ORGANOSULFUR FORMATION IN FOREST SOILS: SITE COMPARISON OF KINETIC PARAMETERS

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Summary—The kinetics of organosulfur formation were analyzed with soil samples collected from various depths of forested sites that should, by virtue of location, have different sulfate deposition histories. Saturation potentials, based on the V_{max} for SO_4^{2-}–S incorporation into organic matter, were found to decrease with increasing depth in 8 out of 12 sites examined. Estimation of the SO_4^{2-} concentration required to saturate a given horizon, was made, and irrespective of depth, a forest located near a coal-fired power plant in Tennessee (the Tarklin site) was the most saturated whereas samples from forests located in relatively non-polluted areas of New York state (Huntington and Whiteface sites) were generally the least saturated. This latter parameter was also found to decrease with increasing depth in 6 out of 12 sites examined. Turnover times for the recently-formed organic S exceeded 10^3 h in 7 out of 12 uppermost (A, E) soil horizons, 5 out of 13 intermediate (primarily A/B) soil horizons, and 7 out of 12 lowermost (B, C) soil horizons. These results indicate that no significant change in the recalcitrance of the newly-formed organic S occurred with increasing depth.

INTRODUCTION

To predict how a forest ecosystem will respond to increasingly greater inputs of anthropogenic sulfate, a thorough understanding of the capacity of forest soil to retain sulfate is mandated. The two principal mechanisms by which the anion can be retained are adsorption (Johnson and Henderson, 1979) and incorporation of sulfate-S into organic matter by enzymes of plant or microbial origin (Fitzgerald *et al.*, 1988). If this latter process occurs in plants, the organic S which is formed may be delivered to the underlying soil during plant litter decomposition (Homann and Cole, 1990). In light of observations that pool sizes of organic S generally exceed those of sulfate at all depths within the soil profile (David *et al.*, 1983, 1987; Bartel-Ortiz and David, 1988), organic S formation may represent a more important S retention mechanism as far as long-term retention is concerned, whereas sulfate adsorption may be more important for short-term retention of S (Autry *et al.*, 1990). To assess the capacity of a site to retain S as organic S, an understanding of the influence of various environmental factors on this process must be ascertained. The influence of temperature (Strickland and Fitzgerald, 1984), moisture content (Watwood *et al.*, 1988a) and pH (Watwood *et al.*, 1986) has been determined for some forest soils. However, comparatively little has been done to assess the response of organic S formation to increasing sulfate concentration or to increasing depth within the soil profile (Swank *et al.*, 1984). In the current work, kinetic parameters for this process were determined for soils collected by horizon from a variety of sites exposed to a wide range of sulfate concentrations.

MATERIALS AND METHODS

Site description and sampling

A partial description of the sites examined is provided in Table 1. Samples of each horizon were collected from excavated pits and were not sieved; however, roots and stones were removed by hand. Samples were maintained, field-moist, in sealed polyethylene bags at about 4°C for about 3 weeks before organic S formation capacities were determined.

Preparation of ^{35}S-labeled standard sulfate solutions

A stock solution of ^{35}S-labelled carrier-free sodium sulfate (approx. 4×10^{10} Bq mmol^{-1}, ICN Pharmaceuticals) having an activity of 1.67×10^3 Bq μl^{-1} was prepared. The final sulfate concentration was then adjusted to 2 μmol μl^{-1} by the addition of unlabeled anion, bringing the sp. act. to 8.33×10^5 Bq mmol^{-1}. This stock solution was then diluted serially, to maintain constant specific activity throughout the dilution scheme, with the most dilute solution having a final sulfate concentration of 5×10^{-3} μmol μl^{-1}. All dilutions were then sterilized by autoclaving and stored at 4°C.

Determination of organic S formation capacities

Triplicate samples (1 g wet wt) were exposed at 20°C for 48 h to sulfate ranging from 0.1 to 400 μmol g^{-1} wet wt. Samples were then extracted to yield water, salt, acid and base extracts. This extraction process, consisting of serial washes with 1 M solutions of Na$_2$SO$_4$, NaH$_2$PO$_4$ and LiCl was shown to quantitatively remove any ^{35}SO$_4^{2-}$ which was adsorbed during treatment. ^{35}S present in each extract was quantified by liquid scintillation, and that present in the acid and base extracts represents the ^{35}S

Table 1. Description of forested study sites investigated

Site	Year sampled	Location	Dominant vegetation	Soils
Becking	1987	Great Smoky Mtns, NC	Red spruce, beech	Inceptisols
B.F. Grant Forest	1988	Eatonton, GA	Loblolly pine	Ultisols
Camp Branch	1987	Falls Creek, TN	Mixed oak	Ultisols
Coweeta hardwood	1988	Coweeta, NC	Oak–hickory	Ultisols
Florida	1988	Gainesville, FL	Slash pine	Spodosols
Huntington Forest	1988	Newcomb, NY	Mixed deciduous	Spodosols
Nolan Divide	1987	Great Smoky Mtns, NC	Red spruce, beech	Inceptisols
Norway spruce	1987	Nordmonen, Norway	Norway spruce	Entisols
Red alder	1987	Thompson Forest, WA	Red alter (mature)	Inceptisols
Tarklin	1987	Walker Branch, TN	Mixed deciduous	Ultisols
Turkey Lakes	1988	Ontario, Canada	Sugar maple–birch	Spodosols
Whiteface Mountain	1987	Lake Placid, NY	Spruce–fir	Histosols, Spodosols

incorporated into organic matter for a given concentration of added sulfate (Fitzgerald et al., 1988).

Kinetic and statistical analysis

The technique used to determine kinetic parameters is a modification of the heterotrophic activity method of Wright and Hobbie (1966), in which a ^{35}S-label is utilized to measure sulfate incorporation into organic matter under conditions in which specific radioactivity is held constant at all concentrations of added sulfate (Autry and Fitzgerald, 1990). This approach, which we believe better reflects in situ conditions, gave results that were not significantly different from those obtained by the original Wright–Hobbie procedure (unpublished data). The method involves a mathematical linearization of the sulfate uptake data and yields the following parameters: V_{max} (rate of sulfate incorporation h^{-1}) and $(Kt + S_n)$, where Kt is the half-saturation constant and S_n is the endogenous sulfate concentration. In this modification, T_t (turnover time) represents the time required for complete mineralization of the recently-formed organic S rather than the time required for complete uptake (immobilization) of the added sulfate. For each horizon examined, organic S formation data were subjected to linearization and then to simple linear regression analysis by the Least Squares Method. Differences in mean amounts of organic S formed were determined by analysis of variance and Duncan's Multiple Range Test ($\alpha = 0.05$). An example of the approach taken for all samples is given in Fig. 1.

RESULTS AND DISCUSSION

Saturation of organic S formation was observed with samples from all depths within the soil profile of the Turkey Lakes site (Fig. 1). An horizon is considered saturated if the mean amounts of organic S formed from increasing concentrations of sulfate are not significantly different over 25% of the concentration range examined. Linearization of the sulfate incorporation data (Fig. 1—insert) yielded a straight line for the A horizon ($r^2 = 0.95$), indicating that only one microbial population was responsible for organic S formation in this horizon. This trend was not evident for the B or C horizons (Fig. 1—insert) where multiphasic kinetics for organic S formation were observed. When extrapolating a straight line off the linear portion of these curves, kinetic parameters, which represent lower limits for the system, were calculated. As noted by Azam and Hodson (1981),

multiphasic uptake kinetics, exemplified by curvilinear relationships in Wright–Hobbie plots, indicate that more than one microbial population is responsible for uptake. Similar kinetic patterns were observed for glucose uptake in marine systems (Azam and Hodson, 1981), for phosphate uptake in freshwater systems (Tarapchak and Herche, 1989), and for sulfate incorporation into organic matter by soils from a forest in Maine (Autry and Fitzgerald, 1990). Multiphasic kinetics were observed with samples from other sites (Table 2), and, in each case, kinetic parameters derived from the linear portion of the curves yielded a lower limit for the system, which closely matched data obtained by visual inspection of the saturation curves for these samples (see Fig. 1). Coefficients of determination > 0.8 were obtained for the linearization of data obtained with samples from all sites.

The Wright–Hobbie linearization yields a V_{max} h^{-1} for sulfate incorporation into organic matter. Watwood et al. (1986, 1988a) and Autry and Fitzgerald (1990) demonstrated the linearity of this process for exposure times up to 70 h. This relationship was also found for amounts of added sulfate within the concentration range examined in the present study (data not shown). It was therefore possible to obtain a saturation potential for organic S formation after 48 h by multiplying V_{max} by 48. All saturation potentials were calculated in this manner.

With uppermost mineral (A, E) horizon soils, substantial variation in saturation potentials was observed, with values ranging from 35 μmol organic S formed by the Huntington Forest A horizon to 0.2 μmol organic S formed in the Tarklin A horizon (Table 2). Samples from most of the sites exhibited high saturation potentials, with 10 out of 12 sites possessing potentials $> 2 \mu$mol organic S formed g^{-1} dry wt. The lone exceptions were the Nolan Divide and Tarklin A horizons. With regard to the intermediate (primarily A/B) horizons, saturation potentials ranged from > 16 for the Camp Branch 2Bt2 horizon to 6×10^{-2} for the Tarklin A/B horizon. As was true for the uppermost horizons, high saturation potentials were also observed with samples from these horizons, where 9 out of 13 horizons examined had potentials $> 2 \mu$mol organic S formed g^{-1} dry wt; the exceptions to this trend included the Florda E, Florida Bh, Huntington E and Tarklin A/B horizons. With samples from the lowermost (B, C) horizons, the same variation in the magnitude of saturation potentials was observed. These ranged from > 57 for the B.F. Grant Forest Bt horizon to about 0.4 for the
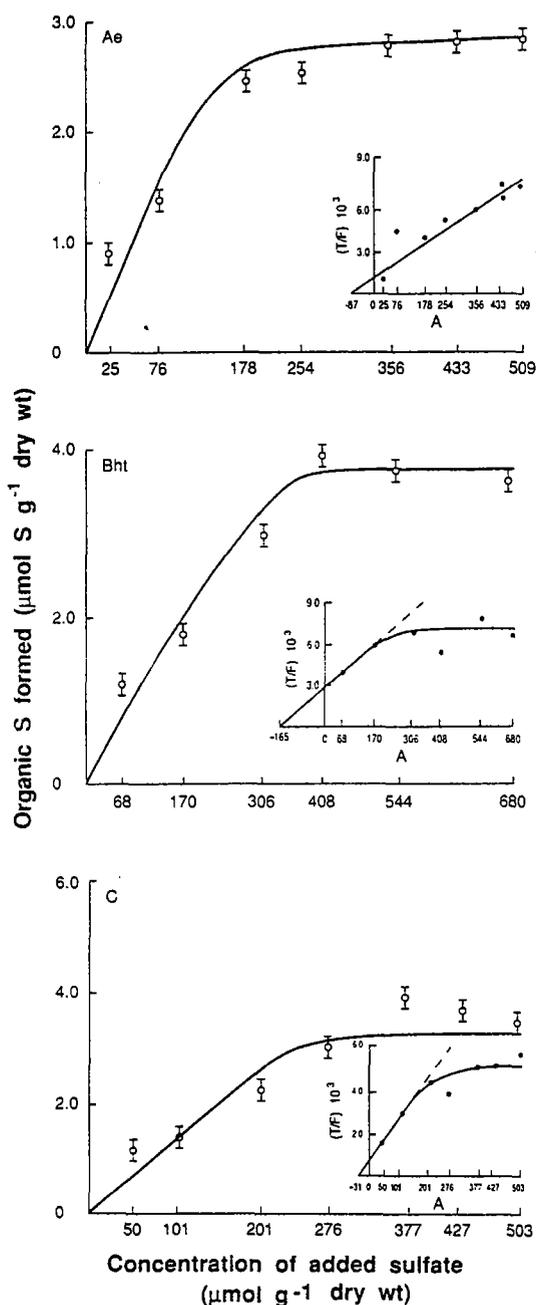
Fig. 1. Relationship between increasing sulfate concentration and organic S formation by soil samples collected from the Turkey Lakes site. (○) Relationship between organic S and sulfate added; (●) Wright–Hobbie plot, where T, F and A are the exposure time, fraction of added S incorporated into organic matter, and concentration of added sulfate, respectively. Soil horizon designation is given in the upper right-hand corner.

Tarklin B horizon. In contrast to the uppermost and intermediate horizons, potentials for the lowermost horizons were generally lower, with 7 out of 12 sites exhibiting saturation potentials $>2\,\mu$mol organic S formed g^{-1} dry wt; the Tarklin B, Norway C, Becking B, Camp Branch C and Florida C horizons were among the exceptions to this trend.

Saturation potentials decreased with increasing sample depth for a given site (Table 2). These results were not unexpected because microbial biomass and activity decrease with increasing depth in soils (Paul and Clark, 1989; Cochran et al., 1989). The exceptions to this trend were the Nolan Divide, Whiteface and B.F. Grant Forest sites, where an increase in saturation potentials was observed, and the Tarklin site, where no obvious trend was observed. A possible reason as to why samples from some sites exhibited increases in saturation potentials in the deeper horizons is that the microbial populations at those depths, albeit smaller in size than those in the upper horizons, may have higher individual capacities for incorporating sulfate into organic matter.

To estimate the sulfate concentration required to bring the soil to saturation, the sum of the half-saturation constant and the endogenous sulfate concentration $(Kt + S_n)$, was doubled to assess an upper limit for this parameter. Azam and Hodson (1981) noted that if S_n is not known, then $(Kt + S_n)$ is a useful limit on Kt, the half-saturation constant. Autry and Fitzgerald (1990) found that doubling $(Kt + S_n)$ can provide a useful and accurate upper limit on the sulfate concentration yielding saturation, which correlates well with that determined by visual inspection of the saturation curves. The concentration of added sulfate required to bring a soil to saturation is a function of the sulfate deposition history for that sample. Thus, the lower the concentration of added sulfate required to saturate the system, the more saturated the system is, and, conversely, the higher the concentration of added sulfate required for saturation, the less saturated the system is. Saturating sulfate concentrations for the uppermost (A, E) horizons ranged from 2 mmol g^{-1} dry wt for the Huntington A horizon to about 2 μmol g^{-1} dry wt for the Tarklin A horizon (Table 2). The unusually large $(Kt + S_n) \times 2$ value for the former site was not surprising, because the endogenous pool size of sulfate (the S_n-value) for this horizon was several orders of magnitude higher than that for surface horizons from the other sites (A. Autry, unpublished). Based on these estimates, the Huntington Forest site was the least saturated, whereas the Tarklin site was the most saturated with respect to organic S formation by surface soils.

Within the intermediate (primarily A/B) horizons, similar variation in the sulfate concentration yielding saturation was observed, with values ranging from >0.6 mmol g^{-1} dry wt for the Whiteface E horizon to 1 μmol g^{-1} dry wt for the Tarklin A/B horizon. With samples collected from the lowermost horizons, the Whiteface Bs horizon was the least saturated, requiring 484 μmol added SO_4^{2-} g^{-1} dry wt, whereas the Tarklin B horizon was the most saturated, requiring only 3.2 μmol added SO_4^{2-} g^{-1} dry wt to reach saturation (Table 2). Collectively, these data suggest that the Tarklin site is the most saturated, while the Whiteface site is the least saturated overall. Depth also influenced this parameter in that the concentration of added sulfate required to yield saturation generally decreased with sample depth. For example, in uppermost horizons, 50% of the sites examined required >200 μmol g^{-1} dry wt to yield saturation (Table 2). This percentage increased to 62 for the

Table 2. Summary of kinetic parameters for organic S formation by samples collected by horizon from various forest sites

Site and horizon	Organic S formed (μmol g^{-1} dry wt)		Sulfate concentration yielding saturation[a] (μmol g^{-1} dry wt)	Turnover time (h)
	Apparent V_{max} (10^{-2} h^{-1})	Potential at saturation (48 h^{-1})		
Tarklin A	0.31	0.18	2.1	352
A/B	0.13	0.06	1.0	380
B	0.76	0.37	3.2	216
Nolan Divide A	3.54	1.69	52	736
B	4.70	2.25	100	1055
Whiteface A[b]	5.71	2.74	86	765
E[c]	9.12	4.38	606	NA[d]
Bs	16.8	8.06	484	1444
Camp Branch A[b]	4.98	2.39	96	968
A/B	5.59	2.68	142	1273
2Bt2[c]	33.5	16.10	471	NA[d]
Bt1[c]	23.3	11.20	473	NA[d]
C	4.13	1.98	114	1397
B.F. Grant Forest A	4.90	2.38	155	1559
A/B[c]	18.1	8.70	441	NA[d]
Bt[c]	119	57.30	481	NA[d]
Turkey Lakes A	6.17	2.96	174	1410
B[b]	7.32	3.51	328	2245
C	4.42	2.12	62	705
Coweeta hardwood A	10.8	5.18	234	1080
Bt[c]	11.5	5.50	510	NA[d]
C	8.70	4.17	85	486
Norway E	17.5	8.38	398	1126
Bs	4.60	2.20	104	1138
C	3.94	1.89	51	656
Becking A	15.0	7.20	422	1403
B	3.43	1.60	72	1075
Florida A[c]	4.52	2.17	481	NA[d]
E[b]	2.10	1.01	297	6917
Bh	4.10	1.97	114	1377
C	2.70	1.30	334	6204
Red alder A	19.5	9.36	562	1442
B21	18.9	9.07	304	804
C	11.9	5.71	301	1303
Huntington A	73.4	35.20	2006	1368
B	8.18	3.90	452	2769

[a]Value given is theoretical upper limit and is equal to $(Kt + S_n) \times 2$, where Kt is the half-saturation constant and S_n is the endogenous sulfate concentration.
[b]Multiphasic system. Data was calculated by regression analysis of the linear portion of the Wright–Hobbie plot.
[c]All data for this horizon is based upon the lower limit, because this horizon was unsaturated.
[d]Not applicable, horizon was unsaturated.

intermediate (primarily A/B) soil horizons and dropped to 42% for the lowermost (B, C) horizons. These observations suggest that the microorganisms in the lowermost horizons are more efficient utilizers of sulfate than are those in the uppermost and intermediate soil horizons (by virtue of the lower estimate of the Kt for samples from these horizons).

With respect to turnover time (T_t), relatively high values ($>10^3$ h in many instances) were noted for samples taken at all depths within individual soil profiles. There were some exceptions to this general trend (e.g. all horizons of the Tarklin site) and in some cases a value for T_t could not be obtained because the sample remained unsaturated over the concentration range examined. These turnover times are not unreasonable for total mineralization of recently-formed organic S, although Strickland and Fitzgerald (1986) noted with A horizon Ultisols collected from the Coweeta basin in North Carolina, that as the age of the organic S increased, the efficacy of the mineralization process decreased. The data of Table 2 also show that as depth increased, no apparent increase in the recalcitrance of recently-formed organic S was observed and in many cases samples from the deepest horizons had shorter turnover times than those for surface horizons. Similar findings with respect to turnover time with increasing depth were noted for the Howland site in northeast Maine (Autry and Fitzgerald, 1990). These results do not agree with results of previous work, where substantial decreases in the mobilization of newly formed organic S with sample depth were documented (Watwood et al., 1988b). It is therefore clear that further attempts must be made to correlate turnover time with in situ organic S mobilization rates for each of these sites to fully understand the regulation of S accumulation at all depths within the soil profile under conditions of sulfate loading.

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