CHAPTER I

SULFUR TRANSFORMATIONS IN FOREST SOILS COLLECTED ALONG AN ELEVATIONAL GRADIENT[1]

Summary – ^{35}S-labeled inorganic sulfate was incorporated into organic matter from A-horizon soils collected along an elevational gradient. These samples formed between 0.7 and 1.7 nmol S (as organic S) g^{-1} dry weight. Soils with high moisture (65%) and high carbon content (8.4%) exhibited higher rates of organic S formation. Of the organic S generated, 33 to 44% was mineralized (mobilized) during 24 h. Samples containing high concentrations of indigenous sulfate exhibited lower rates of mobilization and vice-versa. Assays of samples collected over the gradient demonstrated that pH decreased as carbon content and sulfate adsorption (salt extractable S) increased. Total S, ester sulfate and inorganic sulfate content increased with increasing elevation.

INTRODUCTION

Acidic precipitation represents a major input of sulfate for forested ecosystems (Likens and Borman, 1974) and it is well documented that forests accumulate S from this source (Shriner and Henderson; 1978; Johnson et al., 1981). Adsorption and the formation of organic S are believed to be responsible for the retention of S. Organic S formation is a microbially mediated process in which sulfate is incorporated into organic matter via the formation of covalent linkages (Fitzgerald et al., 1983). Once formed, organic S may be mobilized to yield inorganic sulfate and the other soluble forms of S (Strickland et al., 1984). These forms of S may be utilized for biological uptake, readsorbed within the soil matrix, reincorporated into organic matter, or in the case of sulfate, they may cause the leaching of nutrient cations from soil (Bettany et al., 1980; Johnson et al., 1982; Strickland et al., 1986). Sulfate adsorption can reduce cation leaching (Johnson and Cole, 1977; Johnson, 1980, and Johnson and Cole, 1980) and it is possible that sulfate immobilization resulting from the formation of organic S could have a similar effect.

The fate of ^{35}S-labeled sulfate with respect to adsorption, organic S formation and mobilization was studied using samples collected along an elevational gradient. The influence of associated changes in environmental characteristics were examined in an attempt to determine how these processes are regulated with respect to moisture content, carbon, pH, and intrinsic sulfur pool composition and size.

MATERIALS AND METHODS

Site description

Samples of A horizon soils were collected in August, 1986 from forests in the Sequoia and Kings Canyon National Park, near Three Rivers California. Three sites differing in elevation were investigated: Elk Creek, Log Meadow, and Emerald Lake. The elevation and vegetation characteristics of these sites are listed in Table 1. Three samples representative of each site were collected and mixed. Nine sub-samples of each composite were assayed separately and means with standard errors are reported. Samples were stored (field moist) in air tight bags at 5°C and root material was removed by hand before analysis.

Sulfate adsorption and organic S formation assay

Samples (1g wet weight, not sieved) were exposed at 20°C for 48h to $Na_2{}^{35}SO_4$ (7.5 nmol S, approximately $3x10^{10}$ Bq nmol^{-1}, Amersham)N After exposure, samples were washed three times with 600 µl aliquots of deionized distilled water. These washes were collected by centrifugation and combined to yield a fraction containing soluble S. The samples were then washed sequentially with three 600 µl aliquots of 1$\underline{M}$ Na_2SO_4, NaH_2PO_4, LiCl respectively, and deionized distilled water. These washes were collected as above and combined to yield a fraction containing adsorbed S (salt extractable S). Both procedures quantitatively remove soil sulfate as well as soluble organo-sulfur metabolites. The remaining S was found to be incorporated into organic matter through the formation of covalent linkages (Fitzgerald et al.,

Table 1. Organic sulfur formation potentials and some intrinsic properties of surface
soils collected from sites differing in vegetation and elevation

Site	Vegetation	Elevation (m)	Formation (nmol S g^{-1} dry wt.)	Moisture (%)	Carbon (%)
Elk Creek	Oakwood-Chaparral	680	0.7(0.1)[a]	2.1(0.2)	3.0(0.1)
Log Meadow	Mixed conifer	2250	1.0(0.1)	18.6(4.1)	6.4(1.3)
Emerald Lake	Subalpine	2800	1.7(0.1)	65.7(2.4)	8.4(2.1)

[a]Mean (±SE) n=9.

1985). This fraction was recovered first by treatment with 6$\underline{M}$ HCl at 121°C for 12h, which results in the hydrolysis of ester-linked sulfate. The remaining organic S was recovered after exposure to 2$\underline{M}$ NaOH at room temperature for 12h. This treatment oxidizes C-S linkages (Fitzgerald et al., 1983). The radioactivity of each fraction was counted by liquid scintillation. Total ^{35}S recoveries of 89.4% ($\pm$ 4.4) were obtained for this assay.

Mobilization of recently-formed organic S

Samples (1g wet weight, not sieved) were exposed to 7.5 nmol S as Na$_2$^{35}SO$_4$ to permit the formation of labeled organic S. After exposure at 20°C for 24h unincorporated sulfate was removed by salt extraction (Strickland et al., 1984). Reduction in microbial populations during this initial extraction was minimized by the addition of a 1:5 soil water mixture. After an additional 24h at 20°C, the samples were washed successively with 1$\underline{M}$ Na$_2$SO$_4$, NaH$_2$PO$_4$ and LiCl to extract soluble organic-S or sulfate that was released during mobilization. Treatment of the residue with 6$\underline{M}$ HCl at 121°C followed with 2$\underline{M}$ NaOH for 12h released any organic ^{35}S formed during the initial incubation which had not been mobilized. Total ^{35}S recoveries of 93.5% ($\pm$ 1.2) were obtained. The validity of this assay method has been established by laboratory (Strickland et al., 1984) and field incubations (Strickland et al., 1986).

Intrinsic S pools

Total S was determined by oxidation with hypobromite followed by reduction with hydriodic acid (HI) (Tabatabai and Bremner, 1970).

Total ester-linked sulfate and inorganic sulfate was determined by direct reduction with HI (Freney, 1961). Carbon-bonded S was calculated as the difference between total S and HI-reducible S, whereas amino acid S was determined by reduction with Raney nickel (Freney et al., 1970). The content of sulfonate S was calculated as the difference between carbon-bonded S and amino acid S. Investigations using a wide variety of authentic alkane sulfonates (Fitzgerald and Franklin, 1982), indicated that this linkage was not reduced by HI or by Raney nickel. Carbon-bonded S that is not reduced by these agents is thus considered provisionally to represent sulfonate S. To obtain soluble S, samples (4g, wet weight) were shaken for 15 min with 20 ml of deionized distilled water. The mixture was then centrifuged and the supernatant retained. Adsorbed S was recovered by shaking the residue twice with 20 m$\underline{M}$ Na_2HPO_4 for 30 min and then centrifuged. The combined inorganic and organic sulfate content of each extract was determined by HI reduction, whereas the amount of soluble and adsorbed ester sulfate in each extract was calculated as the difference between HI-reducible S and inorganic sulfate. The latter was determined directly by anion chromatography (Dick and Tabatabai, 1979).

Other intrinsic soil characteristics

Moisture content was determined on samples dried at 50°C for 48h. Total carbon was determined using a Hewlett Packard C analyzer. The pH was determined in a 1:2 soil-water solution.

RESULTS AND DISCUSSION

A positive relationship between elevation, moisture content, carbon content and organic S formation potential was observed with samples from each of the three sites (Table 1). Soil from the highest elevation (Emerald Lake site) had the highest C content (8.4%) as well as the highest organic S formation potential (1.7 nmol S g^{-1} dry wt.). Samples having lower carbon contents exhibited correspondingly lower organic S formation potentials. Organic S formation may also be regulated by moisture content as well as by carbon availability. Thus, the Emerald Lake soils had the highest organic S formation potentials and exhibited a mean moisture content of 65.7% (Table 1). The Log Meadow and the Elk Creek soils exhibited lower organic S formation potentials (0.7 and 1.0 nmol S g^{-1} dry wt., respectively) and mean moisture contents of 18.6 and 2.1%, respectively.

Results of intrinsic S determinations (Table 2) indicate that most (at least 93%) of the total S in soils from all sites is in the form of organic S. In contrast, inorganic sulfate was a minor component of the total S pool. Total S increased somewhat in samples collected from the lowest to the highest elevation (358-588 µg S g^{-1} dry wt.). All three ester sulfate fractions (soluble, adsorbed, and insoluble) also increased with elevation. Roberts and Bettany (1985) noted an increase in organic sulfate (ester sulfate) from lower to upper slope positions within a narrow elevational gradient. However, these researchers found that total S decreased from lower to upper slope positions. Laboratory measurements of organic S formation potentials reflected the content of organic

sulfate present in the soil samples (Table 2). Thus, higher amounts of ester sulfate (soluble, adsorbed and insoluble) were found in samples which exhibited higher formation potentials and vice versa. This relationship was not as clear when the content of carbon-bonded S (amino acid and sulfonate S) was considered indicating that ester sulfate may represent a better indicator of the capacity of these soils for organic S formation than carbon-bonded S content.

Carbon content and soil pH appeared to be related to sulfate adsorption potentials. Thus, as carbon increased, so did sulfate adsorption, and as pH decreased, sulfate adsorption potentials increased accordingly. Sulfate adsorption by agricultural soils was found to be inversely related to pH (Freney and Williams, 1983). Samples from all three sites followed this trend (Table 3). The Elk Creek soils exhibited the lowest sulfate adsorption potential (0.9 nmol S g^{-1} dry wt.) and the highest pH. In contrast, soils from the Log Meadow and Emerald Lake sites had a lower pH (3.5 and 3.2, respectively), and correspondingly higher adsorption potentials. The capacity for sulfate adsorption also increased with elevation and carbon content. Thus, sites with high adsorption also increased with elevation and carbon content. Thus, sites with high adsorption potentials also possessed high carbon content and vice versa. Johnson and et al. (1980) and Johnson and Todd (1983) found the opposite relationship to occur with a variety of B horizon soils. They found that organic matter (measured as total carbon) had a negative influence on sulfate adsorption and suggested (Johnson et al., 1979) that organic matter blocked adsorption sites. In view of our results with A horizon soils, it is possible that

Table 2. Relationship between mean organic sulfur formation
potential and sulfur components of surface soils
collected along an elevational gradient

Process or Sulfur component	Site		
	Elk Creek	Log Meadow	Emerald Lake
Organic S formed (nmol S g^{-1} dry wt.)	0.7(0.1)[1]	1.0(0.1)	1.7(0.1)
Sulfur pools (µg S g^{-1} dry wt.)			
Total S	358.0(30.7)	595.6(59.5)	588.7(86.4)
Total organic	333.1(44.4)	565.0(67.3)	552.6(109.7)
Soluble ester	7.8(0.9)	10.9(1.2)	19.1(2.5)
Adsorbed ester	2.9(1.1)	18.1(6.6)	26.0(7.3)
Insoluble ester	130.3(13.8)	290.7(24.7)	307.3(75.2)
Total ester	141.0	319.7	352.4
Sulfate	24.9(7.0)	30.5(5.5)	36.9(9.3)
Sulfonate	144.9(27.4)	172.9(27.3)	111.0(8.3)
Amino Acid	51.3(1.7)	74.7(7.9)	73.7(12.5)
Total carbon-bonded	196.2	247.6	184.7

[a]Mean (±SE) n=9.

Table 3. Relationship between sulfur processing potentials and some characteristics
of surface soils collected along an elevational gradient

Process or Characteristic	Units	Site		
		Elk Creek	Log Meadow	Emerald Lake
Adsorption	(nmol S g^{-1} dry wt.)	0.9(0.8)[a]	1.7(0.2)	2.5(0.6)
Organic S formed	(nmol S g^{-1} dry wt.)	0.7(0.1)	1.0(0.1)	1.7(0.1)
Organic S mobilized	(% 24h^{-1})	32.9(1.4)	43.7(1.7)	35.0(1.3)
pH	(%)	4.6(0.3)	3.5(0.1)	3.2(0.1)
Carbon	(%)	3.0(0.1)	6.4(1.3)	8.4(2.1)
Total sulfate	(% of total S)	7.0	5.1	6.3

[a]Mean (±SE) n=9.

relationships between adsorption and carbon are either soil type, horizon type or area specific.

An inverse relationship appears to exist between organic S formation and mobilization rate. Thus, the Log Meadow samples exhibited high rates of mobilization and lower organic S formation potentials as compared to the Emerald Lake samples (Table 3). Mobilization rates also exhibited an inverse relationship to sulfate content (soluble and adsorbed) when amounts of this anion were expressed as a percent of total S (Table 3). A high mobilization rate (43.7%), characteristic of the Log Meadow soil, coincided with low amounts of sulfate (5.1% of total S). In contrast, samples from the Elk Creek and Emerald Lake sites had lower mobilization rates (32.9 and 35.0%, respectively) and correspondingly higher contents of sulfate (7.0 and 6.3% of total S). These results suggest that the relative size of the sulfate pool could play a role in controlling the rates of organic S mobilization of a given soil. Treatment of samples with antimicrobial agents (Strickland et al., 1984) failed to inhibit the mobilization of organic S. This process, therefore, does not seem to be linked to the activity of intact microorganisms, but rather it appears to be mediated by enzymes, depolymerases and sulfohydrolases of plant or microbial origin which exist extracellularly in the soil (Strickland et al., 1984). The microbial production of the latter enzyme was shown to be repressed or de-repressed depending upon the availability of sulfate (Fitzgerald, 1976). The amount of this anion may regulate mobilization in the same manner in that higher amounts of sulfate repress the sulfohydrolase enzymes (lowering mobilization rates), while lower

amounts of the anion de-repress the synthesis of these enzymes (raising mobilization rates).

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