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18. Sulfur Pools and Transformations in Litter and Surface Soil of a Hardwood Forest

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Work on sulfur transformations was initiated at Coweeta in 1980 in an effort to de mine the relevance of the plant sulfolipid as a source of sulfate in forest soil. It s became apparent from the results of this study (Strickland and Fitzgerald 1983) tha horizon soil from several watersheds exhibited not only rapid S-mineralization rates this compound, but samples also rapidly incorporated a substantial portion of released sulfate into a fraction which could only be recovered by acid extraction. T work was followed closely by a study of the fate of sulfate in soils of several ecosyst at Coweeta (Fitzgerald et al. 1982). The results confirmed initial observations m with the sulfolipid and suggested that the acid extractable fraction might be compr of soil organic sulfur.

Although work at Coweeta by Johnson et al. (1980) indicated that soil adsorptic an important sulfate retention mechanism, it was also apparent that micro metabolism was also important in sulfur accumulating properties of watersheds loc in the Coweeta basin (Chapter 4). Sulfate arising from atmospheric depositio immobilized by adsorption as well as by conversion to organic forms of sulfur. In co quence, subsequent work has concentrated on sulfate adsorption and on provi quantitative evidence for the incorporation of sulfate-S into organic matter (Fitzge et al. 1983, 1985). Preliminary estimates of the annual flux of sulfate into the fo floor and all mineral horizons of a control hardwood forest (WS 18, 30 Kg S h Swank et al. 1984) suggest that organic S formation is a major pathway in the su cycle at Coweeta.

Because the conversion of sulfate-S to organic S will immobilize the anion, process together with adsorption act as sulfate reservoirs, and should also reduce c leaching and thus lessen the impact of acid precipitation on forest soils of the Cov basin (Swank et al. 1984, 1985). However, organic S formation generates a pool w is subject to mobilization and subsequent mineralization (Strickland et al. 1! Examination of the mobilized S-pool indicated that most of the sulfur (94%) present as sulfate, whereas the remainder consisted of soluble organic S. Since the sequent metabolism of organic S determines the importance of the formation pr as a sulfur retention mechanism, considerable effort (Strickland et al. 1984; Strick and Fitzgerald 1984) has also been made to characterize organic S mobilizati forest soil and litter at Coweeta. The data indicate that organic S mobilization o at rates substantially less than those for organic S formation (Swank et al. 1985), indicating a net accumulation of insoluble organic S.

The purpose of this chapter is to review sulfur research conducted over the p years on control WS 18. Included in this objective is an attempt to provide an ecosy level perspective for organic S formation, mobilization, and accumulation fo forest floor and A_1 horizon soil utilizing data obtained from samples collected monthly basis over at least a year interval in most cases. Data on the in situ leve organic and inorganic S present in forest floor and soil will also be presented tog with a description of the sulfur linkage groups comprising the organic S pool.

Experimental Site and Sulfur Analyses

Data reported in this chapter are based upon analyses of samples taken along a tra of WS 18 established at mid-elevation on the catchment in May 1982. The tran consisting of 10 equally spaced 0.01 ha circular plots, transverses the watershed ridge to stream to ridge. Prior to July 1983, a single sample of the O_1 and O_2 laye three samples of A_1 horizon were taken at random on a monthly basis from each o 10 plots. These samples were analyzed separately. In June 1983 and thereafter, sam taken as above were collected on a quarterly basis. Forest floor components were lyzed separately, but triplicate A-horizon samples from each plot were mixed in e proportions and each mixture was then analyzed. Sample collection dates and quency are given in the text as a footnote for each particular analysis.

Total S was determined by hydriodic acid reduction after oxidation of each samp hypobromite (Tabatabai and Bremner 1970). Ester sulfate content was determine reduction with hydriodic acid (Freney 1961). Since hydriodic acid will not reduce fur which is directly bonded to carbon, the amount of this form of sulfur was calcul as the difference between the total S content of a given sample and the amount of h odic acid reducible S present in that sample. The amount of carbon bonded S pre in each sample as amino acid S was determined by reduction with Raney Ni (Fren al. 1970). Because this catalyst will not reduce the other major form of carbon bo S (e.g., sulfonate-S), the difference between the total carbon bonded S content an Raney Ni reducible S represents the amount of sulfonate-S in a given sample. Sol and adsorbed S was determined by hydriodic acid reduction of extracts obtaine extraction of the samples with water and basic phosphate, respectively. Inorg

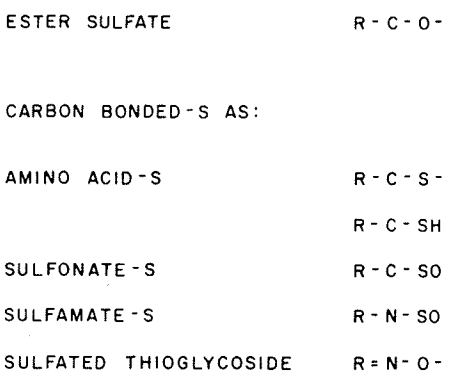

Figure 18.1. Naturally occuring organic sulfur linkage groups.

sulfate in these extracts was determined by anion chromatography (Dick and Tabat 1979). Hydriodic acid reduces ester sulfate and inorganic sulfate; therefore, value soluble and adsorbed ester sulfate were obtained as the difference between hydri acid reducible S and sulfate determined by chromatography. Structures for the var sulfur linkage groups detected by these reagents are shown in Figure 18.1.

In Situ Forms of S and Pool Sizes

Analyses of S forms present in the upper horizons of WS 18 are shown in Table 1 The extent of analyses is comparable to that previously available for agricultural tems (Fitzgerald 1976) and represents, to our knowledge, the only analyses of its for a forest ecosystem. In agreement with observations made for a hardwood fore the Adirondacks (David et al. 1982), carbon bonded S, as opposed to ester sulfat

Table 18.1. Mean Concentrations (μg S g^{-1} Dry Weight), % of Total S[a], and Coefficien Variation (C) for Litter and Soil from WS 18[b]

	O_1 Layer			O_2 Layer			A_1 Horizon	
Form	$\bar{x}$	%	C	$\bar{x}$	% Total S	C	$\bar{x}$	% Total S
Ester sulfate	185	13	0.82	360	27	0.31	118	40
Carbon bonded S	1298	87	0.72	949	72	0.29	161	54
Amino acid S[c]	270	18	0.42	452	34	0.25	69	23
Sulfonate S	1028	69	0.80	497	38	0.33	92	31
Soluble S[d]	0	–	–	1.43	0.1	3.08	1.6	0.5
Adsorbed S[d]	0	–	–	13.8	1.0	0.75	17.1	6
Total S	1482	–	0.74	1325	–	0.31	295	–

[a] Carbon bonded S = amino acid S + sulfonate S.
[b] Samples collected from plots 1–10 in May 1984; n = 20 except as noted.
[c] n = 30
[d] n = 10

Table 18.2. *In Situ* Levels of Soluble and Adsorbed Sulfur in Litter and Soil from WS 18[a]

Horizon	Amount (μg S g^{-1} Dry weight) ± SE and (% E) as: Soluble	Adsorbed
O_1	6.9 ± 2.1 (30.4)	12.2 ± 2.8 (23.0)
O_2	25.2 ± 6.5 (25.8)	40.7 ± 7.7 (18.9)
A_1	4.4 ± 1.1 (25.0)	35.5 ± 3.9 (11.0)

[a] Samples collected from plots 1–10 quarterly, November 1983 through August 1984; $n = 40$.

the dominant component in the forest floor representing 87% and 72% of the tota the O_1 and O_2 layers of WS 18, respectively. However, unlike the Adirondack for which the level of carbon bonded S comprised about 74% of the S in the soil, Table shows that about 54% of the total S of the A_1-horizon of WS 18 consists of sulfur i linkage. After correction for the levels of soluble and adsorbed S in the sample remainder (40%) of the sulfur in soil from WS 18 is comprised of ester sulfate. An ysis of the linkage groups present in the carbon bonded S pool (Table 18.1) indi that sulfonate-S is a major component in all horizons, and especially in the O_1 la which this form comprised 69% of the total S. This finding was not unexpected, the plant sulfolipid in which S is present in a sulfonate linkage is considered to repr a major component of leaf sulfur (Harwood and Nicholls 1979). This observation pled with previous results showing that the A-horizon can mineralize plant sulf and amino acid S (Strickland and Fitzgerald 1983; Fitzgerald and Andrew 1984) gests that carbon bonded S represents an important source of sulfate for forest Soluble and adsorbed S (Table 18.1) represented minor components ($\leq 6\%$) of t

Table 18.3. Nature and Pool Sizes of In Situ Soluble and Adsorbed Sulfur in Litter and Soi WS 18[a]

S-Pool and Determination	Amount (μg S g^{-1} Dry Weight) ± SE in: O_1 Layer	O_2 Layer	A_1 Hc
Soluble S by HI-reduction (SO_4^{2-} + ester SO_4^{2-})	22.9 ± 3.6	85.2 ± 14.1	4.1 ±
Soluble S by IC (SO_4^{2-} only)	20.8 ± 2.5	49.8 ± 8.6	5.8 ±
Soluble ester SO_4^{2-} by difference	5.8 ± 2.5[b]	35.4 ± 5.9	0.4 ±
Adsorbed S by HI-reduction (SO_4^{2-} + ester SO_4^{2-})	0	86.0 ± 19.3	65.9 ±
Adsorbed S by IC (SO_4^{2-} only)	0	28.2 ± 4.1	22.5 ±
Adsorbed ester SO_4^{2-} by difference	0	57.7 ± 16.4	43.3 ±

[a] Samples collected from plots 1–10, August 1984 and extracts analyzed by HI (hydriodic acid) rec and IC (ion chromatography); $n = 10$.
[b,c] HI > IC value for 4 and 2 of the plots, respectively.

found in the forest floor and soil of WS 18 during May of 1984. These sulfur pools exhibited the greatest variability in concentration across the sampling transect, coefficients of variation (CV) ranging from 0.75 to 3.5 compared with the oth forms reported in Table 18.1, where the CV was ≤ 0.75. The 0_1 layer exhibitec highest variability for all forms of sulfur considered. Although soluble and adsorb was undetectable in the O_1 layer of all plots during May 1984 (Table 18.1), some o 0_1 samples collected between November 1983 and August 1984 did have sulfur in t forms (Table 18.2). Variability was large, especially for levels of soluble S in all t horizons. Since hydriodic acid reduces inorganic, as well as ester-linked S, this rea was utilized to quantify soluble and adsorbed S so that forms of S, apart from sul could be detected. Previous work on the fate of sulfate in forest soil (Fitzgerald e 1982) suggested that ester sulfate formed from this anion was not confined solely nonsalt extractable sulfur pool. By analyzing water and salt extracts by hydriodic reduction and by anion chromatography, it is evident (Table 18.3) that ester su represents a major component of the soluble and adsorbed sulfur pools of 0_2 layer s ples collected from the transect during August 1984. A similar observation was n for the adsorbed S pool of the A_1 horizon, whereas, soluble S in this horizon, as as in the 0_1 layer was comprised almost totally of inorganic sulfate (Table 18.3).

Transformations of Amino Acid S

In situ levels of amino acid S have not been documented for other forest ecosyste and in view of the apparent importance of sulfate input to soil from these sourc study of the metabolic fate of ^{35}S-methionine in forest floor and soil from WS 18 conducted (Fitzgerald and Andrew 1984, Fitzgerald et al. 1984). Table 18.4 sum rizes data on rates of S-mineralization and incorporation of methionine into org matter. The rates for the O_1, O_2 forest floor layers and A_1 horizon are compared in situ levels of amino acid S found in each horizon. The relative differences in th situ amino acid levels between each horizon reflect the relative differences in methionine mineralization and incorporation rates for each horizon. Table 18.4 sh that the rate for incorporation exceeds the mineralization rate in both the O_1 anc layers and these horizons contained an in situ level of amino acid S which was at l fourfold greater than the A_1 horizon. As expected from mineralization rates of the p sulfolipid (Strickland and Fitzgerald 1983), the A_1 horizon exhibited a mineraliza

Table 18.4. Metabolism of Added Amino Acid Sulfur in Litter and Soil from WS 18[a]

	(μg S g^{-1} 12h^{-1} at 20°C) $\pm$ SE		μg S g^{-}
Horizon	Amount Mineralized	Amount Incorporated into Organic Matter	In Situ Amino Ac
O_1	0.05 $\pm$ 0.01	0.37 $\pm$ 0.04	270
O_2	0.38 $\pm$ 0.07	0.69 $\pm$ 0.07	452
A_1	0.16 $\pm$ 0.01	0.07 $\pm$ 0.01	69

[a] Added as ^{35}S-methionine; samples collected from plots 1-10 in August 1982; $n = 10$.
[b] Taken from Table 18.1.

rate for methionine which was about twofold greater than the incorporation rate, this difference may account for the low in situ amino acid S level in this horizon rel to the forest floor.

Transformations of Sulfate and Organic S

In view of atmospheric inputs of sulfate to forests of the Coweeta basin, a concent effort has been made over the past 4 years to document the metabolic fate of this a in litter and soil from WS 18. In brief, ^{35}S-labelled sulfate at a concentration simil the average annual input concentration from throughfall and soil solutions is incul with each horizon for 48 hr at 20° C. The samples are then washed with water, extr with salt, and finally extracted with a strong acid and base. The extracts are anal separately to determine the level of ^{35}S which remains nonmetabolized (water extr which has been adsorbed (salt extract) and which is only recovered by acid-base ex tion. Analysis of these extracts by electrophoresis revealed that sulfate was the n ^{35}S-labelled component. Details of the procedure have been published (Fitzgerald 1982, Strickland and Fitzgerald 1984). Results of these analyses on samples coll on a monthly basis for 1 year are summarized in Table 18.5. Total recoveries of the approached 90% for all analyses and in terms of a percentage of ^{35}S which cou recovered, between 9 and 13% of the label remained unmetabolized in all hori Table 18.5 also shows that the potential for adsorption was greatest in the A_1 ho and least in the O_1 horizon (72% and 12% of the ^{35}S present in salt extracts, re tively). On a dry weight basis, the O_1 layer exhibited the greatest potential for i porating the label into acid-base extractable organic matter, whereas the A_1 ho possessed the lowest capacity for incorporation. Nevertheless, approximately 2(the ^{35}S was found in the combined acid-base extract of this latter horizon (Table 1 Moreover, when the quantities of substrate comprising the forest floor and A_1 ho are taken into consideration (Table 18.6), estimates of the annual potential flux o fate into organic matter were greatest for the A_1 horizon. This horizon exhibits a p

Table 18.5. Potential Fates of Added Sulfate in Litter and Soil from WS 18[a]

	Amount (nmole SO_4^{2-} g^{-1} 48 h^{-1} at 20°C) ± SE in		
Fraction	O_1 Layer	O_2 Layer	A_1 Ho
Water extract	2.48 ± 0.40 (8.8)[b]	2.48 ± 6.1 (12.5)	0.74 ± (8
Salt extract	3.29 ± 0.41 (11.7)	6.34 ± 0.44 (32.0)	6.03 ± (71
Acid-base	22.4 ± 0.91 (79.5)	11.0 ± 0.66 (55.5)	1.67 ± (19
Total percent recovery	88.3 ± 0.80	89.3 ± 0.68	89.6 ±
n	120	120	36

[a] Samples collected from plots 1-10 monthly May 1982 through June 1983, inclusive.
[b] Percent of total ^{35}S recovered is given in parentheses.

Table 18.6. Estimates of Annual Potential Flux of Sulfate into Salt Extractable (Adsorbed S) and Acid plus Base Extractable Organic S in Litter and Soil from WS 18[a]

	Flux (Kg SO_4^{2-} − S ha^{-1} y^{-1}) into:	
Horizon	Adsorption	Organic Matter
O_1	0.12	0.78
O_2	0.22	0.38
A_1	41.38	11.46

[a] Calculations made using means shown in Table 18.5.

tial at least 10-fold greater than that for the forest floor. Similar considerations apply to the potential capacity for sulfate adsorption (Table 18.6) but, in this case, potential for the A_1 horizon was more than 100-fold greater than the forest floor. W data are placed within an ecosystem perspective, it is clear that sulfate adsorption incorporation into organic matter are important soil processes in the sulfur cycle of forest. The importance of the incorporation process is not confined solely to the horizon. Based upon a soil profile study conducted during August 1982, Swank and workers (1984, 1985) estimated a flux of sulfate into organic matter in the B_W hori which was equivalent to that estimated for the A_1 horizon for this sampling date.

Characterization of Acid-Base Extractable ^{35}S

Since the incorporation of sulfate into organic matter is based on incorporated S can only be recovered by acid and base extraction, some attention has been give characterizing this conversion in an effort to prove that the sulfur is incorporated organic matter by covalent bond formation. Recovery of ^{35}S under conditions extract organic matter does not, of necessity, mean that ^{35}S was originally preser organic S. An alternative possibility is that the ^{35}S was simply adsorbed to organic r ter and could not be released by salt extraction. Several lines of evidence rule out alternative, and these will be reviewed briefly. The incorporation of ^{35}S into the a base extractable pool was shown to be time- and temperature-dependent (Fitzge and Johnson 1982; Fitzgerald et al. 1983), and subject to stimulation by incre energy availability (Strickland and Fitzgerald 1984). While these characteristics dc unequivocally rule out adsorption of S in favor of covalent bond formation, the show that the process is microbially mediated with characteristics unlikely for a pu physical phenomenon such as adsorption. We were subsequently able to isolate labelled organic matter by pyrophosphate extraction at pH 8 (Fitzgerald et al. 19 Unlike acid-base extractions (Fitzgerald et al. 1983), organic S can be extracted this reagent with minimal destruction of the sulfur linkage groups. Unequivocal p for the incorporation of sulfate-S into organic matter was obtained when ^{35}S retained after dialysis of the pyrophosphate extract under conditions which would c pletely release adsorbed sulfate. Moreover, the dialyzed extract reacted with reag specific for organic S linkage groups (Fitzgerald et al. 1985).

Table 18.7. Mobilization of Organic Sulfur Formed from Sulfate in Litter and Soil WS 18[a]

Horizon	Organic S Mobilized 24 h^{-1} (%) ± SE[a]	Organic S Formed 48 h^{-1} (n mole S g^{-1})[b]	Organic Retained (n mole S
O_1	13.2 ± 0.71	22.4	19.4
O_2	33.6 ± 2.3	11.0	7.3
A_1	41.3 ± 2.0	1.7	1.0

[a] Samples collected March through November 1983; $n = 42$.
[b] Taken from Table 18.5.

Mobilization and Accumulation of Organic S

Studies of the capacity of soil to mobilize organic S were initially conducted on ples collected from Coweeta control WS 2 (Strickland et al. 1984). The met ology for these determinations is complex and will not be described here. Det descriptions have been published elsewhere (Strickland et al. 1984; Strickland Fitzgerald 1984). Although most of the mobilized S consists of sulfate, some of sulfur is released as soluble organic S. Based upon these findings, Strickland co-workers (1984) suggested that the mobilization process involves depolyme tion of the insoluble organic S matrix to yield soluble forms which then undergo d fation (mineralization).

Potential capacities to mobilize organic S are now available for the forest floor A_1 horizon of WS 18. Means of data for samples collected over a 9-month period the entire transect are shown in Table 18.7. The A_1 horizon and the O_2 forest floor mobilized between 34 and 41% of the available organic S during a 24-hr time inte and only 13% was mobilized in the O_1 layer. Variability was low in all cases wit of estimates <7%; this small variation corresponds with the low variation of sulf incorporation observed in the A_1 horizon (Table 18.5). Moreover, the low variat in the two processes, irrespective of the season in which samples are taken, pro some justification for an attempt to estimate potential organic sulfur accumulati the forest floor and A_1 horizon of WS 18. By utilizing the amount of organic S for within a 48-hr interval (Table 18.5) and by applying the percentage of this sulfur w could be mobilized during a subsequent 24-hr interval, it can be seen (Table 18.7) during this latter period substantial quantities of organic S may be retained within horizon. These calculations are based on a number of assumptions which will be mined in future studies and are presented to provide a perspective of the relative im tance of incorporation and mobilization processes.

Conclusions and Future Considerations

Results derived from the initial study of the capacity to mineralize plant sulfoli conducted some 5 years ago have opened many previously unexpected avenues of s research at Coweeta. The most prominent of these may be transformations invol

inorganic sulfate. Clearly, a long term study of organic S formation and mobiliza
will be absolutely essential for better understanding of soil nutrient dynamics and
true effects of acidic deposition on forests. These processes together with sul
adsorption dominate the sulfur cycle at Coweeta. The extraordinary lack of spa
variability of the respective activities in forest soil indicates that when other fac
which regulate these processes are quantified, a realistic and predictable model
these transformations at the ecosystem level will be tractable. In view of the long-t
data base on nutrient and water budgets which already exists for the Coweeta basin,
influence of sulfur transformations on net accumulation of sulfate (by adsorption
organic S formation) and stream chemistry can be tested. This combined effort sh
lead to a more accurate interpretation of atmospheric deposition impacts.