Potential for organic sulfur accumulation in a variety of forest soils at saturating sulfate concentrations

A. R. Autry and J. W. Fitzgerald

Department of Microbiology, University of Georgia, Athens, GA 30602, USA

Summary. Increasing the sulfate concentration and concomitant increases in the organic S concentration failed to exert any effect on organic S mobilization in samples collected from all depths within the mineral soil profile, from 15 sites differing in soil type, vegetation, and geographic location. Mobilization capacities at saturating concentrations of sulfate for organic S formation generally tended to increase with increasing depth. The potentials for the accumulation of organic S with various sulfate inputs exhibited saturation kinetics similar to those observed for organic S formation; values for the former parameter ranged from 3×10^{-3} to 12.6 µmol S g^{-1} dry weight 24 h^{-1} for the uppermost (A, E) soil horizons, 3 nmol to 10 µmol S g^{-1} dry weight 24 h^{-1} for intermediate (primarily AB) soil horizons, and from 3 nmol to 13.4 µmol S g^{-1} dry weight 24 h^{-1} for the lowermost (B, C) soil horizons. Irrespective of depth, the Fullerton, Tarklin, and Loblolly sites in Tennessee and the Florida site showed the least net accumulation of organic S at saturation (< 0.2 µmol S g^{-1} dry weight 24 h^{-1} for all horizons examined), while the Duke Forest (North Carolina), Douglas Fir (Washington), Whiteface (New York) and the Howland (Maine) sites had the highest potential net accumulation of organic S at saturation (> 1.0 µmol S g^{-1} dry weight 24 h^{-1} for most horizons examined).

Key words: Sulfate concentration – Saturation kinetics – Organic S – Mobilization – Potential – Net accumulation – Saturation – Acidic precipitation

A thorough comprehension of S transformations and retention mechanisms in forested ecosystems is necessary in order to predict the responses of these ecosystems to an increasing input of atmospheric sulfate, for example from acidic precipitation. An accumulation of S derived from exogenous sources has been reported for forests in various locations in the United States (Henderson et al. 1977; Swank and Douglas 1977; Johnson et al. 1982). In view of observations that numerous sites have greater organic S than inorganic S pools in litter (Strickland et al. 1987) and at all depths within the mineral soil profile (David et al. 1983, 1987; Bartel-Ortiz and David 1988), it is possible that organosulfur formation is a major S-retention mechanism in forest soils. Autry and Fitzgerald (1990) applied a modification of the "heterotrophic activity method" of Wright and Hobbie (1966) to assess the saturation potential for organic S formation in forest soils. This potential is an index of the maximum amount of organic S that a given soil can form, irrespective of the size of the sulfate input. While the saturation potential is a good indicator of the capacity of a soil to accumulate organic S, obviously, the backward reaction, organic S mobilization, must be taken into account when seeking an accurate estimate of biologically mediated S accumulation. As defined by Strickland and coworkers (1984), the term "mobilization" refers to the depolymerization of large-molecular-weight organic S species, leading to the release of smaller, soluble components which may then be subject to S mineralization. In contrast to the organic S formation process, organic S mobilization is mediated not as a direct result of microbial metabolism, but rather by pre-existing depolymerases and sulfohydrolases present in the soil matrix (Strickland et al. 1984; Fitzgerald and Strickland 1987).

Because biological S accumulation at any concentration of added sulfate is a function of the capacity of the soil to form and mobilize organic S, the present study was designed to investigate the effect of increasing the sulfate concentration, with associated increases in the organic S concentration, on organosulfur mobilization at all depths within the soil profile for a variety of sites in order to assess the maximum potential capacity of these soils to accumulate S as organic S.

Materials and methods

Sample collection

Samples were collected from excavated pits dug at each site. Roots and stones were removed, and the samples were maintained, field-moist, at 4°C in sealed polyethylene bags before analysis.

Preparation of Na$_2$^{35}SO$_4$ standard solutions

A stock solution of ^{35}S-labelled Na$_2$SO$_4$ (approximately 4×10^{10} Bq mmol^{-1}, Pharmaceuticals), with a radioactivity of 1666 Bq µl^{-1}, was prepared. The final concentration was brought to 2 µmol µl^{-1} by the addition of unlabelled sulfate. This stock solution, with a specific radioactivity of 8.33×10^5 Bq mmol^{-1}, was diluted to achieve sulfate concentrations ranging from 0.0375 nmol µl^{-1} to 2.0 µmol µl^{-1}. Constant specific radioactivity was maintained throughout the dilution scheme.

Determination of organic ^{35}S mobilization capacities

Organic S mobilization was determined according to the method of Strickland and coworkers (1984). To allow organic S to form, samples (1 g wet weight, not sieved) were placed in scintered glass filtersticks and incubated in triplicate at 20 °C for 24 h, with concentrations of sulfate ranging from 7.5 nmol to 400 µmol g^{-1} wet weight. Following incubation, the samples were extracted with $1 M$ NaH$_2$PO$_4$ to remove any ^{35}SO$_4^{2-}$ that had not been incorporated into organic matter. To the soil residue remaining after this extraction was added 200 µl of a 1:5 soil:water solution to reconstitute any microbial populations removed by the phosphate extraction. Following reincubation for 24 h, the samples were washed with $1 M$ Na$_2$SO$_4$, NaH$_2$PO$_4$, and LiCl, yielding a soil salt extract, containing ^{35}S released from mineralization of organic S. The samples were then autoclaved with $6 M$ HCl and later treated with $2 M$ NaOH, to release any ^{35}S that was not mobilized during the incubation. The percentage mobilization was calculated by dividing the ^{35}S present in the salt extract by that present in the sum of the salt, acidic, and basic extracts for each concentration of added sulfate.
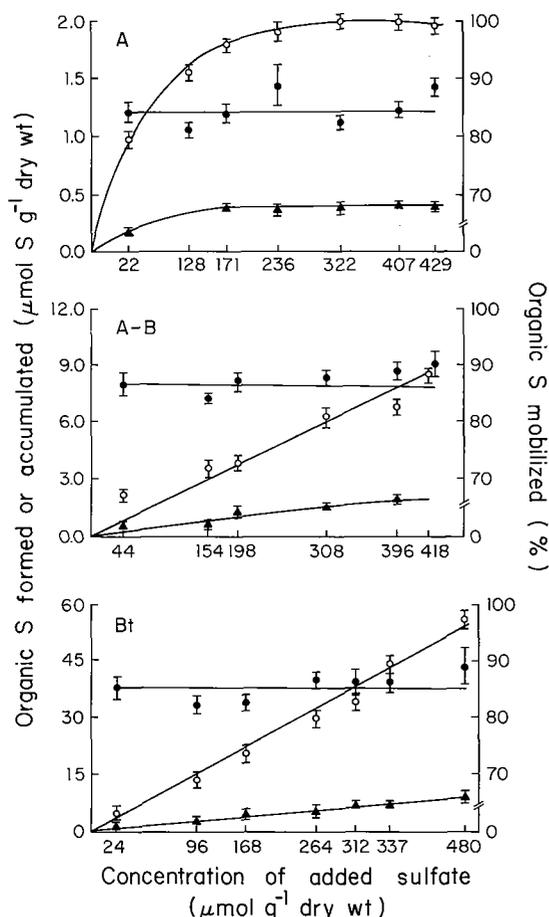

Fig. 1. Relationship between sulfate concentration, % mobilization (●), organic S formed (○), and potential net accumulation of organic S (▲) for the B. F. Grant Forest site. Horizon designations are shown at upper left; *dry wt.*, dry weight

Data analysis

Data derived from the mobilization studies were assessed by both simple linear regression analysis by the least squares method and analysis of variance using Duncan's multiple range test ($\alpha = 0.05$).

Results

Figure 1 illustrates the effect that increasing sulfate concentration, with concomitant increases in organic S concentration, had on the mobilization of recently formed organic S for all depths within the mineral soil profile for the B. F. Grant Forest site. The values for mobilized organic S, over the wide range of sulfate concentrations used, were not significantly different (analysis of variance, Duncan's multiple range test, $\alpha = 0.05$) for all concentrations of added sulfate examined. Moreover, a regression analysis of these plots of mobilized organic S (Fig. 1) showed that the slope of the fitted regression line was not significantly different from 0 ($P < 0.005$), indicating that the organic S concentration had no influence on the rate of organic S mobilization for any depth at this site. The capacity for this process at saturation was calculated for each horizon by determining the y-intercept of the regression line. Identical treatment was applied to mobilization data for samples collected from the other sites, and in no case did an increase in the organic S concentration exert an influence on organic S mobilization. The saturation potential for organic S formation was calculated as described by Autry and Fitzgerald (1990), using the linearization method of Wright and Hobbie (1966), for the Howland, Tarklin, Florida, B. F. Grant, Whiteface, Camp Branch, Coweeta Hardwood, Nolan Divide, Becking, Norway, and Huntington Forest sites. The saturation potential for each sample collected from the remaining sites was calculated by regression analysis of the portion of the sulfate saturation curve showing zero-order kinetics, because Wright Hobbie linearization was ineffective with organic-S formation data for these sites. The potentials for the accumulation of organic S at saturation were calculated by subtracting the amount of organic S mobilized at saturation from the amount of organic S formed at saturation. The potential for organic S formation, mobilization and accumulation for each site is given in Table 1, together with brief site descriptions.

More than 70% of the newly formed organic S was mobilized in 9 out of 15 uppermost (A, E) soil horizons, the exceptions being the Nolan Divide A, Fullerton Ap, Loblolly A, Tarklin A, Douglas Fir A, and Howland E horizons, where 48, 66, 61, 60, 65, and 65% of the organic S was mobilized, respectively, after 24 h. In intermediate (primarily AB) soil horizons, the mobilization capacity at saturation generally increased, ranging from 66% for the Fullerton and Loblolly AB horizons to 93% for the Duke EB horizon; 15 out of the 19 horizons examined showed a mobilization capacity of >70%. The only exceptions were the Fullerton AB, Loblolly AB, and Douglas Fir B21 and B22 horizons. Results for the lowermost (B, C) soil horizons were similar; the mobilization capacity at saturation ranged from 64% for the Douglas Fir C horizon to 88% for the Fullerton B horizon, with 12 out

Table 1. Organic S formation, mobilization, and accumulation in response to saturating concentrations of sulfate in soil samples collected from various forested sites

Site, location, vegetation; and soil classification	Horizon	Potential for formation of organic S at saturation (μmol S g^{-1} dry weight 48 h^{-1})	Mobilization of organic S at saturation (% 48 h^{-1})	Potential net accumulation of organic S at saturation (μmol S g^{-1} dry weight 48 h^{-1})
Fullerton, Walker Branch, TN; mixed deciduous; Ultisols	Ap	0.01	65.65	0.01
	AB	0.01	65.50	0.00
	B	0.02	87.50	0.00
Tarklin, Walker Branch, TN; mixed deciduous; Ultisols	A	0.07	60.76	0.03
	AB	0.06	78.13	0.01
	Bt	0.37	73.78	0.10
Loblolly, Oak Ridge, TN; loblolly pine; Ultisols	A	0.18	60.12	0.07
	AB	0.04	65.50	0.01
	B	0.72	75.00	0.18
Florida, Gainesville, FL; slash pine; Spodosols	A	>2.17[a]	88.64	>0.25[a]
	E	1.01	87.50	0.13
	Bh	1.97	91.30	0.17
	C	1.30	86.31	0.18
Duke Forest, Mebane, NC; loblolly pine; Ultisols	Ap	0.71	84.30	0.11
	EB	3.50	92.74	0.25
	B1	51.00	80.37	10.01
	B2	100.00	86.57	13.43
Huntington, Newcomb, NY; mixed deciduous; Spodosols	A	35.20	91.31	3.06
	E	1.40	88.08	0.17
	Bs	1.89	86.39	0.26
Coweeta, Otto, NC; oak-hickory; Ultisols	A	5.18	87.31	0.66
	B	5.50	85.86	0.78
	C	>4.17[a]	85.46	>0.61[a]
Grant Forest, Eatonton, GA; loblolly pine; Ultisols	A	2.38	84.32	0.37
	AB	>8.70[a]	89.40	>0.92[a]
	Bt	>57.30[a]	85.20	>8.48[a]
Camp Branch, Falls Creek, TN; mixed oak; Ultisols	A	2.39	75.44	0.59
	AB	2.68	70.75	0.78
	Bt1	>11.20[a]	78.00	>2.46[a]
	2Bt2	>16.10[a]	85.09	>2.40[a]
	C	1.98	70.74	0.58
Whiteface, Lake Placid, NY; spruce-fir; Histosols, Spodosols	A	2.74	80.82	0.53
	E	>4.38[a]	71.05	>1.27[a]
	Bs	8.06	65.07	2.82
Noland Divide, Smoky Mountains, NC; red spruce, beech; Inceptisols	A	1.69	48.02	0.88
	B	2.25	84.23	0.35
Becking, Smoky Mountains, NC; red spruce, beech; Inceptisols	A	7.20	75.89	1.74
	B	1.60	76.55	0.38
Norway, Nordmonen, Norway; norway spruce; Entisols	E	8.38	75.77	2.03
	Bs	2.20	78.92	0.46
	C	2.25	73.16	0.60
Howland, Orono, ME; mixed spruce-fir; Histosols	E	5.13	64.79	1.81
	Bs	16.27	80.77	3.13
	BC	4.27	71.11	1.23
	C	4.18	69.76	1.26
Thompson Forest, WA; douglas fir; Inceptisols	A	36.00	65.04	12.59
	B21	20.16	67.16	6.62
	B22	12.00	65.64	4.12
	C	14.00	63.99	5.04

[a] Value given is lower limit, because horizon is unsaturated (Autry and Fitzgerald 1990)

of 15 sites having a capacity of >70%. The exceptions to this trend included the Howland C, Whiteface Bs, and Douglas Fir C horizons, where 70, 65, and 64% of the organic S was mobilized, respectively, within 24 h.

The potential net accumulation of S as organic S at saturation varied widely. This parameter, which accounts for constant mobilization of organic S while the system is saturated with respect to organic S formation, should be the best index of a given forest's maximum capacity to retain S as organic S. In uppermost (A, E) horizons, values for potential organic S accumulation at saturation ranged from 3 nmol S g^{-1} dry weight 24 h^{-1} for the Fullerton Ap horizon to 12.6 μmol S g^{-1} dry weight 24 h^{-1} for the Douglas Fir A horizon. In most of the sites examined (10 out of 15) the potential net accumulation at saturation was <1.0 μmol S g^{-1} dry weight 24 h^{-1}, with the Becking A, Howland E, Norway E, Huntington A, and Douglas Fir A horizons being the exceptions to this trend.

Values for the intermediate (primarily AB) soil horizons were similar, with the potential net accumulation of organic S ranging from 3×10^{-3} to $10\,\mu mol\ S\ g^{-1}$ dry weight $24\ h^{-1}$ for the Fullerton AB and Duke Forest B1 horizons, respectively. As with the uppermost soil horizons, the bulk of the intermediate soil horizons (11 out of 19) exhibited values for potential net accumulation of organic S at saturation of $<1.0\,\mu mol\ S\ g^{-1}$ dry weight $24\ h^{-1}$; exceptions included the Howland Bs and BC, Camp Branch Bt1 and 2Bt2, Douglas Fir B21 and B22, Whiteface E, and Duke Forest B1 horizons. For the lowermost (B, C) horizons, similar results were obtained, the values ranging from 3×10^{-3} to $13.4\,\mu mol\ S\ g^{-1}$ dry weight $24\ h^{-1}$ for the Fullerton B and Duke Forest B2 horizons, respectively, and as with the uppermost and intermediate soil horizons, in the lowermost horizons the values for potential accumulation of organic S at saturation were $<1.0\,\mu mol\ g^{-1}$ dry weight $24\ h^{-1}$ for 10 out of 15 sites examined. Again, the exceptions to the trend included the Howland C, Whiteface Bs, Douglas Fir C, and Duke Forest B2 horizons.

Discussion

The observation that increasing concentrations of organic S (derived from increasing concentrations of sulfate) had no effect on organic S mobilization is consistent with the finding that this process is mediated by preformed enzymes (depolymerases and sulfohydrolases) present in the soil matrix (Strickland et al. 1984; Fitzgerald and Strickland 1987), and suggests that the K_m for these enzymes is significantly lower than the concentrations of organic S generated by the addition of sulfate. Moreover, these enzymes operate at the collective V_{max} over the concentration range examined. The capacity of the soil to mobilize organic S increased as depth increased. This finding is in conflict with results obtained by Swank and colleagues (1985) and Watwood and coworkers (1988), who found that the organic S mobilization capacity decreased with increasing depth in the mineral soil profile. Total C for the sites examined in the present study also decreased with increasing depth (A. R. Autry and J. W. Fitzgerald (1989), unpublished data). The reduction in C implies a reduction in biomass and thus reduced enzyme levels at these depths, and hence lower mobilization rates. A possible explanation for the different findings is that the sulfohydrolases present at these lower depths are more efficient at hydrolyzing organic S than similar enzymes present in the upper horizons.

The effect of an increasing sulfate concentration on the potential accumulation of organic S is directly related to the saturating sulfate concentration with respect to organic S formation, the potential for organic S formation at saturation, and the mobilization capacity of a given soil at saturation. Collectively, the data suggest that unlike organic S formation and mobilization, increasing depth does not influence the potential accumulation of organic S at saturation. It is clear, however, that, regardless of depth, the Fullerton, Tarklin, Loblolly, and Florida sites have the lowest potential for net accumulation of organic S at saturation, while the Duke Forest, Douglas Fir, Whiteface, and Howland sites have the highest potential for net accumulation of organic S at saturation.

References

Autry AR, Fitzgerald JW (1990) Application of the heterotrophic activity method to organosulfur formation in forest soils. Soil Biol Biochem 22:743–748

Bartel-Ortiz LM, David MB (1988) Sulfur constituents and transformation in upland and floodplain forest soils. Can J For Res 18:1106–1112

David MB, Schindler SC, Mitchell MJ, Strick JE (1983) Importance of organic and inorganic sulfur to mineralization processes in a forest soil. Soil Biol Biochem 15:671–677

David MB, Mitchell MJ, Scott TJ (1987) Importance of biological processes to the sulfur budget of a northern hardwood ecosystem. Biol Fertil Soils 5:258–264

Fitzgerald JW, Strickland TC (1987) Mineralization of organic sulphur in the O_2 horizon of a hardwood forest: Involvement of sulphatase enzymes. Soil Biol Biochem 19:779–781

Henderson GS, Hunley A, Selvidge W (1977) Nutrient discharge from Walker Branch Watershed. In: Correll DL (ed) Watershed Research North America: A workshop to compare results. Smithsonian Institution Press, Edgewater, Maryland, pp 307–320

Johnson DW, Henderson GS, Huff DD, Lindberg SE, Richter DD, Shriner DS, Todd DE, Turner J (1982) Cycling of organic and inorganic sulphur in a chestnut oak forest. Oecologia 54:141–148

Strickland TC, Fitzgerald JW, Swank WT (1984) Mobilization of recently formed forest soil organic sulfur. Can J For Res 14:63–67

Strickland TC, Fitzgerald JW, Ash JT, Swank WT (1987) Organic sulfur transformations and sulfur pool sizes in soil and litter from a southern Appalachian hardwood forest. Soil Sci 6:453–458

Swank WT, Douglass JE (1977) Nutrient budgets for undisturbed and manipulated hardwood ecosystems in the mountains of North Carolina. In: Correll DL (ed) Watershed Research in Eastern North America: A workshop to compare results. Smithsonian Institution Press, Edgewater, Maryland, pp 343–363

Swank WT, Fitzgerald JW, Strickland TC (1985) Transformations of sulfur in forest floor and soil of a forest ecosystem. In: Johansson I (ed) Hydrological and hydrochemical approaches to the acidification of ecological systems. Nordic Hydrological Program Rep 10, Coordinating Committee for Hydrology in Norden, Uppsala Sweden, pp 137–145

Watwood ME, Fitzgerald JW, Swank WT, Blood ER (1988) Factors involved in potential sulfur accumulation in litter and soil from a coastal pine forest. Biogeochemistry 6:3–19

Wright RT, Hobbie JE (1966) Use of glucose and acetate by bacteria and algae in aquatic ecosystems. Ecology 47:447–464