Reprint Series
13 January 1984, Volume 223, pp. 182–184

SCIENCE

Microbial Transformation of Sulfate in Forest Soils

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Abstract. *Incubation of forest soils containing sulfate labeled with sulfur-35 showed rapid conversion of the added sulfate to organic sulfur forms by microbial populations. Activity rates were highest in the forest floor, but significant activity was observed throughout the soil profile. The annual potential sulfur incorporation for forest floor and soil combined is estimated to be 30 kilograms per hectare. The metabolism of inorganic sulfate to organic forms can be a major process in the sulfur cycle, influencing sulfate accumulation and mobility in forest ecosystems.*

Sulfate has been identified as a major constituent of acid precipitation in several regions of the United States (*1, 2*). However, the fate of exogenous sulfate in forest ecosystems is not well known. Studies have shown that soil adsorption of sulfate can be an important component of the sulfur cycle for some forests (*3, 4*). In agreement with results obtained by Freney *et al.* (*5*) for agricultural soils, our research has suggested that the metabolism of sulfate to organic sulfur forms may also act as a major pathway in the sulfur cycle of forest ecosystems (*6*). In this report we present quantitative evidence substantiating the importance of biological transformations of sulfate in these ecosystems.

The study site is located at the Coweeta Hydrologic Laboratory in western North Carolina. The U.S. Department of Agriculture Forest Service facility is a site of long-term hydrologic and ecological research with collaboration between university and government scientists. During the past 14 years, the program has emphasized research on biogeochemical processes important in forest ecosystems, with the gaged watershed used as the unit of ecosystem investigation. On the basis of 80 gage-years (eight gages in operation for 10 years) of bulk precipitation chemistry measurements taken over the 2185-ha basin, sulfate makes up 68 percent of precipitation anions and the average annual *p*H of bulk precipitation is 4.45. During the period 1973 to 1982, the mean annual input of sulfate-sulfur in bulk precipitation was 10.0 kg ha^{-1} $year^{-1}$ and export in streamflow for mixed hardwood-cover watersheds was 1.5 kg ha^{-1} $year^{-1}$; thus there was a large apparent accumulation of sulfate-sulfur of 8.5 kg ha^{-1} $year^{-1}$.

Our preliminary research with surface soil from four Coweeta watersheds indicated that exogenous $^{35}SO_4^{2-}$ was rapidly converted into nonsalt-extractable ester sulfate and carbon-bonded sulfur (*6*). More intensive sampling was initiated on watershed 18 in May 1981 to quantitatively assess the importance of transformations relative to sulfate accumulation within a watershed. Watershed 18 is a 12.5-ha north-facing catchment that has been a primary site of long-term research on nutrient cycling. Overstory vegetation is dominated by *Quercus*, *Carya*, and *Acer* species with *Rhododendron* and *Kalmia* in the understory. Several soil types occur over the watershed, but the dominant soil is a sandy loam Ashe, a

member of the mesic Typic Dystrochrept family with an A_1 horizon that averages about 10 cm in thickness. Permanent plots were established, and on each sampling date three samples of the A_1 horizon were taken at random on each plot (*7*).

Subsamples of soil (each 1 g, wet weight) were incubated with a range of 2.7 to 10.4 nmole of $Na_2{}^{35}SO_4$ (3.3×10^{10} becquerels per millimole) for 48 hours at 20°C (*8*). Soil water, salt extracts, acid hydrolyzates, and base fractions were obtained by established procedures (*9*). Fractions were analyzed by electrophoresis and radioactive components located with a scanner were determined with a scintillation counter. Details of the laboratory methodology have been reported (*6*).

Our earlier research has demonstrated that extraction procedures yield ≥ 90 percent of added ^{35}S. About 60 percent of the $^{35}SO_4{}^{2-}$ was found in the salt extract, which indicates a substantial sulfate adsorption capacity for these soils. This result is consistent with other research showing large adsorption capacity for this anion in Coweeta soils (*4*). However, between 8 and 27 percent of the radioactivity was recovered in the nonsalt-extractable fraction. As determined by electrophoresis, the major sulfur constituent was sulfate, an indication that ester sulfate was recovered by hydrolysis (*9*). Fitzgerald and Johnson have presented evidence demonstrating that this transformation is mediated by bacteria and fungi (*10*).

Table 1. Temporal variability in the formation of nonsalt-extractable sulfur in the A_1 soil horizon (0 to 10 cm) of Coweeta watershed 18; S.E., standard error. Values are based on three random samples taken at each of ten permanent plots ($N = 30$) distributed over the watershed. The incubation temperature was 20°C. We tested the means, using analysis of variance (Duncan's multiple range test, $P < 0.05$). Means followed by an asterisk or by a dagger or by a double dagger or by a section sign are not significantly different from each other.

Date	Mean (± S.E.) $^{35}SO_4{}^{2-}$ incorporated into nonsalt-extractable sulfur (nmole g^{-1} per 48 hours)
May 1982	1.25* (0.05)
June	1.28* (0.05)
July	1.49†‡ (0.06)
August	2.13§ (0.11)
September	1.70† (0.06)
October	1.56†‡ (0.07)
November	1.41*‡ (0.05)
December	1.57†‡ (0.05)
January 1983	1.60†‡ (0.06)
Average	1.55

Table 2. Formation of nonsalt-extractable sulfur within the forest floor and soil horizons on Coweeta watershed 18 in late August 1982.

Forest floor or soil horizon	Depth (cm)	Incorporation of $^{35}SO_4{}^{2-}$ (nmole g^{-1} per 48 hours)
O_1	4	11.68
O_2	2	8.73
A_1	0 to 10	1.85
A/B	11 to 25	0.61
B_W	26 to 65	0.44
C_r	66+	0.08

The activity for the surface soil on watershed 18 over a 9-month period in 1982 showed seasonal differences with highest activities occurring during August and September (Table 1). Substantial incorporation into nonsalt-extractable sulfur was also observed in samples collected in late spring and winter.

In late August 1982, activity was determined within the soil profile for one of the permanent plots and for the O_1 and O_2 forest floor layers at all ten plots (Table 2). The concentrations of $^{35}SO_4{}^{2-}$ added to soil samples varied by soil horizon because the incorporation rates were concentration-dependent. The determination of ambient litter and soil sulfate concentrations were based on annual concentrations of water collected by porous-cup lysimeters (*11*). The activity of sulfate incorporation was about sixfold greater in O_1 and O_2 layers than in the A_1 horizon, and activity declined rapidly with soil depth. The incorporation rate in the C_r horizon was only about 4 percent of that in the A_1 horizon (Table 2).

In an effort to place activity data in quantitative perspective from the standpoint of annual ecosystem flux, we made the following assumptions: (i) the activity data from incubation studies are representative of in situ data; (ii) the average activity of the A_1 horizon for the 9-month period (Table 1) can be extrapolated to the three remaining months; and (iii) activity data for the remaining soil horizons are representative of transformation rates over the entire year. Combining activity data with prior estimates of forest floor weights and soil bulk densities provides an estimate for the potential annual incorporation into nonsalt-extractable sulfur (Fig. 1). Although the forest floor exhibited high activity, the annual potential incorporation of sulfate is less than 0.5 kg ha^{-1} $year^{-1}$ because of the quantity of substrate involved (6×10^6 g ha^{-1} of O_1 and O_2). In contrast, each of the A_1 and B_W horizons provides a potential flux of about 11 kg ha^{-1} $year^{-1}$. When combined, the total annual flux of sulfate-sulfur in litter and soil is estimated as 30 kg ha^{-1} $year^{-1}$.

We emphasize that the fluxes in Fig. 1 represent potential rates since activity data are based on a standard incubation temperature of 20°C. Preliminary research has suggested lower incorporation rates at 5°C (*10*). Thus, it is reasonable to expect lower in situ fluxes than shown in Fig. 1, particularly below 10 cm in the soil profile, where ambient soil temperatures are 5° to 10°C lower than the assay temperature. Activity data are representative of other in situ variables important in regulating sulfate incorporation, such as moisture and soil sulfate concentrations. More precise estimates of annual in situ incorporation for an ecosystem must await detailed, long-term studies.

The annual incorporation of sulfate into organic sulfur forms substantially exceeds the sulfate-sulfur input in bulk precipitation (10 kg ha^{-1} $year^{-1}$) at Coweeta and the sulfur sequestered in vegetation (2 kg ha^{-1} $year^{-1}$) as documented for a similar forest ecosystem (*12*). The process of organic sulfate formation could account for part of the apparent sulfur accumulation indicated by ecosystem budgets. Moreover, the presence of this biological pathway in the sulfur cycle of a forest has implications for the interpretation of atmospheric sulfuric acid effects on forest ecosystems. That is, processes that reduce the mobility of sulfate also reduce cation leaching from forest soils (*13*). Incorporation of sulfate to organic forms could provide a buffer against the impacts of acid precipitation on forest soils. Conversely, the formation of organic sulfur

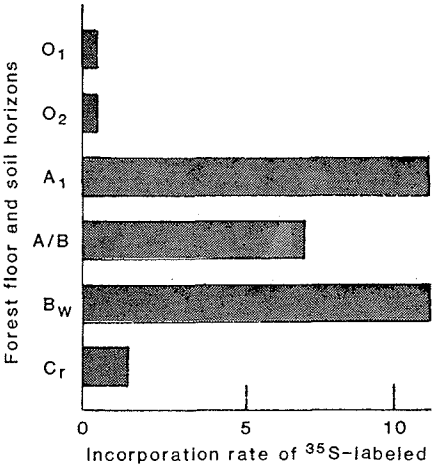

Fig. 1. Estimates of annual potential fluxes of inorganic sulfate converted to organic sulfur forms by microbial populations in forest floor and soil horizons of a forest ecosystem at Coweeta Hydrologic Laboratory.

provides a potential soil pool that could subsequently be remineralized. If mineralization rates were accelerated by chemical, physical, or biological factors, the released sulfate would increase cation leaching. Our analyses indicate that the formation of soil organic sulfur is of sufficient magnitude to warrant investigation in a variety of forest soils. Further study is also needed on the composition and turnover dynamics of this sulfur fraction.

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References and Notes

1. J. N. Galloway, G. E. Likens, E. S. Edgerton, *Science* **194**, 722 (1976).
2. P. L. Brezonik, E. S. Edgerton, C. D. Hendry, *ibid.* **208**, 1027 (1980).
3. D. W. Johnson and G. S. Henderson, *Soil Sci.* **128**, 38 (1979).
4. D. W. Johnson, J. W. Hornbeck, J. M. Kelly, W. T. Swank, D. E. Todd, in *Atmospheric Sulfur Deposition: Environmental Impact and Health Effects*, D. S. Shriner, C. R. Richmond, S. E. Lingberg, Eds. (Ann Arbor Press, Ann Arbor, Mich., 1980), pp. 507–520.
5. J. R. Freney, G. E. Melville, C. H. Williams, *Soil Biol. Biochem.* **3**, 133 (1971).
6. J. W. Fitzgerald, T. C. Strickland, W. T. Swank, *ibid.* **14**, 529 (1982).
7. A 280-m transect was established across the watershed at mid-elevation and was transversed from ridge to stream to ridge. The transect was segmented into ten equally spaced 0.01-ha circular plots. Samples of the A_1 horizon were routinely collected on a monthly basis; forest floor and other soil horizons were sampled in late summer.
8. The incubation time was determined from a time series study of varying incubation periods which showed that $^{35}SO_4^{2-}$ incorporation into organic sulfur was complete after 48 hours.
9. After incubation, the soils were washed three times with water and the water was pooled and designated as soil water. Soils were then extracted three times each with 1*M* Na_2SO_4, NaH_2PO_4, and LiCl and washed three times with water to yield a salt extract. The sample was then hydrolyzed in 6*N* HCl at 121°C for 12 hours; the residue was washed in water once, and the sample was then held in contact with 2*N* NaOH for 12 hours at room temperature and was once again washed with water. In this report, the acid and base fractions were combined to yield a fraction designated as nonsalt-extractable sulfur.
10. J. W. Fitzgerald and D. W. Johnson, in *Sulphur 82*, A. I. More, Ed. (British Sulphur Corporation, London, 1982), vol. 1, pp. 411–426.
11. The average annual sulfate concentrations of soil water collections in a mixed hardwood forest at Coweeta decreased from 105 μeq liter^{-1} at a soil depth of 5 cm to 69 and 17 μeq liter^{-1} at 30 and 100 cm, respectively.
12. D. W. Johnson *et al.*, *Oecologia (Berlin)* **54**, 141 (1982).
13. D. W. Johnson, D. W. Breuer, D. W. Cole, *J. Environ. Qual.* **8**, 246 (1979).
14. Support for this research was provided in part by NSF grant BSR-8215259 and in part by the Southeastern Forest Experiment Station, U.S. Department of Agriculture, Forest Service. We thank D. W. Johnson, J. E. Douglass, and T. C. Strickland for helpful comments.

25 July 1983; accepted 5 October 1983