TRANSFORMATIONS OF SULFUR IN FOREST FLOOR AND SOIL OF A FOREST ECOSYSTEM

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ABSTRACT

Incubation of forest floor and soils containing sulfate labeled with sulfur-35 showed rapid metabolism of the added sulfate to organic sulfur forms by microbial populations. Rates of incorporation were regulated by exogenous sulfate concentrations and temperature. Mobilization experiments also using a ^{35}S label showed substantial release of incorporated sulfur; the mobilization process appeared to be mediated by preformed enzymes with temperature-sensitive activities. Initial results indicate that incorporation rates exceed mobilization rates resulting in a net accumulation of organic sulfur in the soil. This is reflected in watershed budgets which show net sulfate accumulations. These transformation processes are dynamic and strongly influence the supply and mobility of sulfate in soil solution which is important in understanding the impact of acid precipitation on leaching losses of ions.

INTRODUCTION

Acid precipitation represents a major source of the sulfate (SO_4^{2-}) anion in several regions of the United States (Likens & Bormann 1974, Brezonik et al. 1980). At the Coweeta Hydrologic Laboratory in western North Carolina, SO_4^{2-} comprises 68 % of precipitation anions and the bulk precipitation has a mean annual pH of 4.45 (Swank et al. 1984). A few studies have examined S cycling in forest ecosystems (Johnson et al. 1982, Likens et al. 1977) but the fate of exogenous SO_4^{2-} is not well known; particularly transformations in the soil.

In some mixed hardwood forests in the southeastern United States, the hydrologic budgets of SO_4^{2-} indicate that precipitation inputs exceed streamflow export (Johnson et al. 1980). The average annual hydrologic budget for hardwood-covered watersheds at Coweeta during the past 10 years shows apparent accumulations of SO_4^{2-} ranging from 5.9 to 8.9 kg ha^{-1}

TABLE 1. Mean annual (1973-1982) sulfate-sulfur budgets for four mixed hardwood watersheds at Coweeta Hydrologic Laboratory

Watershed number	Area (ha)	Sulfate-Sulfur (kg ha^{-1} $year^{-1}$)		
		Input	Output	Net difference
2	12.1	9.5	1.3	+ 8.2
18	12.5	10.5	1.6	+ 8.9
27	38.8	13.2	7.0	+ 6.2
36	48.6	11.7	5.8	+ 5.9

year^{-1} of elemental S (Table 1). Studies by Johnson et al. (1980) indicate that SO_4^{2-} adsorption to Fe and Al oxides in the soil profile accounts for part of the ecosystem S accumulation. Other research by Fitzgerald et al. (1983) and Swank et al. (1984) suggest that microbial metabolism of SO_4^{2-} to organic S forms in soil is also a major process of potential S immobilization. Sulfur incorporated into organic matter is also subject to mobilization (Strickland et al. 1984).

The objective of this paper is to summarize evidence for SO_4^{2-} incorporation and mobilization in the forest floor and soil of a hardwood forest ecosystem and some of the factors which regulate these transformation processes.

STUDY SITE AND METHODS

The study was located at the Coweeta Hydrologic Laboratory in the mountainous region of western North Carolina, USA. The 2280-ha laboratory, a facility of the U.S. Department of Agriculture, Forest Service, is a site of long-term hydrologic and ecological collaborative research between university and government scientists. Although S transformation research has been conducted in a number of forest soils at Coweeta (Fitzgerald et al. 1982), studies have focused on Watershed (WS) 18. This 12.5-ha north-facing catchment with side slopes of about 50 % has also been a site of long-term research on nutrient cycling. Annual precipitation averages about 180 cm and is rather evenly distributed with at least 10 cm in each month. Overstory vegetation is dominated by 60- to 125-year-old Quercus, Carya, and Acer species with Rhododendron and Kalmia in the understory. The dominant soil is a sandy loam Ashe, a member of the mesic Typic Dystrochrept family. The A_1 horizon is well developed and averages about 10 cm in thickness, with A/B and B_w horizons of about 15 cm and 40 cm thickness, respectively. The C horizon is fractured and strongly weathered.

The basic sampling design consisted of ten 0.01 ha circular plots equally spaced along a 280 m transect that ran across the catchment at mid elevation from ridge to stream to ridge. Forest floor samples were collected by hand and soil samples were collected using a 5-cm diameter auger or by sample removal from the side of a soil pit. All samples were stored in sealed plastic bags and maintained at 4°C.

Incorporation of SO_4^{2-} into organic matter was assayed by adding 2.7 to 10.4 nmol of $Na_2{}^{35}SO_4$ (approximately 3.3 x 10^{10} becquerels mmol^{-1}) to unsieved subsamples of forest floor and soil (1 g, wet weight) which were incubated for 48 h at temperatures indicated in the text. Following incubation, the sample was washed three times with water and the water was combined to yield a soil water fraction. Samples were then extracted three times each with 1 M Na_2SO_4, NaH_2PO_4, and LiCl and washed three times with deionized water to yield a salt extract. The sample was then hydrolyzed in 6 N HCl at 121°C for 12 h; the residue was washed in water once, and the sample was then held in contact with 2 N NaOH for 12 h at room temperature and was once again washed with water. In this paper, the acid and base fractions were combined to yield a fraction designated as non-salt extractable S. Fractions were analyzed by electrophoresis and radioactive components located with a scanner were determined with a scintillation counter. The non-salt extractable ^{35}S has been characterized to be in organic forms of ester sulfate and carbon-bonded S (Fitzgerald et al. 1982).

Mobilization of the non-salt extractable fraction was determined by extraction of samples with 1.0 M NaH_2PO_4 and washing with deionized water to remove unmetabolized sulfate followed by addition of 200 ul of the soil water mixture (1 soil/5 water) to each sample followed by incubation for a second period (24 h). After incubation, samples were extracted successively with 1.0 M Na_2SO_4, NaH_2PO_4, and LiCl followed by two extractions with water. The sample residue was treated with 2.0 N NaOH, extracted by centrifugation, and the residue was again extracted with NaOH and washed with water. The residue was then hydrolyzed in 6 N HCl and washed with water. The quantity of sulfur mobilized from the non-salt extractable fraction was determined as a percentage of the sulfur incorporated into this fraction. Further details of laboratory methodology for incorporation (Fitzgerald et al. 1982,Fitzgerald et al. 1983) and mobilization (Strickland et al 1984) assays have been reported.

Factors examined which potentially influence rates of incorporation and mobilization were soil temperature, concentrations of exogenous SO_4^{2-}, and a number of effectors including antibiotics.

RESULTS AND DISCUSSION

Incorporation

Total recovery of ^{35}S from forest floor and soil samples of WS 18 usually exceeded 90% of the added ^{35}S (Table 2). Similar total recoveries were found in several other ecosystems at Coweeta (Fitzgerald et al 1982) and the inability to completely recover ^{35}S probably reflects experimental error. The O_1 and O_2 layers of the forest floor showed small but measurable quantities of adsorbed sulfate but more than 60% of the ^{35}S in the A_1 was found in the salt extract. This indicates a substantial soil sulfate adsorption capacity which is consistent with other research showing large adsorption capacity for this anion Coweeta soils (Johnson et al. 1980).

TABLE 2. Recovery of ^{35}S in various fractions from the O_1 and O_2 forest floor layers and A_1 soil horizon of WS 18.*

Fraction	% Recovery		
	O_1	O_2	A_1
Soil water	38	49	15
Salt extract	6	15	65
Acid + base extracts	49	26	15
All fractions	93	90	95

* After Fitzgerald et al (1983)
Samples incubated for 48h with 7.8 nmol $Na_2{}^{35}SO_4$ at 20°C

In contrast, the O_1 and O_2 layers showed substantially more sulfur incorporation into the acid and base fractions (non-salt extractable S) compared with the A_1 horizon soil. Inorganic sulfate accounted for at least 90% of the radioactivity of the acid fraction and comprised all of the ^{35}S found in the base extract (Fitzgerald et al. 1983). As suggested by Fitzgerald et al.(1982), the recovery of $^{35}SO_4^{2-}$ in the acid and base extracts could

originate from the hydrolysis of ester linkages and (or) the oxidation of sulfonate linkages of organic matter. Thus, incorportion of sulfur into non-salt extractable fractions involves the formation of covalent sulfur linkages which is microbially mediated.

Further evidence for microbial incorporation of sulfate into organic matter was obtained from samples treated prior to incubation with compounds which stimulate or inhibit microbial metabolism (Table 3). The amendment of glucose to A_1 horizon samples doubled the level of incorporation. Treatment with erythromycin (an antibacterial antibiotic) and candicidin (an anti-fungal-antialgal antibiotic) indicated that bacteria have a major role in incorporation in the O_2 and A_1 layers while the incorporation process is dominated by fungi and/or algae in the O_1 layer. Additions of sodium azide strongly inhibited incorporation which suggests the transformation process is carried out by aerobically respiring organisms.

TABLE 3. Incorporation of sulfate into non-salt extractable organic sulfur in the forest floor and A_1 soil horizon of WS 18 as influenced by a variety of effectors 1.

Effectors	Incorporation (nmol ^{35}S g^{-1} dry wt 48 h^{-1} at 20°C)		
	O_1	O_2	A_3
None	26.3	4.0	1,8
Erythromycin[2]	19.2	1.0	0,7
Candicidin[3]	4.2	2.6	1.7
Sodium azide[4]	1.0	0.6	0.4
Glucose[5]			
1.2	--	--	3.5
4.8	--	--	4.4

1) After Strickland & Fitzgerald (1984)
2) Concentration of 0.5 mg g^{-1} sample dry wt
3) Concentration of 10.7 mg g^{-1} sample dry wt
4) Concentration of 128 mg g^{-1} sample dry wt
5) Concentration of mmol g^{-1} sample dry wt

The effects of temperature and amount of exogenous sulfate on incorporation rates of the A_1 horizon soil are shown in Fig. 1. The increased activity with an increase in temperature from 5 to 20°C would be expected for a biologically mediated process. Incorporation is a linear function of exogenous sulfate concentrations; at a given temperature, the amount of incorporation is a constant percentage of sulfate supplied. The dependency of incorporation activity on temperature has also been shown for other Coweeta soils. (Fitzgerald et al. 1983).

Incorporation of $^{35}SO_4^{2-}$ into organic forms of sulfur for A_1 soil samples collected on WS 18 throughout the year showed seasonal differences with highest activities occurring during August and September and lowest rates

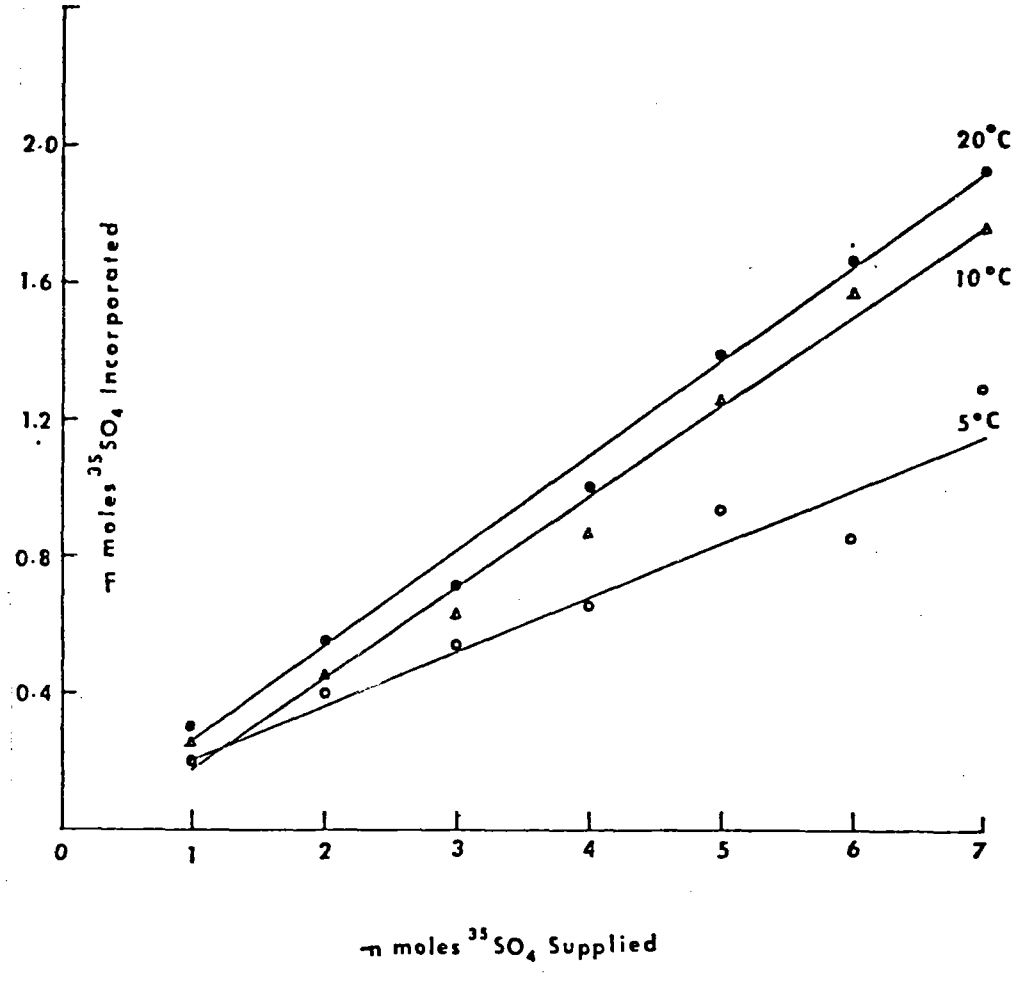

FIG. 1. Rates of sulfate incorporation into organic matter in A_1 soil samples as a function of temperature and exogenous sulfate concentrations.

of formation occurring in late spring and winter months. The average activity for the year was 1.53 nmol sulfate g^{-1} soil 48 h^{-1} (Swank et al. 1984). The potential capacity for incorporation in O_1 and O_2 layers and throughout the soil profile was determined on samples using ambient soil water sulfate concentrations. Incorporation rates declined rapidly in the soil profile and reached a minimum in the C horizon at a depth of 65+ cm with an activity of only 4% of that found in the A_1 horizon (Swank et al. 1984). Activity data were used to obtain a preliminary estimate of potential annual incorporation rates in forest floor and soil compartments (Table 4) based on several assumptions of the representativeness of the data (Swank et al. 1984). Although rates of sulfate incorporation in the O_1 and O_2 layers were 5 to 6 times greater than in the A_1 horizon, annual incorporation of sulfur is less than 0.5 kg ha^{-1} $year^{-1}$ because the quantity of substrate is small.

Table 4. Estimates of annual rats of sulfate incorporation into organic sulfur forms by microbial populations in forest floor and soil horizons of a forest ecosystem at Coweeta Hydrologic Laboratory.[1)]

Location	Incorporation rate (kg S ha^{-1} $year^{-1}$)
O_1	0.2
O_2	0.2
A_1	10.7
A/B	6.1
B_w	11.1
C_r	1.5
Total	29.8

1) After Swank et al. (1984).

The potential flux for both the A_1 and B_w horizons is about 11 kg ha^{-1} $year^{-1}$ and the total annual incorporation for litter and soil is 30 kg ha^{-1} $year^{-1}$ (Table 4). These estimates represent potential rates based on a standard incubation temperature of 20°C; therefore, lower in situ fluxes could be expected, particularly below 10 cm in the soil profile where ambient temperatures are 5 to 10°C below the assay temperature. Nevertheless, this assessment places the incorporation process into perspective from an ecosystem viewpoint; i.e., sulfate incorporation into organic sulfur forms exceeds the bulk precipitation input of 9 to 13 kg ha^{-1} $year^{-1}$.

The presence of this biological pathway in the sulfur cycle of a forest could provide a buffer against the impacts of acid precipitation on forest soils by reducing the mobility of the sulfate anion which, in turn, would reduce cation leaching. However, such interpretations are partly dependent upon the mobilization dynamics of the incorporated sulfate.

Mobilization

Research at Coweeta has shown that sulfur incorporated into organic matter can be mobilized (Strickland & Fitzgerald 1984, Strickland et al. 1984). The term mobilization is used here to describe the solubilization process involving depolymerization and/or desulfation of the non-salt extractable sulfur pool. Rates of mobilization were substantial in forest floor and soil horizons on WS 18 and tended to decrease with an increase in soil depth (Table 5). Incorporation data for the same samples used in mobilization assays are also shown in Table 5, but values differ from Table 4 because a constant amount of $^{35}SO_4^{2-}$ was added to all samples (7.6 nmol g^{-1} soil) and rates are normalized to a 24-h period. Less than 20% of the sulfate incorporated in the O_1 layer was moblized while values increased to nearly 50% in the O_2. In soil horizons, about 49% of incorporated sulfate was mobilized in the A_1 with a decrease in subsequent horizons to 43% in the C horizon.

Additional experiments provided evidence that, unlike incorporation of sulfate into organic matter, mobilization is not directly linked to microbial metabolism. For example, additions of unlabeled sulfate ranging from 4 to 46 nmol sulfate g^{-1} soil failed to inhibit mobilization of incorporated sulfur (Strickland et al. 1984). If the process was microbially mediated, inhibition would be expected since sulfohydrolase synthesis by microorganisms is repressed by sulfate (Fitzgerald 1976). Furthermore, treatment of samples with the same antibiotics used in incorporation experiments (Table 3) and other effectors such as methionine, cysteine, glucose, and ammonium nitrate failed to inhibit or stimulate mobilization of organic sulfur (Strickland & Fitzgerald 1984).

TABLE 5. Incorporation and mobilization of sulfur[1] in forest floor and soil of Coweeta WS 18.[2]

Horizon	Depth (cm)	^{35}S incorporated[3] (nmol g^{-1})	^{35}S mobilized[4] (nmol g^{-1})	^{35}S mobilized (%)
O_1[5]	--	6.85	1.30	19
O_2[5]	--	2.11	0.96	46
A_1	0-5	0.83	0.41	49
A/B	15-30	0.67	0.31	46
B	30-50	0.58	0.26	45
C	70-180	0.43	0.18	43

1) 7.6 nmol $^{35}SO_4^{2-}$ added g^{-1} of sample
2) Collected August 1982 and incubated at 20°c.
3) Incubated 48 h but data expressed on 24-h basis.
4) Incubated 24-h.
5) Incubated 24-h during incorporation.

Strickland et al. (1984) suggested that the mobilization process is mediated by depolymerases and sulfohydrolases, enzymes that are present extracellularly in the soil. These enzymes have different temperature activity optima as evidenced by mobilization responses at different incubation temperatures ranging from 5 to 30°C. For example, A_1 horizon soil samples incubated at 5°C for 24 h mobilized only 30% of the incorporated sulfur while samples incubated at 20°C mobilized 75% (Strickland et al. 1984).

CONCLUSIONS

Collectively, our research with Coweeta soils shows that incorporation of sufate into organic matter is mediated by microbial populations and is a major process in the sulfur cycle that is regulated by exogenous sulfate concentrations and temperature. Preliminary estimates of annual potential incorporation quantities in combined forest floor and soil is 30 kg ha^{-1}; although incorporation rates are highest in the organic layers, the mineral soil is the compartment of most incorporation. Mobilization experiments show substantial release of incorporated sulfur and the process appears to be mediated by preformed enzymes with temperature-sensitive activities.

The net balance between the highly dynamic processes of incorporation and mobilization is important in regulating movement of the sulfate anion in soil solutions. Initial results indicate that incorporation exceeds mobilization; thus, there is a net accumulation of organic sulfur in the soil which reduces the supply and mobility of sulfate. Quantification of the balance between processes at an ecosystem level must await more intensive sampling and longer term research to integrate variables which regulate the processes. However, we suggest that biological transformations of sulfur in forest soils are important in understanding and assessing the impact of acid precipitation on leaching losses of ions.

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