

Fitzgerald, J.W., and T.L. Andrew. 1984. Mineralization of methionine sulphur in soils and forest floor layers. Soil Biol. Biochem. 16:565–570.

Fitzgerald, J.W., J.T. Ash, T.C. Strickland, and W.T. Swank. 1983. Formation of organic sulfur in forest soils: A biologically mediated process. Can. J. For. Res. 13:1077–1082.

Fitzgerald, J.W., and B.L. Franklin. 1982. The primary alkylsulfatase of *Pseudomonas aeruginosa*: Inducer specificity and induction kinetics. Can. J. Microbiol. 28:1296–1299.

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

Fitzgerald, J.W., W.T. Swank, T.C. Strickland, J.T. Ash, D.D. Hale, T.L. Andrew, and M.E. Watwood. 1987. Sulfur pools and transformations in litter and surface soil of a hardwood forest. *In* W.T. Swank and D.A. Crossley (ed.) Forest hydrology at Coweeta. Springer-Verlag, New York.

Fitzgerald, J.W., M.E. Watwood, and F.A. Rose. 1985. Forest floor arylsulphatase: Hydrolysis of tyrosine sulphate, an environmentally relevant substrate for the enzyme. Soil Biol. Biochem. 17:885–887.

Freney, J.R. 1961. Some observations on the nature of organic sulphur compounds in soil. Aust. J. Agric. Res. 12:424–432.

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

Freney, J.R., G.E. Melville, and C.H. Williams. 1971. Organic sulphur fractions labelled by addition of ^{35}S-sulphate to soil. Soil Biol. Biochem. 3:133–141.

Johnson, C.M., and N. Nishita. 1952. Microestimation of sulfur in plant material, soil, and irrigation waters. Anal. Chem. 24:736–742.

Johnson, D.W., and G.S. Henderson. 1979. Sulfate adsorption and sulfur fractions in a highly weathered soil under a mixed deciduous forest. Soil Sci. 128:38–40.

Johnson, D.W., G.S. Henderson, D.D. Huff, S.E. Lindberg, D.D. Richter, D.S. Shriner, D.E. Todd, and J. Turner. 1982. Cycling of organic and inorganic sulphur in a chestnut oak forest. Oecologia 54:141–148.

Johnson, D.W., J.W. Hornbeck, J.M. Kelly, W.T. Swank, and D.E. Todd. 1980. Regional patterns of soil sulfate accumulation: Relevance to ecosystem sulfur budgets. p. 507–520. *In* D.S. Shriner et al. (ed.) Atmospheric sulfur deposition: Environmental impact and health effects. Ann Arbor Publ., Ann Arbor, MI.

Johnson, D.W., and D.E. Todd. 1983. Relationships among iron, aluminum, carbon, and sulfate in a variety of soils. Soil Sci. Soc. Am. J. 47:792–800.

McLaren, R.G., J.I. Keer, and R.S. Swift. 1985. Sulphur transformations in soils using sulphur-35 labelling. Soil Biol. Biochem. 17:73–79.

Mitchell, M.J., M.B. David, D.G. Maynard, and S.A. Telang. 1986. Sulfur constituents in soils and streams of a watershed in the Rocky Mountains of Alberta. Can. J. For. Res. 16:315–320.

Schindler, S.C., M.J. Mitchell, T.J. Scott, R.D. Fuller, and C.T. Driscoll. 1986. Incorporation of ^{35}S-sulfate into inorganic and organic constituents of two forest soils. Soil Sci. Soc. Am. J. 50:457–462.

Shriner, D.S., and G.S. Henderson. 1978. Sulfur distribution and cycling in a deciduous forest watershed. J. Environ. Qual. 7:392–397.

Strick, J.E., S.C. Schindler, M.B. David, M.J. Mitchell, and J.P. Nakas. 1982. Importance of organic sulfur constituents and microbial activity to sulfur transformations in an Adirondak forest soil. Northeast. Environ. Sci. 1:161–169.

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

Strickland, T.C., J.W. Fitzgerald, and W.T. Swank. 1986a. *In situ* measurements of sulfate incorporation into forest floor and soil organic matter. Can. J. For. Res. 16:549–553.

Strickland, T.C., J.W. Fitzgerald, and W.T. Swank. 1986b. *In situ* mobilization of ^{35}S-labeled organic sulphur in litter and soil from a hardwood forest. Soil Biol. Biochem. 18:463–468.

Swank, W.T., and D.A. Crossley, Jr. (ed.) 1988. Forest hydrology and ecology at Coweeta. Springer-Verlag, New York.

Swank, W.T., and J.E. Douglass. 1977. Nutrient budgets for undisturbed and manipulated hardwood forest ecosystems in the mountains of North Carolina. p. 343–363. *In* D.L. Cornell (ed.) Watershed research in eastern North America: A workshop to compare results. Vol. I. Smithsonian Inst., Edgewater, MD.

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

Tabatabai, M.A., and J.M. Bremner. 1970. An alkaline oxidation method for determination of total sulfur in soils. Soil Sci. Soc.

Am. J. 34:62–65.
Watwood, M.E., J.W. Fitzgerald, and J.R. Gosz. 1986. Sulfur processing in forest soil and litter along an elevational and vegetative gradient. Can. J. For. Res. 16:689–695.
Watwood, M.E., J.W. Fitzgerald, and W.T. Swank. 1988a. Effects of moisture content on sulfate generation and retention in hardwood forest upper soil horizons. Can. J. For. Res. (in press).
Watwood, M.E., J.W. Fitzgerald, W.T. Swank, and E.R. Blood. 1988b. Factors involved in potential sulfur accumulation in litter and soil from a pine forest. Biogeochemistry (in press).