

Microbial incorporation of sulfate into organic matter in forest soils

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ABSTRACT The metabolism of inorganic sulfate into organic matter by microbial populations can be an important process in the sulfur cycle of forest ecosystems. Potential annual incorporation in forest floor and soil horizons of a mixed deciduous forest in the Southern Appalachian Mountains was estimated to be 30 kg ha⁻¹. The process is partially responsible for the apparent sulfur accumulation indicated by ecosystem budgets and affects sulfate mobility, cation leaching, and hence the interpretation of atmospheric sulfuric acid effects on forest ecosystems.

Incorporation microbienne des sulfates dans la matière organique du parterre forestier

RESUME La métabolisation du sulfate inorganique dans la matière organique par la population microbienne peut s'avérer importante dans le cycle du soufre en milieu forestier. L'incorporation annuelle potentielle dans la litière et les sols d'une forêt décidue mixte du sud des Appalaches a été estimée à 30 kg ha⁻¹. Le processus est partiellement responsable de l'accumulation apparente de soufre indiqué par les budgets au niveau de l'écosystème, et affecte la mobilité du sulfate, le lessivage cationique, et, par conséquent, l'interprétation des effets de l'acide sulfurique atmosphérique sur l'écosystème forestier.

INTRODUCTION

The deposition of sulfate from acid precipitation and ocean aerosols has been identified as the major sources of the sulfate anion in forest ecosystems (Kellogg et al., 1972; Likens & Bormann, 1974). However, the fate of this anion after it enters a forest ecosystem is not well known. Several studies have shown that soil sulfate

adsorption can be an important component of the sulfur cycle for some forests (Johnson & Henderson, 1979; Johnson et al., 1980).

Our research over the past several years suggests that microbial metabolism of sulfate to organic sulfur may also act as a major pathway in the sulfur cycle of forest ecosystems (Fitzgerald et al., 1982). The objective of this paper is to provide evidence that substantiates the quantitative importance of biological transformations of sulfate in a forest ecosystem at the Coweeta Hydrologic Laboratory in western North Carolina. Based on 10 years of bulk precipitation chemistry measurements taken over the 2185 ha basin, sulfate comprises 68% of precipitation anions. The average annual hydrologic input in bulk precipitation and export in streamflow for control, hardwood-covered watersheds during the past 10 years shows large apparent accumulations of sulfate, ranging from 6.1 to 9.1 kg ha⁻¹year⁻¹ (Table 1).

TABLE 1 Physical characteristics and mean annual (1973-1982) sulfate-sulfur budgets for four experimental watersheds at Coweeta Hydrologic Laboratory

Watershed number	Area (ha)	Midarea elevation (m)	Input	Sulfate-sulfur	
				Output	Net difference (kg ha ⁻¹ year ⁻¹)
2	12.1	840	9.7	1.4	+8.3
18	12.5	810	10.7	1.6	+9.1
27	38.8	1330	13.2	7.1	+6.1
36	48.6	1300	11.8	5.5	+6.3

STUDY SITE AND METHODS

The study was conducted on Coweeta Watershed 18, a 12.5 ha north-facing catchment which has been a primary site of long-term nutrient cycling research. Overstory vegetation on this relatively undisturbed area is dominated by *Quercus*, *Carya* and *Acer* species with *Rhododendron* and *Kalmia* in the understory (Day & Monk, 1977). Annual precipitation averages 180 cm and is rather evenly distributed throughout the year with at least 8 cm in most months. Catchment elevations range from 730 m to 985 m and side slopes average about 50%. Several soil types occur over the catchment but the dominant soil is a sandy loam Ashe, a member of the mesic Typic Dystrochrept family with an A horizon that averages about 10 cm in thickness.

A permanent transect 280 m in length was established across the catchment at midelevation and traversed from ridge to stream to ridge. The transect was segmented into 10 equally spaced 0.01 ha circular plots. Three samples of the A₁ horizon were randomly collected on each plot on a monthly basis; forest floor and other soil horizons were sampled in late summer.

One gram (wet weight) of unsieved soil samples were incubated at 20°C for 48 h with a range of 2.7 to 10.4 nmol Na₂ ³⁵SO₄²⁻ (3.3 x 10¹⁰ becquerel mmol⁻¹). The incubation time was determined from a time series study of varying incubation periods which showed that ³⁵SO₄²⁻ incorporation into organic matter was complete after 48 h. A range of ³⁵SO₄²⁻ concentration was used to represent the average ambient sulfate concentrations of water in the forest floor and soil horizons. For example, the average annual sulfate concentrations of soil water collections in a mixed hardwood forest at Coweeta decreased from 105 µeq l⁻¹ at 5 cm in the soil profile to 69 and 17 µeq l⁻¹ at 30 and 100 cm, respectively. A more detailed description of incubation procedures can be found in Fitzgerald *et al.* (1982).

After incubation, the soils were washed three times with water and the solutions were pooled to represent water soluble organosulfur metabolites. Soils were then extracted three times each with 1 M Na₂SO₄, NaH₂PO₄, and LiCl and washed three times with water to yield a salt extract fraction. This procedure removes all adsorbed inorganic sulfate (Fitzgerald *et al.*, 1982). The extracted sample was then hydrolyzed in 6 N HCl at 121°C for 12 h; the centrifuged residue was washed with water and the sample was then held in contact with 2 N NaOH for 12 h at room temperature and again washed with water. Acid treatment causes hydrolysis of ester sulfate (Fitzgerald, 1976) and solubilization of low molecular weight fulvic acid containing carbon-bonded sulfur and sulfonate sulfur. Base treatment extracts sulfur as sulfonate and/or carbon-bonded sulfur with large molecular weight components (Fitzgerald *et al.*, 1983). Total sulfur incorporated into organic matter was calculated as the sum of the amounts extracted by the acid and the base treatments and was termed "non-salt extractable" organic sulfur. The total extraction procedure consistently yields >90% recovery of the added ³⁵SO₄²⁻. Soil water, salt extracts and acid plus base fractions were analyzed by electrophoresis and radioactive components located with a scanner were determined with a scintillation counter. Complete details of laboratory methodology have been reported elsewhere (Fitzgerald *et al.*, 1982).

RESULTS AND DISCUSSION

The influence of varying amounts of ³⁵SO₄²⁻ added to A₁ soil samples on quantitative recovery of the various fractions is illustrated in Fig.1. All fractions showed a linear recovery rate over a wide range of ³⁵SO₄²⁻ amendments. Of the total ³⁵SO₄²⁻ recovered from soil samples, the water soluble, salt extractable, and acid plus base (non-salt extractable) fractions consistently accounted for 50, 40 and 10%, respectively. These recoveries are similar to results previously obtained for A₁ soil samples taken from four other forest ecosystems at Coweeta (Fitzgerald *et al.* 1982).

Additional recovery data for salt extractable and non-salt extractable fractions for forest floor and A₁ horizons are shown in Fig.2. Again, recovery rates of both fractions in all horizons were a linear function of the amount of exogenous sulfate added. The O₁ and O₂ layers of the forest floor showed small but measurable quan-

tities of adsorbed sulfate (salt extractable fraction). The A₁ horizon is a much more active zone of adsorption, accounting for about 40% of the $^{35}\text{SO}_4^{2-}$ recovered from the samples. Significant sulfate adsorption in the A₁ horizon on Watershed 18 was also observed by Johnson et al. (1980).

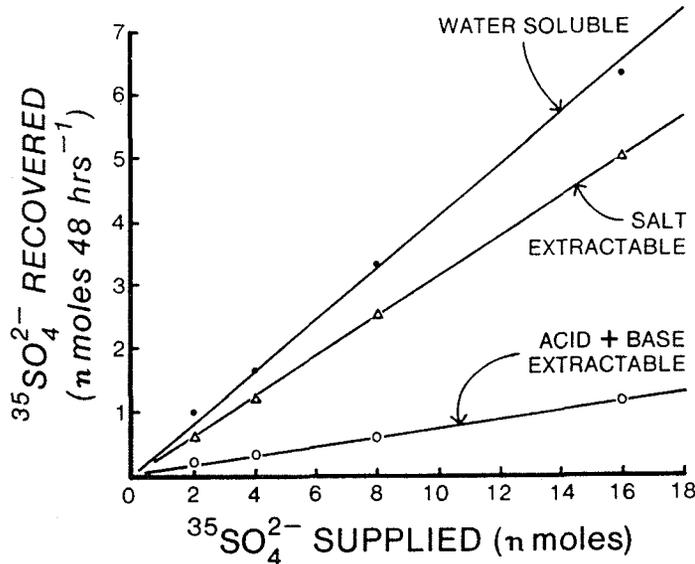


FIG.1 Recovery of $^{35}\text{SO}_4^{2-}$ in various extracted fractions following incubation of Coweeta Watershed 18 A₁ soil samples supplied with different amounts of $^{35}\text{SO}_4^{2-}$.

In contrast to the adsorption results, $^{35}\text{SO}_4^{2-}$ incorporation activity was substantially higher in the O₁ component of the forest floor with about 55% of the added sulfate recovered in the non-salt extractable fraction over a wide range of amended sulfate concentrations. Incorporation activity was much lower in both the O₂ and A₁ with 13 and 8% recoveries, respectively. Previous studies indicate that the non-salt extractable fraction consists of organic matter (Fitzgerald et al., 1982, Strick et al., 1982) and that the incorporation processes are carried out by aerobically respiring organisms (Strickland & Fitzgerald, 1984).

Incorporation of $^{35}\text{SO}_4^{2-}$ into organic forms of sulfur for A₁ soil horizons on Watershed 18 showed seasonal differences with highest activities occurring during August and September (Fig.3). Lower rates of formation occurred in late spring and in winter months but samples still exhibited significant incorporation. The average activity for the year was 1.53 nmol sulfate g⁻¹ soil 48 h⁻¹. The potential capacity for incorporation throughout the soil profile was determined on samples collected in late August 1982 from a soil pit located at Plot 5 on the transect and for O₁ and O₂ samples collected from all 10 plots (Table 2). Concentrations of $^{35}\text{SO}_4^{2-}$ added to soil samples were based on previously measured ambient sulfate concentrations because incorporation rates are concentration dependent (Fig.2). Rates of sulfate incorporation in the O₁ and O₂

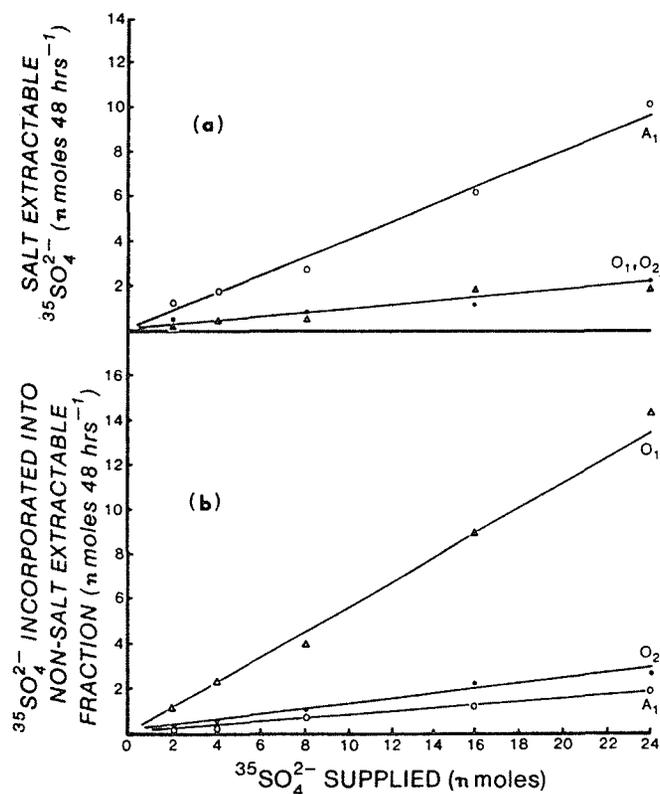


FIG.2 Recovery of $^{35}\text{SO}_4^{2-}$ in salt extractable (a) and non-salt extractable (b) fractions for O_1 and O_2 layers and A_1 soil horizon on Coweeta Watershed 18 for samples incubated with different amounts of $^{35}\text{SO}_4^{2-}$.

layers (Table 2) were 5 to 6 times greater than in the A_1 horizon. Incorporation rates declined rapidly in the soil profile and reached a minimum in the C horizon at a depth of 66+ cm with an activity of only 4% of that found in the surface soil.

TABLE 2 Formation of non-salt extractable sulfur within the forest floor and soil horizons on Coweeta Watershed 18 in late August 1982 (Swank et al., 1984)

Forest floor and soil horizons	Depth (cm)	$^{35}\text{SO}_4^{2-}$ incorporation (nmol g $^{-1}$ soil 48 h $^{-1}$)
O_1	4	11.68
O_2	2	8.73
A_1	0-10	1.85
A/B	11-25	0.61
B_w	26-65	0.44
C_r	66+	0.08

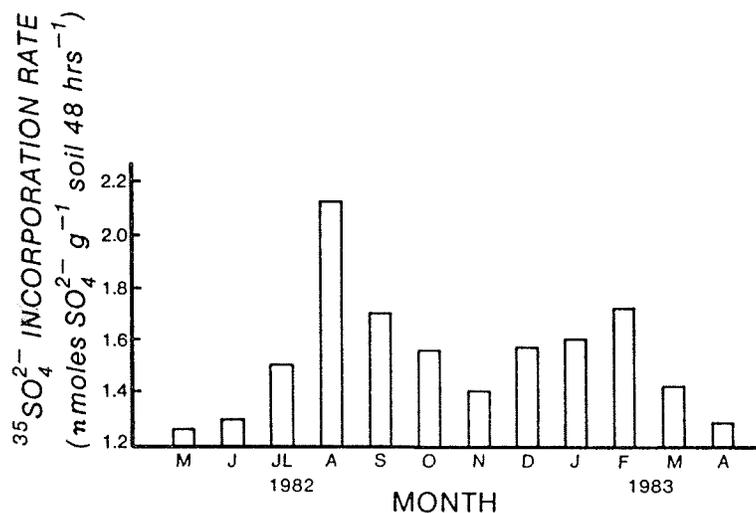


FIG. 3 Monthly rates of $^{35}\text{SO}_4^{2-}$ incorporation into organic matter for A_1 soil samples from Coweeta Watershed 18. Standard error of estimates ($n = 10$) were $\leq \pm 0.06$ for most months.

TABLE 3 Estimates of annual potential fluxes of inorganic sulfate to organic sulfur forms by microbial populations in forest floor and soil horizons of a hardwood forest ecosystem at Coweeta Hydrologic Laboratory in North Carolina (Swank et al., 1984)

Forest floor or soil horizon	Incorporation rate (kg sulfur 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

Swank et al. (1984) obtained preliminary estimates of annual potential incorporation rates in forest floor and soil compartments by combining activity data with estimates of forest floor weights and soil bulk densities along with several assumptions (Table 3). Although high activity was observed in the forest floor, the annual potential incorporation of sulfur is less than $0.5 \text{ kg ha}^{-1}\text{year}^{-1}$ because the quantity of substrate is small. In contrast, the potential flux for both the A_1 and B_w horizons is about $11 \text{ kg ha}^{-1}\text{year}^{-1}$. The total annual incorporation for litter and soil is $30 \text{ kg ha}^{-1}\text{year}^{-1}$ (Table 3). These estimates represent potential rates and

are probably overestimates since the activity data are based on a standard incubation temperature of 20°C. Other preliminary research conducted in our laboratory indicates that incorporation rates are lower at 5°C (Fitzgerald & Johnson, 1982; Fitzgerald *et al.*, 1983). 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. On the other hand, incubation conditions are representative of other *in situ* variables important in regulating sulfate incorporation such as moisture and sulfate concentrations.

CONCLUSIONS

A more precise quantification of annual *in situ* incorporation will require long term studies in all forest floor and soil horizons. The present assessment serves to place the incorporation process into perspective from an ecosystem viewpoint. The magnitude of sulfate incorporation into organic sulfur forms exceeds the bulk precipitation sulfate-sulfur input of 9 to 13 kg ha⁻¹year⁻¹. Moreover, incorporation greatly exceeds the 2 kg ha⁻¹year⁻¹ of net sulfur uptake by vegetation as documented by Johnson *et al.* (1982) for a forest ecosystem similar to that at Coweeta. It appears that organic sulfur formation by microbial populations is a major process responsible for part of the apparent sulfur accumulation indicated by ecosystem budgets. The quantitative contribution of incorporation to apparent system accumulations of sulfur are unclear because other research we have conducted indicates that incorporated sulfur is subject to mineralization although the potential turnover rates are lower than incorporation rates (Strickland & Fitzgerald, 1984; Strickland *et al.*, 1984).

The presence of a biological pathway of sulfate transformation into organic matter has implications in the interpretation of atmospheric sulfuric acid effects on forest ecosystems. Processes which reduce the mobility of the sulfate anion also reduce cation leaching from forest soils (Johnson 1981, Lee & Weber 1982). Thus, incorporation in the forest floor and soil could provide a buffer against exogenous sulfate. Incorporation of sulfate into organic matter could also provide a soil pool of organic sulfur; if mineralization rates were accelerated, the released sulfate anion would increase cation leaching. Microbial incorporation of sulfate into organic matter, coupled with evidence for the importance of organic sulfur in forest soils (David *et al.*, 1982) and soil sulfate adsorption demonstrate the complexity of sulfate dynamics in the soil solution. Simultaneous examination of geochemical and biological processes are needed in a variety of forest ecosystems to more fully evaluate the source, fate, and impact of sulfate in acidification processes.

ACKNOWLEDGEMENTS Support for this research was provided in part by National Science Foundation Grant BSR-8215259 and in part by the Southeastern Forest Experiment Station, US Department of Agriculture, Forest Service.

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