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SULFUR PROCESSING IN SOIL FROM HIGH AND LOW ELEVATION FORESTS IN THE SOUTHERN APPALACHIANS OF THE UNITED STATES

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Summary—Samples of A, E, and B horizons, collected from a high and a low elevation watershed, were analyzed for their capacity to adsorb sulfate, generate organic S and mobilize organic S. Sulfate adsorption potentials were significantly greater in soil from the high elevation watershed compared to that from the low elevation watershed. Only A horizon samples from the two watersheds were statistically different in their capacity to synthesize organic S, although when these samples were incubated at ambient soil temperatures, no statistical difference in organic S formation was observed. Soil moisture, carbon, pH and S constituents were quantified and relationships between these variables and S processing potentials were determined. With high elevation samples, carbon content was positively correlated with organic S formation rates ($r = 0.90$; $P \leqslant 0.005$). Sulfonate S was the major S constituent of these soils; however, insoluble ester was the major S constituent in samples from the low elevation watershed. A 60–70% increase in organic S formation rates was observed after amendment with cellobiose with samples from all horizons of the low elevation watershed, indicating that soil from this watershed may be energy deficient in terms of organic S formation. Attempts were made to determine which S processes may be involved in ecosystem-level responses currently being observed with these watersheds.

INTRODUCTION

Studies have been initiated at the Coweeta Hydrologic Laboratory in Western North Carolina to determine the fate of sulfate derived from atmospheric deposition on forest soils. Analysis of bulk soils revealed that sulfate can account for 74% of the anions in precipitation at Coweeta (Swank and Waide, 1988). The budget for this anion (precipitation input–stream output) indicates that sulfate is accumulating in the soil of these ecosystems (Johnson *et al.*, 1980; Swank and Waide, 1988). Two mechanisms which have been proposed for this accumulation are sulfate adsorption, a chemical process in which sulfate adsorbs to sesquoxides (Johnson, 1980), and organic S formation, a biological process where sulfate is incorporated into organic matter (Fitzgerald *et al.*, 1988). In addition, two mechanisms have been identified which negatively influence S accumulation by releasing sulfate. These are desorption and organic S mobilization (Strickland and Fitzgerald, 1984). Although sulfate-S has accumulated at a rate of 6–11 kg ha^{-1} yr^{-1} in soil, analysis of stream waters indicate that sulfate concentrations (volume-weighted means yr^{-1}) are increasing (Swank and Waide, 1988). Amounts of the anion in streams from the higher elevation watersheds (WS 27 and 36) are greater than those present in streams from the lower elevation watersheds (WS 2 and 18) and the dominant anion in streams from the high elevation watersheds has shifted from HCO_3^- to SO_4^{2-}. On this basis, it was suggested by Swank and Waide (1988) that the high elevation streams in the Southern Appalachians may be in the initial stages of acidification.

The objective of this study was to evaluate environmental factors that influence transformations in soil from a high and a low elevation watershed. Most of the research on these transformations has been conducted with samples from low elevation watersheds (Fitzgerald *et al.*, 1988), and little is known about sulfur processing on these higher elevation watersheds. An understanding of the process level response to sulfate inputs and factors that influence these processes across elevational gradients might help to explain the ecosystem level responses that are currently being observed.

MATERIALS AND METHODS

Site description

Samples were collected from watersheds 27 and 2 of the Coweeta basin. The former (WS 27, 1061–1455 m; minimum and maximum elevation, respectively) is a 39 ha north-facing catchment with a mean annual precipitation of 245 cm and an average land slope of 55%. Watershed 2 (709–1004 m; minimum and maximum elevation, respectively) is a 12.3 ha

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south-facing catchment with an average of about 180 cm of precipitation per yr^{-1}, and has an average slope of 50%. These undisturbed watersheds are covered by mixed mature hardwood forests, with Rhododendron dominating the understory in the study sites selected. Although several soil types exist, sampling was restricted to the Tuckasegee–Cullasaja complex, a member of the mesic Typic Haplumbrept family. Three soil pits on each watershed were sampled by horizon in September 1989 and in April 1990. These pits were arranged in a triangular pattern *ca* 2 m apart. All samples were stored (field moist) in sealed plastic bags at about 5°C until assayed.

Sulfur processing assays

Sulfate adsorption and organic S formation potentials were determined by exposing, in triplicate, 1 g samples (wet weight, not sieved) to 7.5 nmol $Na_2{}^{35}SO_4$ (3.3×10^{10} Bq $mmol^{-1}$, ICN) at 20°C for 48 h. After exposure, samples were extracted to yield a water soluble, a salt-extractable, and an acid and base-extractable fraction. The salt extraction (1 M Na_2SO_4, NaH_2PO_4, LiCl and deionized distilled water) removed S that had been adsorbed, while treatment with acid (6 M HCl at 121°C for 12 h) and base (2 M NaOH at about 25°C for 12 h) released organic S. Although the latter two treatments are destructive, previous research has shown that these fractions contain predominately ester sulfate and carbon-bonded S, respectively (Fitzgerald *et al.*, 1985). Total recoveries of ^{35}S were >90%.

The potential for these soils to mobilize (solubilize and mineralize) organic S was determined by exposing triplicate samples (1 g wet wt, unsieved) to 7.5 nmol of $Na_2{}^{35}SO_4$ at 20°C for 24 h to enable organic S formation to occur (Strickland and Fitzgerald, 1984). After exposure, samples were washed with 1 M NaH_2PO_4 followed by centrifugation to remove S that had not incorporated into organic matter. The microbial populations removed by this wash were re-established by adding an untreated soil–water mixture (1:5) to the phosphate-extracted samples. Following a second exposure at 20°C for 24 h, samples were washed successively with 1 M Na_2SO_4, NaH_2PO_4. LiCl and deionized water to recover S that had been mobilized from organic S. Insoluble S (the remaining non-mobilized organic S) was released by treatment with acid (6 M HCl at 121°C for 12 h) and base (2 M NaOH at about 25°C for 12 h). The capacity for organic S mobilization was calculated on a % base as ^{35}S solubilized from the organic ^{35}S formed during the initial 24 h exposure. Total ^{35}S recoveries were >92%.

Humic acid extraction

Humic acid was extracted from composite samples from each horizon and watershed using the method of Hayes *et al.* (1975). Briefly, soil was treated with 0.5 M NaOH to solubilize humic acid which, after centrifugation, was precipitated from supernatants with dilute HCl until a pH of 1 was obtained. Precipitates were collected by centrifugation and washed with deionized distilled water until chloride-free by anion chromatography. The pH of the humic acid was raised with NaOH to the pH of the soil sample extracted, initially. In all cases, solubilization of humic acid occurred and the sulfate content of each extract was negligible by anion chromatography. Organic carbon content was measured using a Shimadzu Total Carbon Analyzer.

Table 1. Organic carbon content of humic acid extracts of soil

Watershed	Horizon	Organic carbon* (mg C g^{-1} of soil)
27	A	207.3 (2.5)
	E	95.9 (4.9)
	B	74.2 (2.8)
2	A	100.9 (0.6)
	E	38.3 (0.3)
	B	26.2 (0.6)

*Values are expressed as means ($n = 3$); standard error is given in parentheses.

Carbon amendments

Sulfate adsorption and organic S formation potentials were determined as before, except that samples were first amended with either 25 or 50 mg of cellobiose g^{-1} of soil. Controls were amended with an equivalent volume (200 μl) of deionized distilled water. In separate assays, soil was also exposed to cellobiose or humic acid for 48 h at 20°C before the addition of the ^{35}S-label. A concentration of humic acid (in 200 μl of deionized water) equal to that which was determined for each sample (Table 1) was added, thus doubling the humic acid content of that sample.

Soil respiration

Soil respiration rates were determined by a modification of the technique of Anderson (1982). Soil (10 g wet wt) was added to 100 cm^3 bottles and mixed with cellobiose (50 mg g^{-1} of soil), whereas controls were amended with an equivalent volume of deionized distilled water. Evolution of CO_2 was linear after 5 h and this time of exposure was used for all assays. Evolved CO_2 was trapped in 1 M NaOH and quantified by titration. All samples were assayed in triplicate.

Characterization of sulfur pools

Total S was determined by hypobromite oxidation (Tabatabai and Bremmer, 1970) followed by hydriodic acid (HI) reduction. HI-reducible S (ester 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. Amino acid S (a constituent of the carbon-bonded S pool) was quantified by reduction with Raney nickle (Freney *et al.*, 1970). The remaining carbon-bonded S which was resistant to reduction by HI and Raney nickle was considered, on the basis

of previous work (Autry and Fitzgerald, 1990), to represent sulfonate S.

Soluble S was recovered by shaking samples (4 g) with 20 ml of deionized distilled water for 30 min followed by centrifugation, while adsorbed S was obtained by shaking the resulting residue twice with 20 ml of 20 mM Na_2HPO_4 for 30 min followed by centrifugation. The resulting supernatants from both extraction procedures were subjected to HI reduction and anion chromatography to determined the amount of ester sulfate and inorganic sulfate, respectively.

Other soil variables

Moisture was determined by drying samples at 50°C for 48 h. Total carbon content was quantified by total combustion using a Leco Total Carbon Analyzer, while pH was measured in a 1:2 soil–water solution. Average temperature (at 10 cm) in the month of September, 1989 for soil from watersheds 27 and 2 was estimated using a model of soil temperature profiles (Vose and Swank, 1991).

Statistical analysis

Analysis of variance was performed on the data and Duncan's multiple range test was used to determined statistical differences among values ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Organic and inorganic S constituents

Significant differences in amounts of organic sulfur were found in samples collected in September 1989 from watershed (WS) 27 and WS 2 (Table 2). In every horizon, samples from WS 27 contained substantially greater amounts of total S, amino S and sulfonate S when compared to those from WS 2. These trends were confirmed with the April 1990 sampling. Sulfonate S was the dominant organic S pool in soil from WS 27 (ranging from 14 to 56% of total S), whereas the amount of sulfonate in samples from WS 2 ranged from ~6 to 23% of total S. The observation that sulfonate S was the dominant organic S pool in soil from WS 27 (the high elevation watershed) is consistent with results of other studies using lower elevation watersheds at Coweeta (Watwood and Fitzgerald, 1988) and elsewhere (Autry and Fitzgerald, 1990). The main source of sulfonate in forest soil is from plant sulfolipids (Harwood and Nicholls, 1979), although in $^{35}SO_4$-labeling experiments, sulfonate was also formed microbiologically (Watwood and Fitzgerald, 1988). Research (Strickland and Fitzgerald, 1983) using 6-sulfoquinovose (the sulfonate-bearing component of this lipid) indicated rapid mineralization of this form of S by forest soil, which suggests the dynamic nature of this pool. Inputs of sulfonate to soil of WS 27 probably exceed mineralization, thus causing an accumulation of this form of S. If one assumes similar inputs from plant material during and after deciduous senescence, soils from WS 2 are probably capable of mineralizing sulfonate-S more rapidly than those from WS 27.

Although soil from WS 27 contained significantly more amino acid S than WS 2, this form of S was a minor constituent from both watersheds irrespective of soil horizon (3–11% of total S, WS 27; 6–11%, WS2). Amounts of other organic S constituents generally were not significantly different in soil from the two watersheds. Of these organic pools, insoluble ester sulfate typically was the largest component comprising between 15 and 28% (WS 27) and 15–60% of total (WS 2). Other studies of forest soils showed amino acid S to be a minor constituent of total S (Watwood and Fitzgerald, 1988; Autry and Fitzgerald, 1990). Results of previous research at Coweeta (Fitzgerald and Andrew, 1984; Hale and Fitzgerald, 1990) demonstrated that methionine and cysteine were subject to mineralization, which might explain why S-containing amino acids were only minor S constituents in the soils of the current study. Previous research (Watwood and Fitzgerald, 1988), might explain why insoluble ester sulfate was the largest ester pool in the current study. In experiments using a $^{35}SO_4$-label, these investigators followed transformations in A horizon soils between ester sulfate pools over time. Results suggested a transfer of S from soluble and adsorbed ester sulfate to insoluble ester sulfate.

Notable differences in inorganic S constituents were found in soil from these two watersheds (Table 2). Larger amounts of soluble and adsorbed sulfate were found in samples from WS 27 and with samples from both watersheds; adsorbed sulfate was greater than soluble sulfate. No clear pattern could be established between these S constituents and horizon. Other inorganic S components were analyzed and thiosulfate content was $<1\%$ of total S while tetrathionate was undetectable (data not shown). Wainwright (1979) also found small amounts of these S components in a number of S-polluted soils.

Moisture, carbon content and soil pH

Moisture content was considerably higher in samples from WS 27 when compared to those collected from WS 2 (Table 3). With samples from the former, moisture content ranged from 117 to 50% and decreased with soil depth, whereas with samples from WS 2 moisture ranged from only 33 to 26%. These values decreased with soil depth. Watershed 27 receives about 30% more precipitation on an annual basis than does WS 2. Higher elevation watersheds at Coweeta typically have shallow soils and lower evapotranspiration rates compared to their lower elevation counterparts (Swift *et al.*, 1988). Therefore, higher soil moisture on WS 27 is generally expected. Swank and Waide (1988) observed that ecosystem carbon flow for higher watersheds tended to be less than that for the lower watersheds due to lower primary production, decomposition and root respiration, which leads to an accumulation of carbon in

Table 2. Sulfur forms and pool sizes from high and low elevation watersheds*

Watershed (elevation)	Horizon	Month sampled	Total S	Amino acid-S	Sulfonate†	Adsorbed† ester SO_4^{2-}	Soluble† ester SO_4^{2-}	Adsorbed SO_4^{2-}	Soluble SO_4^{2-}	Insoluble† ester SO_4^{2-}
			$\mu g\ S\ g^{-1}$ dry wt							
27 (1280 m)	A	September	592.7 a (5.1)	64.5 a (14.0)	252.5 a	67.0 a	8.3 a	75.1 a (6.0)	8.5 a (8.1)	166.2 a
		April	631.0 a (9.5)	45.4 a (18.5)	354.9 a	15.8 a	14.7 a	39.4 a (28.0)	6.1 a (16.2)	152.8 a
	E	September	373.3 a (7.2)	42.8 a (13.9)	121.9 a	68.3 a	8.5 a	60.4 a (18.0)	7.2 a (5.9)	93.0 a
		April	457.5 a (4.3)	14.5 a (5.6)	179.7 a	31.8 a	8.7 a	92.4 a (8.5)	9.0 a (18.9)	129.5 a
	B	September	631.1 a (13.2)	21.0 a (12.9)	86.5 a	285.6 a	13.5 a	110.8 a (23.6)	7.8 a (7.2)	92.6 a
		April	476.7 a (6.7)	13.7 a (13.9)	123.3 a	ND‡	7.2 a	122.6 a (23.6)	8.3 a (16.9)	98.5 a
2 (625 m)	A	September	238.2 (3.2)	21.0 b (7.3)	14.7 a	48.2 a	6.3 a	7.2 b (6.6)	3.8 b (12.9)	128.6 a
		April	192.9 b (4.5)	17.8 b (14.0)	34.3 b	20.5 a	3.1 b	14.5 b (13.1)	3.7 b (5.4)	29.0 b
	E	September	206.5 b (4.5)	15.7 b (9.8)	10.9 b	50.4 a	5.7 a	16.0 b (9.9)	4.4 a (8.7)	121.2 a
		April	149.2 b (7.6)	15.7 b (6.1)	35.3 b	11.2 a	9.8 a	33.8 b (7.4)	3.5 b (5.3)	73.9 a
	B	September	236.2 b (4.3)	19.2 a (12.9)	14.8 b	109.7 a	10.1 a	10.9 b (13.6)	4.0 b (9.6)	125.7 a
		April	188.2 b (10.6)	12.2 a (9.6)	32.0 b	ND	7.7 a	77.3 b (5.4)	3.1 b (7.4)	54.2 b

*Means are reported, $n = 9$; % error is given in parentheses. Within a given horizon and month, values followed by the same letter are not significantly different ($P = 0.05$).
†Calculated by difference, see text.
‡Not detected.

Table 3. Moisture, carbon, pH and sulfur processing potentials in soil from high and low elevations watersheds*

Watershed (elevation)	Horizon	Month sampled	Moisture content (%)	Carbon	pH	SO_4^{2-} adsorption potential (nmol S g^{-1} dry wt 48 h^{-1})	Organic S formation (nmol S g^{-1} dry wt 48 h^{-1})	Mobilization (% 24 h^{-1})	Organic S† accumulation (nmol S 24 h^{-1})
27 (1280 m)	A	September	117.1 (7.9)	9.8 a (2.1)	4.1 a (2.4)	9.8 a (2.1)	2.5 a (8.0)	55.5 a (0.5)	1.1 a
		April	105.4 a (6.2)	13.2 a (11.4)	4.2 a (2.2)	8.2 a (3.7)	4.6 a (10.4)	62.0 a (2.5)	1.8 a
	E	September	82.8 a (8.3)	5.0 a (11.8)	4.3 a (2.3)	9.1 a (7.7)	1.8 a (22.2)	56.9 a (2.1)	0.8 a
		April	70.7 a (3.4)	6.2 a (6.5)	4.4 a (2.1)	8.7 a (2.7)	1.7 a (6.9)	73.6 a (2.9)	0.5 a
	B	September	65.1 a (14.5)	2.7 a (13.8)	4.5 a (2.2)	10.1 a (5.9)	0.8 a (22.2)	61.0 a (2.0)	0.3 a
		April	49.4 a (3.0)	2.6 a (17.6)	4.3 a (1.2)	8.1 a (4.8)	1.0 a (15.4)	68.5 a (3.4)	0.4 a
2 (625 m)	A	September	29.2 b (3.1)	2.1 b (4.4)	4.7 b (2.1)	6.8 b (1.5)	1.4 b (7.1)	49.6 b (1.2)	0.7 b
		April	33.3 b (1.3)	2.3 b (3.4)	4.7 b (1.1)	6.7 b (2.1)	1.6 b (4.3)	62.4 b (1.6)	0.6 b
	E	September	25.5 b (3.1)	1.2 b (9.2)	4.9 b (0.1)	7.1 b (1.4)	1.3 a (7.7)	50.7 b (1.4)	0.6 a
		April	28.5 b (1.3)	1.0 b (4.1)	4.9 b (5.7)	7.1 b (2.7)	1.5 a (8.6)	64.8 b (2.5)	0.5 a
	B	September	24.6 b (2.8)	0.6 b (5.0)	5.0 b (2.0)	7.3 b (1.4)	0.9 a (11.1)	50.6 b (0.8)	0.5 a
		April	27.4 b (1.3)	0.6 b (5.0)	4.6 b (1.7)	6.8 b (3.2)	1.9 b (9.9)	67.9 a (2.5)	0.6 a

*Means are reported, $n = 9$; % error is given in parentheses. Within a given horizon and month, values followed by the same latter are not significantly different ($P = 0.05$).
†Calculated by difference, see text.

soils. The carbon data from the current study tends to support this observation. With samples from every horizon, C-content for WS 27 was significantly greater than that for WS 2. This parameter decreased with depth in both watersheds. Soil pH was also significantly different between the two watersheds for all horizons, with samples from WS 27 being more acidic by 0.5 pH units than those from WS 2.

Sulfate adsorption potentials and pH

Sulfate adsorption potentials were also greater with soil from WS 27 compared to that from WS 2. Within each watershed, no pattern could be established between adsorption potentials and horizon. Collectively, soil from WS 27 had a lower pH and higher adsorption potentials compared to WS 2 soil, which had higher pH values with lower adsorption potentials (Table 3). A positive relationship was established between pH and adsorption potentials in soil from WS 2 ($r = 0.93$; $P \leqslant 0.001$). Harrison *et al.* (1989) observed a similar relationship with a number of subsoils. This relationship contrasts with the results of other studies in which soil pH was artificially lowered and an increase in sulfate adsorption was observed and vice versa (Kamprath *et al.*, 1956; Patil *et al.*, 1989). Soil pH was not artificially created in the current study or in the study by Harrison *et al.* (1989). Instead, pH differences were achieved through normal soil development, and it was pointed out by Harrison *et al.* (1989) that it is difficult to compare data when pH was not achieved in the same manner. No correlation was detected between this variable and sulfate adsorption potentials with samples from WS 27, although a positive relationship was established between adsorbed ester sulfate and sulfate adsorption potential ($r = 0.60$; $P \leqslant 0.007$). With these samples, adsorption potentials might be reflecting existing pools of adsorbed ester sulfate. For example, with September samples, B horizon soil had the largest sulfate adsorption potential (10 nmol S g^{-1} dry wt), which corresponded to the greatest amount of adsorbed ester sulfate (287 μg S g^{-1} dry wt). In experiments utilizing $^{35}SO_4$, Watwood and Fitzgerald (1988) found that some of the label added to soils was converted to adsorbed ester sulfate after 2 days exposure.

Although different soils were examined, results of other sulfate adsorption research with high (WS 27) and low (WS 18) elevation watersheds suggests that soils at Coweeta have the potential to accumulate further inputs of sulfate. Soil from WS 27 contained more adsorbed sulfate than that from WS 18 and also had a higher potential to adsorb additional inputs of sulfate (Johnson *et al.*, 1980). This trend was observed with the soils from the current study. Thus, soil from WS 27 had greater amounts of adsorbed sulfate compared to WS 2, and samples from the former also had greater sulfate adsorption potentials (Tables 2 and 3). According to Swank and Waide (1988), the high elevation watersheds are starting to respond to acidic precipitation, as indicated by increasing amounts of sulfate in streams. Observations made in the current study and those of Johnson *et al.* (1980) suggest that, at the process level, these soils still exhibit some buffering capacity to sulfate inputs. The question is to what extent do S processes contribute to higher sulfate concentrations in streams of high elevation watersheds.

Organic S formation and mobilization potentials

Organic S formation potentials in samples from both watersheds tended to decrease with depth (Table 3). Only the rates for A horizon samples from the two watersheds were significantly different (with the exception of the B horizon soil from WS 2, April sampling). These potentials for samples from WS 27 were positively correlated to carbon content ($r = 0.90$; $P \leqslant 0.005$). A process which negatively influences the amount of organic S accumulated in soils is organic S mobilization. Rates for this process with all samples from both watersheds were $> 50\%$ 24 h^{-1} (Table 3). Although samples from the E horizon (WS 27) for both sampling dates had greater mobilization rates than those for WS 2, no effect on the accumulation of organic S was observed. Mobilization rates for samples from WS 27 were positively correlated to amounts of soluble soil sulfate ($r = 0.57$; $P \leqslant 0.05$). By combining mobilization and formation rates, it was possible to determine the potential for organic S accumulation. In comparing the sites, only the A horizon soils were significantly different in accumulating organic S (Table 3).

Spatial and seasonal variability

The variation between each pit sampled (indicated by % error) for all assays was low ($< 10\%$ in most cases), which indicates a lack of spatial variability for these S processes and S constituents. Similar conclusions were drawn from results obtained with samples collected over a year along a transect of a low elevation watershed (WS 18) in the Coweeta basin (Fitzgerald *et al.*, 1988). The second sampling of WS 2 and 27 (April 1990) was therefore initiated to confirm differences or similarities observed with samples collected initially, especially for the high elevation WS. In all but a few cases, the same relationships were observed (Tables 2 and 3).

Influence of temperature on organic S formation

Samples collected in September, 1989 were utilized for the temperature experiments. Predicted soil temperatures of 15 and 20°C were used for WS 27 and 2, respectively; samples from WS 27 were also exposed to 20°C. There was no significant influence of this variable on sulfate adsorption by soils from WS 27 (Table 4), and potentials for WS 27 were still greater than those for WS 2. When organic S mobilization, using samples from WS 27, was examined under these two temperature regimes, no significant difference was observed (data not shown). The only S

Table 4. Effect of temperature on sulfate adsorption and organic S formation in soil from high and low elevation watersheds*

Watershed (elevation)	Horizon	Temperature (°C)	Sulfate adsorption	Organic S formation
			nmol S g^{-1} dry wt 48 h^{-1}	
27 (1280 m)	A	15	12.7 a1 (3.6)	1.6 a1 (9.4)
		20	12.0 a (4.3)	2.7 b (7.3)
	E	15	11.5 a1 (4.6)	1.9 a1 (15.6)
		20	10.6 a (6.4)	2.2 a (14.7)
	B	15	10.3 a1 (4.4)	1.3 a1 (8.3)
		20	9.9 a1 (3.7)	1.3 a (2.9)
2 (625 m)	A	20	7.3 2 (1.2)	1.4 1 (2.9)
	E	20	6.3 2 (2.3)	1.3 1 (5.3)
	B	20	6.2 2 (3.7)	1.4 1 (6.6)

*Means are reported, $n = 9$; % error is given in parentheses. Data reported are for samples collected in September 1989. Within a given horizon, values followed by the same letter or number in each column are not significantly different ($P = 0.05$).

process which temperature influenced was organic S formation. A 48 h exposure time was chosen because formation rates were linear through 72 h of exposure (data not shown). Samples from the A horizon (WS 27) showed a significant decrease in organic S formation rates when exposed to 15°C compared to 20°C (about a 2-fold decrease; Table 4). Time course experiments illustrated that formation rates for these samples were significantly lower at 15°C, not only after 48 h (as shown in Table 4), but at all exposure times up to 72 h (data not shown). When rates at 15°C for similar samples from WS 27 were compared to those from WS 2, no significant difference was observed.

Influence of cellobiose and humic acid amendments

Samples from all horizons of WS 2 amended with cellobiose together with ^{35}S-labeled sulfate were capable of adsorbing significantly greater amounts of sulfate compared to controls [Fig. 1(a)]. This increased adsorption was also observed when soil was exposed for 48 h to cellobiose prior to the addition of the ^{35}S-label. Only A horizon soil from WS 27 exhibited increased adsorption compared to controls when samples were not pre-exposed to cellobiose, whereas both the A and E horizon samples exhibited significant increases in adsorption when exposed for 48 h to cellobiose prior to the addition of label [Fig. 1(b)]. A 48 h pre-exposure to humic acid caused a significant increase in sulfate adsorption in soil from WS 2 [Fig. 1(c)], whereas only the A horizon soil from WS 27 was influenced by the addition of this source of organic matter. After a 1 week exposure, a significant increase in adsorption was observed with samples from all horizons of the latter watershed [Fig. 1(d)]. Results of studies by Johnson and Todd, 1983 and Fuller *et al.*, 1985 showed that soils with high organic matter had a negative influence on sulfate adsorption, and it was proposed that organic

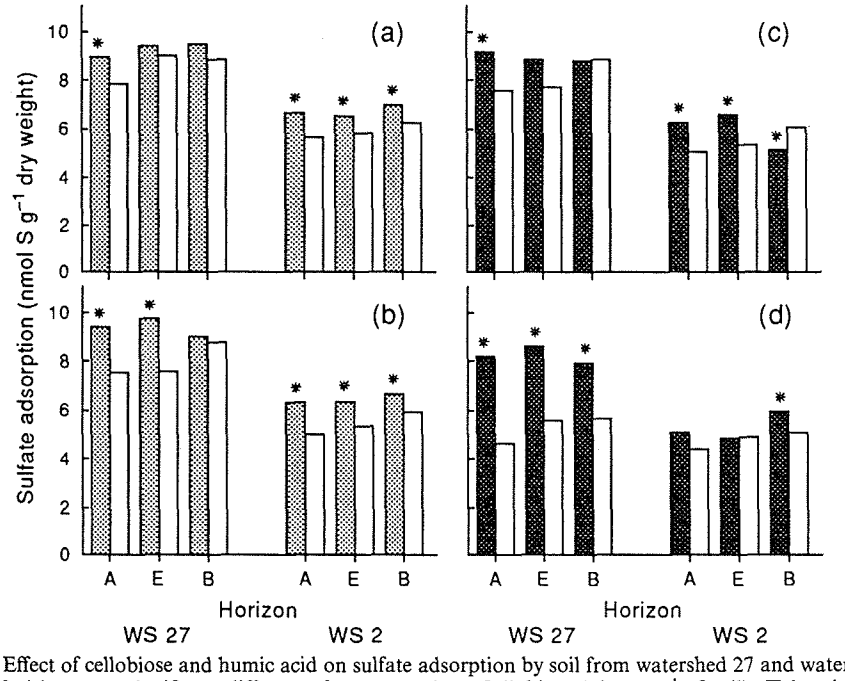

Fig. 1. Effect of cellobiose and humic acid on sulfate adsorption by soil from watershed 27 and watershed 2. $n = 9$; *denotes a significant difference from control. ⊡ Cellobiose (50 mg g^{-1} of soil); ■ humic acid (for concentration see Table 1 and text); □ no added carbon source (control). (a) Cellobiose added together with ^{35}S-labeled sulfate; (b) samples exposed to cellobiose for 48 h prior to the addition of ^{35}S-labeled sulfate; (c) soil exposed to humic acid for 48 h prior to the addition of ^{35}S-label; (d) soil exposed to humic acid for 1 week prior to the addition of ^{35}S-label. Samples were collected in September 1989.

matter blocked adsorption sites. Other work (Patil *et al.*, 1989) revealed a positive influence of organic matter on the adsorption of sulfate. In the current study cellobiose had a positive influence on sulfate adsorption especially with samples from WS 2. The adsorption capacity of soil from WS 27 changed over time [see e.g. Fig. 1(a) and (b), E horizon], indicating that metabolism of this carbon source might be causing an increase in adsorption capacity. The same explanation may be given for increases in adsorption with samples from WS 2 even though no differences were observed between the two exposure times. Adsorption sites also did not appear to be blocked by humic acid, rather sulfate adsorption appeared to increase in the presence of increased amounts of this soil colloid. Sulfate adsorption capacities increased in soil from WS 27 when samples were pre-exposed for 1 week (compared to 48 h) to humic acid, suggesting that as decomposition of humic acid was occurring, more adsorption sites were made available. Samples from the A and E horizons from WS 2, however, did not exhibit an increase in adsorption after exposure for 1 week to this colloid.

When the influence of cellobiose on organic S formation was examined with samples from these two watersheds, soil from WS 2 exhibited a significant increase in organic S formation after amendment with cellobiose and [^{35}S]sulfate. These increases ranged from 60 to 70% compared to controls containing no added carbon [Fig. 2(a)]. Formation rates in samples from WS 27, however, did not change with the addition of this carbon source. When soil was exposed to cellobiose for 48 h before the addition of the label, samples from WS 2 still formed more organic S than controls, although to a lesser extent than when no pre-exposure was undertaken [Fig. 2(b)]. After pre-exposure, A horizon soil from WS 27 was able to form greater amounts of organic S compared to controls. When soil from both watersheds was incubated with humic acid (pre-exposed for 48 h), only rates with A horizon samples increased [Fig. 2(c)]. After exposure to humic acid for 1 week, an increase in organic S formation occurred in soil samples from all horizons of WS 27 [Fig. 2(d)].

The positive influence of cellobiose on organic S formation suggests that soil from WS 2 might be energy deficient in terms of the capacity to form organic S. Soil from WS 27 had greater amounts of carbon compared to WS 2 and may be energy sufficient, which may explain why cellobiose had no effect with samples from this watershed. The lack of response of samples to humic acid was not unexpected due to the recalcitrant nature of this colloid, although after a 1 week pre-exposure, organic S formation increased over controls in samples from WS 27. This observation suggests that organisms in these samples were eventually capable of utilizing this substrate. Soil from WS 2, generally remained unresponsive to humic acid. These differences might be due to the type of humic acid in each soil, or the organisms involved in organic S formation might be different. For example, organisms in soil from WS 2 might not be able to utilize humic acid as a carbon source compared to those from WS 27.

To further confirm some of the relationships that were established with soil from these watersheds, experiments using cellobiose were performed with samples collected in April 1990. The influence of two concentrations of this carbon source on sulfate adsorption and organic S formation was determined [Fig. 3(a), (b)]. Again, cellobiose increased sulfate adsorption in soil from both watersheds by amounts

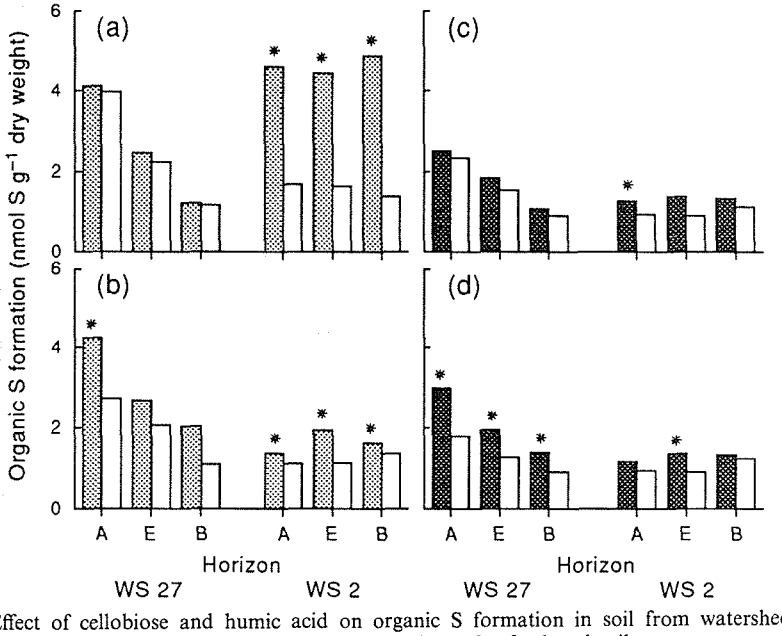

Fig. 2. Effect of cellobiose and humic acid on organic S formation in soil from watershed 27 and watershed 2. See caption to Fig. 1, for further details.

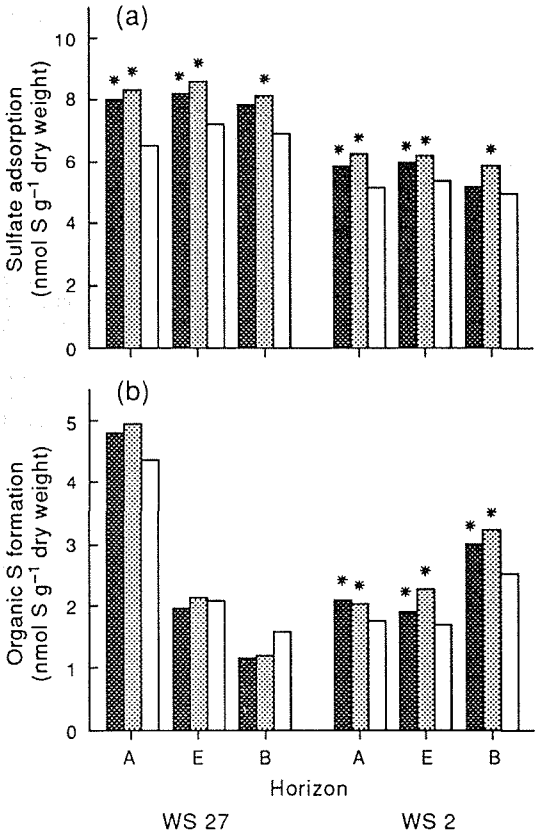

Fig. 3. Effect of cellobiose on (a) sulfate adsorption and (b) organic S formation in soil from watershed 27 and watershed 2 collected in April, 1990 ($n = 9$). Soil was exposed to carbon source together with ^{35}S-labeled sulfate; *denotes significant differences from control. ▩ Cellobiose (50 mg g^{-1} of soil); ⬚ cellobiose (25 mg g^{-1} of soil); □ no added cellobiose.

similar to those recorded for samples collected in September. This influence was not concentration dependent except for B horizon soil from both watersheds, which exhibited a significant increase in adsorption when 25 mg (g^{-1} of soil) of cellobiose was added, whereas no response was observed when 50 mg was used. Similar results concerning organic S formation were observed with April and September samples [Fig. 3(b)]. Soil from WS 2 had a greater rate when amended with either 25 or 50 mg of cellobiose compared to controls, but the concentration used had no effect on the amount of organic S formed. Samples from WS 27 did not exhibit increases in organic S formation compared to controls. Increases in respiration rates were observed with soil from WS 2 amended with cellobiose (Table 5). These increases corresponded to an increase in organic S formation. No significant increases in respiration rates were observed with samples from WS 27.

Table 5. Effect of cellobiose on respiration rates in soil from watershed 27 and watershed 2*

Watershed	Sampling date	Horizon	No addition	Cellobiose†
			μmol CO_2 g^{-1} dry wt h^{-1}	
27	September	A	77.2 (8.2) a	62.6 (4.6) a
	April		134.7 (4.2) a	149.6 (4.5) a
	September	E	73.1 (2.7) a	72.9 (1.1) a
	April		98.9 (8.4) a	68.4 (1.8) b
	September	B	65.1 (7.2) a	41.7 (4.5) a
			69.7 (2.6) a	54.5 (3.3) a
2	September	A	119.9 (2.2) a	184.6 (15.1) b
	April		46.8 (3.9) a	85.5 (4.8) b
	September	E	91.6 (5.6) a	130.7 (4.0) b
	April		33.9 (3.0) a	64.1 (4.9) b
	September	B	99.6 (9.3) a	114.2 (9.6) b
	April		26.1 (3.3) a	65.9 (4.0) b

*Values are expressed as means ($n = 9$); standard error is given in parentheses. Within a given horizon and samples date, values followed by the same letter are not significantly different ($P = 0.05$).
†50 mg g^{-1} of soil.

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