Sulfate adsorption and microbial immobilization in northern hardwood forests along an atmospheric deposition gradient

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While a number of studies have investigated adsorption and microbial immobilization as sulfate (SO_4^{2-}) retention mechanisms, few have investigated these processes under field-like conditions on a regional, ecosystem basis. Adsorption and microbial immobilization of SO_4^{2-} were studied in four northern hardwood stands that span an atmospheric deposition gradient in the Lake States region (5 to 10 kg $S \cdot ha^{-1} \cdot year^{-1}$). Soil cores collected in spring, summer, and autumn were labeled with $^{35}SO_4^{2-}$ to trace the flux of S between physical and biological sinks, and to investigate seasonal variation in sink strength. Intact soil cores were injected with $Na_2{}^{35}SO_4$ and incubated for 8 d in the laboratory at field temperature to study rates of adsorption and microbial immobilization. The amount of ^{35}S recovered within these pools was significantly different between surface and subsurface soil horizons. Microbial immobilization was the dominant S sink in the A+E horizon, whereas adsorption was the most important S sink in the B horizon. During the 8-d incubation, the proportion of ^{35}S that was immobilized in the A horizon (49% of applied ^{35}S) was equivalent to the proportion of ^{35}S adsorbed in the B horizon (47% of applied ^{35}S). Microbial immobilization sequestered an additional 25% of the applied ^{35}S in the B horizon. Adsorption and microbial immobilization were not significantly different among sampling dates. Sulfur retention in forested ecosystems should be viewed as a combination of geochemical and microbially mediated processes. However, given current levels of S deposition at these sites, neither process seems to represent a significant mechanism for long-term S retention.

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Bien qu'un certain nombre d'études aient évalué l'adsorption et l'immobilisation microbienne comme mécanismes de rétention du sulfate (SO_4^{2-}), peu d'études ont évalué ces processus dans des conditions similaires au champ sur une base régionale et d'écosystèmes. L'adsorption et l'immobilisation microbienne de SO_4^{2-} ont été étudiées dans quatre peuplements de feuillus nordiques présentant un gradient de dépositions atmosphériques dans la région des Grands Lacs (5 à 10 kg $S \cdot ha^{-1} \cdot an^{-1}$). Des carottes de sol récoltées au printemps, à l'été et à l'automne ont été marquées avec du $^{35}SO_4^{2-}$ afin de suivre les flux de S entre les sinks physiques et biologiques, et d'évaluer la variation saisonnière dans la force des sinks. Des carottes de sol intactes ont été injectées avec du $Na_2{}^{35}SO_4$ et incubées pendant 8 jours au laboratoire, à la température du champ, pour étudier les taux d'adsorption et d'immobilisation microbienne. La quantité de ^{35}S récupérée dans les différents pools était significativement différente entre les horizons de surface et de sous-surface. L'immobilisation microbienne était le sink dominant de S dans l'horizon A+E, tandis que l'adsorption était le sink le plus important dans l'horizon B. Pendant l'incubation de 8 jours, la proportion de ^{35}S qui a été immobilisée dans l'horizon A (49% du ^{35}S ajouté) était équivalante à la proportion de ^{35}S adsorbée dans l'horizon B (47% du ^{35}S ajouté). L'immobilisation microbienne a séquestré un 25% additionnel du ^{35}S ajouté dans l'horizon B. L'adsorption et l'immobilisation microbienne n'étaient pas significativement différentes entre les dates d'échantillonnage. La rétention de S dans les écosytèmes forestiers devrait être vue comme une combinaison de processus géochimiques et de processus contrôlés microbiologiquement. Toutefois, étant donné les niveaux de déposition de S dans ces stations, aucun des processus ne semble représenter un mécanisme significatif de la rétention de S à long terme.

[Traduit par la rédaction]

Introduction

Forest ecosystems in the Great Lakes region receive atmospheric additions of SO_4^{2-} that range from 3 kg SO_4^{2-}-$S \cdot ha^{-1} \cdot year^{-1}$ in northern Minnesota to over 12 kg SO_4^{2-}-$S \cdot ha^{-1} \cdot year^{-1}$ in Ohio, and several studies have suggested that ecosystem-level patterns of S retention and loss have been altered by these sustained inputs (David *et al.* 1988; MacDonald *et al.* 1991, 1992; Pregitzer *et al.* 1992). For example, foliar S concentrations in northern hardwood forests, and the quantity of S returned to the forest floor in annual leaf fall, are positively correlated with rates of atmospheric deposition (Pregitzer *et al.* 1992). However, overstory uptake and assimilation appear to represent only a limited net sink for the quantities of SO_4^{2-} entering these ecosystems, as SO_4^{2-}-S leaching across the gradient is highly correlated with S deposition ($r > 0.95$, MacDonald *et al.* 1992).

The retention of SO_4^{2-} by forest soils has received considerable attention (Johnson 1980, 1984; Johnson *et al.* 1982; Reuss *et al.* 1987) and is viewed as a major process regulating the leaching of SO_4^{2-} and associated cations. Retention in forest soils was once thought to be a geochemical process, composed almost entirely of adsorption (Johnson and Henderson 1979; Johnson *et al.* 1979, 1982; Johnson 1984). However, microbial immobilization, the conversion of SO_4^{2-} to organic forms of S via microbial metabolism, is of additional importance (David *et al.* 1982; Fitzgerald *et al.* 1983; Swank *et al.* 1984; Autry *et al.* 1990). Fitzgerald *et al.* (1988) used ^{35}S to trace the flux of SO_4^{2-} into organic compounds and found that approximately 20% of the applied label was immobilized. Moreover, rates of microbial SO_4^{2-} immobilization were highly dependent on soil temperature, moisture, and substrate availability, factors that can exhibit marked seasonal variation. While these factors vary seasonally, few studies

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have addressed the seasonal influence they may have on adsorption or microbial immobilization.

Sulfate, like many other inorganic nutrients, is required for microbial metabolism and is incorporated in a proportional relationship with substrate to form new microbial cells. Sulfur represents approximately 1% of microbial biomass (Smith and Paul 1990), most of which is contained in amino acids and sulfolipids (Paul and Clark 1989). Seasonal patterns of soil temperature and moisture availability, as well as patterns of substrate input resulting from above- and below-ground litter production, should have an important influence on the rate at which soil microbes sequester SO_4^{2-} from soil solution (Freney *et al.* 1971).

Schindler *et al.* (1986) suggested that the combined effects of microbial activity and physiochemical adsorption need to be considered when evaluating the influence of anthropogenic SO_4^{2-} additions on forest soils. We reasoned that microbial immobilization of SO_4^{2-} should represent an important S sink in surface forest soils, particularly during portions of the growing season when soil physical factors and substrate availability are favorable for microbial activity. Microbial immobilization should be relatively less important in subsurface horizons, where lower labile organic matter contents and higher Fe and Al contents favor SO_4^{2-} adsorption. Because adsorption and microbial immobilization have generally been studied on a point-specific basis, we endeavored to understand how these processes vary seasonally and spatially within northern hardwood forests. Our primary objective was to investigate the relative importance of microbial immobilization and SO_4^{2-} adsorption in sequestering S from soil solution. To accomplish this, we studied seasonal patterns of microbial immobilization and adsorption in northern hardwood forests that lie along a SO_4^{2-} deposition gradient in the Lake States region.

Site descriptions

The four northern hardwood stands we studied span a S deposition gradient that ranges from 5 to 10 kg SO_4^{2-}-S·ha^{-1}·year^{-1} (Fig. 1). Climate, soils, and vegetation of the four stands are summarized in Table 1. The overstory composition of each stand was approximately 80% sugar maple (*Acer saccharum* Marsh.). Three 30 × 30 m plots were located within each stand for a related study (MacDonald *et al.* 1991), and we selected one plot in each stand with level topography and a sandy soil texture throughout the solum to study SO_4^{2-} adsorption and microbial immobilization. Soils were well drained and belonged to closely related subgroups of the Spodosol order (Entic Haplorthods and Typic Haplorthods). Percent silt + clay and soil pH were very similar across all sites in both surface and subsurface horizons. A more detailed discussion of the edaphic properties of these sites can be found in MacDonald *et al.* (1991).

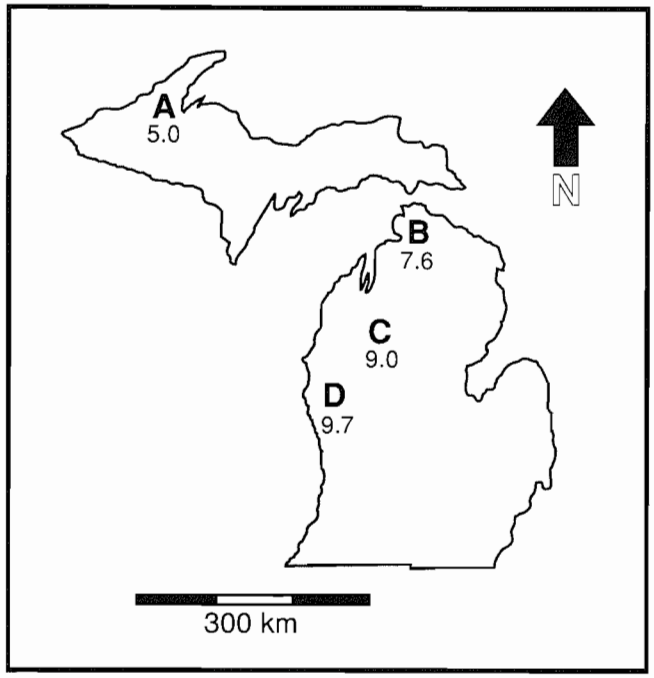

FIG. 1. Location of study sites along the regional acidic deposition gradient. Numbers represent estimates of mean annual sulfate-S inputs (kg·ha^{-1}·year^{-1}) including both wet and dry deposition (October 1987 through September 1990). Note that 10 kg/ha = 1 g/m^2. (Revised from MacDonald *et al.* 1992).

Methods

Soil sampling

Soil cores from surface and subsurface horizons were collected from each stand on May 9, July 10, and October 23, 1990. On the first sampling date, soil samples were collected at eight random azimuths and distances from the center of the study plot. Five random sample points were used thereafter based on within-stand variance in S pools. The Oi, Oe, and Oa horizons were removed, and two surface soil cores and two subsurface soil cores were collected from each sampling point. A metal core, 5.0 cm in diameter and 15 cm in length, was used to remove the intact soil cores from surface and subsurface soil horizons. Surface cores were collected from the upper 10 cm of mineral soil; these cores were composed of A and E horizon material (A+E). Subsurface cores were collected from the upper 10 cm of the underlying Bhs horizon. A rubber stopper was used to seal the bottom of each core, and the cores were enclosed in polyethylene bags and kept upright at all times to avoid disturbing the soil. Immediately following removal of cores from the soil profile, the field temperature of each sampling depth was recorded using a digital thermometer. Soil cores were stored at approximately 4°C until they were returned to the laboratory, usually within 24 h. In the laboratory, the intact cores were stored at 2°C until analyses began, always within 7 d of collection. One set of soil cores (A+E and B) from each sampling point was used to determine soil S pools, while the other was labeled with ^{35}S to trace SO_4^{2-} among physical and biological sinks.

Sulfur pools

On each sampling date, one set of surface soil cores were combined within each site and homogenized prior to analysis; subsurface soils received the same treatment. For each analysis three field-moist subsamples were taken from each homogenized sample. Soils were analyzed for water-extractable SO_4^{2-}-S, $H_2PO_4^-$-extractable SO_4^{2-}-S, microbial biomass S, and total S. Water- and $H_2PO_4^-$-extractable SO_4^{2-}-S were determined by extraction with deionized water and 0.016 M NaH_2PO_4, respectively, using a 5:1 solution–soil ratio. Samples were shaken for 1 h, and the resulting suspensions were filtered through Whatman No. 42 filter paper. Any remaining sediment was removed by centrifugation at 3000 rpm for 10 min. Sulfate S in the water and $H_2PO_4^-$ extracts was determined using a Dionex 4000i ion chromatograph.

Microbial biomass S content was measured using a modification of the Strick and Nakas (1984) procedure. Subsamples (20 g) were fumigated with alcohol-free chloroform in a vacuum desiccator for 18 h, while another set of subsamples was covered with Parafilm™ and maintained at room temperature during the fumigation. Both the control and fumigated samples were inoculated with 0.5 g of the fresh soil, covered with Parafilm™, and stored at room temperature in the

TABLE 1. Climate, soil, and vegetation of four northern hardwood stands distributed along a SO_4^{2-} deposition gradient in the Lake States region

	Site A	Site B	Site C	Site D
Climate				
Longitude (W)	88°53′	84°52′	85°50′	86°09′
Latitude (N)	46°52′	45°33′	44°23′	43°40′
Mean annual temperature (°C)	4.2	5.2	5.8	7.6
Mean annual precipitation (cm)	87	83	81	85
Soil*				
Silt + clay (%)				
A+E	14.8	10.6	10.6	12.7
B	13.7	13.4	11.1	11.0
pH (1:1 soil–H_2O)				
A+E	4.83	5.03	4.47	4.66
B	5.24	5.30	5.49	5.26
Bulk density (Mg/m^3)				
A+E	1.55	1.43	1.36	1.34
B	1.48	1.44	1.30	1.29
Total horizon thickness in profile (cm)				
A+E	11.1	17.9	21.8	17.6
B	49.6	71.6	64.0	73.7
Vegetation				
Overstory age (years)	79	73	74	78
Overstory biomass (Mg/ha)	262	263	275	234
Annual basal area increment (m^2/ha)†	0.09	0.23	0.29	0.26

*A+E and B horizon soil properties calculated for 10 cm sampling increments from soil pit data of MacDonald *et al.* (1991).

†Total stand net increment of basal area growth during 1988.

dark for 10 d. Control and fumigated samples were then extracted with 0.016 M NaH_2PO_4, as described above. A 30-mL aliquot of the resulting suspension was evaporated to ca. 3 mL and was digested in a mixture of concentrated HNO_3 and $HClO_4$ prior to total S determination (Wall *et al.* 1980). Samples were subsequently analyzed for total S by inductively coupled plasma atomic emission spectrometry (ICPAES).

To determine the efficiency of the microbial biomass assay, 600 g of soil were collected from site C during autumn of 1989. The soil was labeled with 45 mL of $Na_2{}^{35}SO_4$ (36×10^{-12} mmol, ca. 5×10^{13} Bq·mmol^{-1}) and homogenized by hand. For a period of 8 d, $H_2PO_4^-$-extractable ^{35}S was determined daily from two 20-g subsamples by liquid scintillation counting. Samples were corrected for radioactive decay to day 0, and the decline in radioactivity (over 8 d) was assumed to equal microbial ^{35}S assimilation. Microbial biomass ^{35}S was then determined on additional subsamples, using procedures described above. When microbial ^{35}S assimilation was compared with the values from the microbial biomass ^{35}S assay, recovery was found to be 35% (i.e., $K_s = 0.35$). This value is similar to those reported by others (Saggar *et al.* 1981; Strick and Nakas 1984; Castellano and Dick 1991). Therefore, we calculated microbial S by dividing the difference in S extracted between fumigated and control samples by 0.35 (e.g., (fumigated S – control S)/K_s).

Total S content of the soil was determined using a modification of the procedure described by Wall *et al.* (1980). Subsamples (0.25 g) of air-dried soil were digested with HNO_3 and $HClO_4$ for 4 h. Following digestion, each sample was diluted to 50 mL, and total S was measured using ICPAES. Total S content was calculated using bulk density estimates (Table 1). Organic S content was determined by subtracting the sum of water-extractable SO_4^{2-}, surface-adsorbed SO_4^{2-}, and microbial biomass S from total S content. Thus, we have defined organic S as the sum of all organic S forms in the soil other than that which is contained in microbial biomass. Microbial biomass S will refer to S that has been assimilated by soil microbes and exists in microbial cells.

Pools and fluxes of sulfur

The second set of surface and subsurface soil cores was used to determine the partitioning of S among physical and biological sinks. While still within the metal core, and sealed at the bottom, field-moist samples were labeled with 12 mL of $Na_2{}^{35}SO_4$ (9.1×10^{-12} mmol, ca. 5×10^{13} Bq/mmol) using a disposable 5-mL syringe with a modified 15 cm long parasympathetic nerve needle (20 gauge). This quantity of $^{35}SO_4^{2-}$ was proportional to 0.1% of the water-extractable SO_4^{2-} pool. The solution was added in water–soil (w/w) ratio of 1:20. The needle was equipped with a 1.2-cm pointed stainless steel tip with six ports (0.075 mm in diameter) that delivered a fine spray perpendicular to the needle shaft. Each core received three 4-mL injections, and isotope was delivered as the needle penetrated the soil. Radioactively labeled cores were covered with Parafilm™ and incubated for 8 d at field temperature (the mean temperature of all sites on the date of collection). Cores were incubated at 9, 15, and 7°C for the May, July, and October sampling dates, respectively.

At the end of the incubation, the content of each core was placed in a polyethylene bag and homogenized by hand. Subsamples were prepared for analysis for water- and $H_2PO_4^-$-extractable $^{35}SO_4^{2-}$-S in the same manner as described above, except that these samples were centrifuged but not filtered. Samples to be analyzed for microbial biomass ^{35}S were prepared as described above. Recoveries of ^{35}S in soil pools were expressed as a percentage of the added ^{35}S, and so total ^{35}S digests (prepared as described above) served merely as a quality assurance check. Mean recovery of ^{35}S in total S digests was 88.86% (±15.8); this value was not significantly different from 100%.

The activity of ^{35}S was measured in each pool by liquid scintillation counting using a Packard TriCarb 1900TR. Internal standards were used to adjust for counting efficiency, and samples were quench corrected. Aqueous extracts were suspended in Scintiverse™ (Fisher Scientific, Inc.), whereas acidic total ^{35}S digests were suspended in Hionic-FluorTM (Packard Instruments, Inc.). Results are reported on a dry mass basis, corrected for ^{35}S decay to day 0, and expressed as the percentage of the added ^{35}S.

Values for water-extractable $^{35}SO_4^{2-}$ were subtracted from $H_2PO_4^-$-extractable $^{35}SO_4^{2-}$ to approximate surface-adsorbed $^{35}SO_4^{2-}$ (Johnson and Todd 1983; Schindler *et al.* 1986). Organic ^{35}S was determined by subtracting the sum of water-extractable $^{35}SO_4^{2-}$-S, surface-adsorbed $^{35}SO_4^{2-}$-S, and microbial biomass ^{35}S from total ^{35}S content. Organic ^{35}S refers to ^{35}S that has been assimilated by soil microbes and has subsequently turned over. Immobilized ^{35}S was estimated as the sum of microbial biomass ^{35}S and organic ^{35}S.

Statistical analyses

A two-way analysis of variance (ANOVA) for a randomized complete block design was used to determine the influence of site, soil horizon, and the interaction of these factors on soil S pools. For this analysis, sampling dates were treated as block effects. Differences in recoveries of adsorbed $^{35}SO_4^{2-}$-S and immobilized ^{35}S for dates, sites, and horizons were tested using a four-way ANOVA with interaction terms. Analyses of variance were performed using SAS (SAS Institute Inc. 1988) and SYSTAT (Wilkinson 1989). Treatment means were compared using a protected Fisher's LSD procedure, and significance for all analyses was accepted at $\alpha = 0.05$.

Results

Organic and inorganic sulfur pools

When averaged over sampling dates, water-extractable SO_4^{2-}-S in the A+E horizon and B horizons represented only a small portion of total S (1 and 2%, respectively; Table 2). Significant differences in water-extractable SO_4^{2-} were present among the sites. The smallest pools were measured in the

TABLE 2. Organic and inorganic S content of the upper 10 cm of surface and subsurface soil horizons

	Horizon	Content (g/m^2) Site A	Site B	Site C	Site D
Water-extractable SO_4^{2-}-S (LSD = 0.03)	A+E	0.20 (0.06)	0.15 (0.03)	0.17 (0.03)	0.19 (0.02)
	B	0.19 (0.07)	0.15 (0.01)	0.23 (0.04)	0.20 (0.01)
Adsorbed SO_4^{2-}-S (LSD = 0.04)	A+E	0.13 (0.09)	0.13 (0.10)	0.13 (0.10)	0.16 (0.15)
	B	0.31 (0.10)	0.21 (0.09)	0.39 (0.08)	0.40 (0.15)
Microbial biomass S	A+E	0.73 (0.21)	1.29 (0.26)	0.85 (0.15)	0.47 (0.32)
	B	0.48 (0.55)	1.49 (1.63)	0.15 (0.07)	0.24 (0.10)
Organic S (LSD = 6.55)	A+E	20.12 (6.51)	20.88 (9.94)	14.40 (6.23)	14.83 (1.17)
	B	15.72 (4.77)	10.87 (4.34)	9.32 (1.93)	9.82 (1.11)
Total S (LSD = 4.76)	A+E	20.81 (5.48)	22.18 (8.29)	14.18 (5.58)	15.40 (2.79)
	B	16.69 (4.40)	12.72 (3.21)	10.08 (1.96)	10.66 (1.21)

NOTE: Values for each site represent means (SD) across dates. LSD compares S content between sites and horizons.

TABLE 3. Recovery of ^{35}S in organic and inorganic S pools in the upper 10 cm of surface and subsurface horizons

	Horizon	% of total ^{35}S May 9	July 10	October 23
H_2O-extractable S	A+E	57.0 (10.1)	59.8 (12.7)	53.0 (14.9)
	B	50.3 (14.4)	49.9 (9.0)	41.2 (10.0)
Adsorbed S	A+E	6.3 (8.9)	9.1 (11.8)	11.7 (13.1)
	B	39.3 (19.7)	48.3 (13.1)	53.4 (14.0)
Immobilized S	A+E	49.2 (14.4)	49.0 (14.5)	47.4 (30.1)
	B	26.5 (11.4)	14.8 (10.3)	29.6 (20.5)
Microbial biomass S	A+E	41.8 (19.6)	45.4 (17.9)	44.1 (30.8)
	B	22.8 (13.2)	13.8 (9.7)	28.5 (21.5)
Organic S	A+E	7.4 (10.8)	3.6 (10.5)	3.3 (7.3)
	B	3.7 (7.9)	1.0 (3.2)	1.1 (2.0)

NOTE: Values for each date represent means (SD) across all four sites.

A+E and B horizons (0.15 g S/m^2) of site B, and the largest pools occurred in the B horizon of site C (0.23 g S/m^2; Table 2). Adsorbed SO_4^{2-}-S represented only a small fraction of total S in the A+E horizon (1%), but a relatively larger fraction of total S in the B horizon (3%; Table 2). Significant differences in adsorbed SO_4^{2-}-S were present among the B horizons of the four sites, but the adsorbed SO_4^{2-}-S of the A+E horizons demonstrated little variation. The average amount of microbial biomass S represented 5% of the total S in A+E horizons and ranged from 0.73 to 1.29 g S/m^2 (Table 2). In the B horizon, microbial S represented a similar proportion of total S, but values displayed a larger degree of variation (0.48 to 1.49 g S/m^2) compared with the A+E horizon. Organic S represented 97% of the total S in A+E horizons and 91% of the total found in B horizons; it was an order of magnitude greater than water-extractable, adsorbed, and microbial S pools. Both organic S and total S differed among horizons.

Recovery of ^{35}S in soil pools

Recoveries of ^{35}S in water-extractable pools were slightly higher in the A+E compared with the B horizon, but these differences were not significant (Table 3). Moreover, approximately 50% of the applied isotope either entered immobilized or adsorbed pools, suggesting the 8-d incubation was sufficient time for the isotope to move from solution into soil S pools. Recoveries of ^{35}S in adsorbed and immobilized S pools were not significantly different among sampling dates or sites (Table 4). However, the significant interaction between soil S pools and horizons revealed that the portion of ^{35}S entering the adsorbed pool was five times greater in the B horizon compared with the A+E horizons (Fig. 2 and Table 3). In contrast, immobilized S was two times greater in the A+E horizon compared with amounts recovered in the B horizon.

Discussion

Atmospheric deposition and sulfur retention mechanisms

The presence of an atmospheric S deposition gradient in the Lake States region has been well documented (Armentano and Loucks 1983; Glass and Loucks 1986; MacDonald *et al.* 1991). David *et al.* (1988) and MacDonald *et al.* (1991) found evidence to support the hypothesis that soil S levels have increased across the Great Lakes region as a result of atmospheric S deposition. In our study, quantities of adsorbed SO_4^{2-}-S tended to increase from north to south as S deposition increased. In surface and subsurface horizons, both water-extractable and adsorbed SO_4^{2-}-S increased as a percentage of total S from sites A to D. This was most evident in the B horizon, in which adsorbed SO_4^{2-}-S increased from 1.9% at site A to 3.8% at site D. These trends were consistent with previous work at the same sites, where variation in SO_4^{2-}-S pools was statistically related to a combination of soil properties and SO_4^{2-}-S deposition (MacDonald *et al.* 1991). Total S concentrations in the A+E horizon were an order of magnitude lower than those found in Spodosols of a Canadian northern hardwood forest (Autry and Fitzgerald 1990), but were similar to those found in a northern hardwood forest of the eastern United States (David *et al.* 1982; David and Mitchell 1987). However, total S concentrations in the B horizon of a northeastern United States Spodosol were more than six times higher than those found in the upper B horizon of our sites (David and Mitchell 1987).

TABLE 4. Results of analysis of variance for effects of date, site, and horizon on recoveries of ^{35}S (in adsorbed and immobilized pools)

Source	df	MSE*	Significance of F
Date	2	0.074	0.059
Site	3	0.022	0.458
Horizon	1	0.247	0.002
Pool	1	0.392	0.000
Date × site	6	0.022	0.522
Date × horizon	2	0.043	0.187
Date × pool	2	0.088	0.034
Site × horizon	3	0.005	0.904
Site × pool	3	0.049	0.132
Horizon × pool	1	5.678	0.000
Date × site × horizon	6	0.010	0.884
Date × site × pool	6	0.045	0.113
Date × horizon × pool	2	0.045	0.175
Site × horizon × pool	3	0.015	0.635
Date × site × horizon × pool	6	0.028	0.380
Error	218	0.026	

*Error mean square.

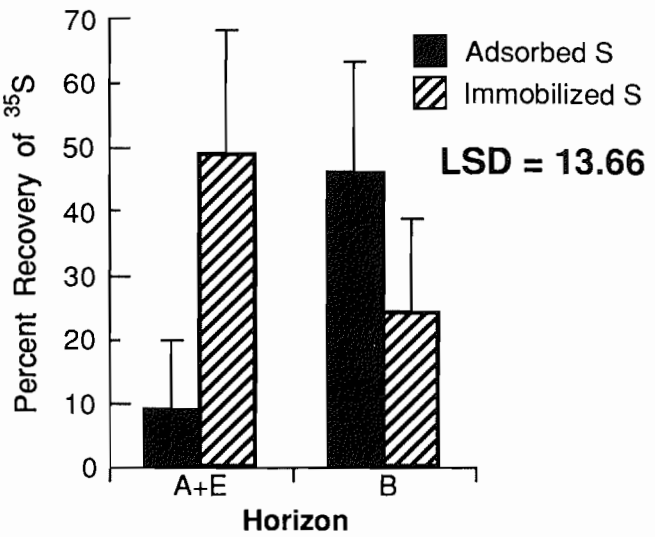

FIG. 2. Recovery of ^{35}S in adsorbed and immobilized S pools from A+E and B soil horizons. Values are mean recoveries, and each bar represents 1 SD.

Sulfate adsorption (Johnson *et al.* 1979; Johnson and Cole 1977; Johnson and Henderson 1979) and microbial immobilization (Strickland *et al.* 1986; Fitzgerald *et al.* 1983; Swank *et al.* 1984; Autry *et al.* 1990) have been proposed as important S retention mechanisms. Adsorption was an important process in the B horizon, where 47% of the applied ^{35}S was recovered. In contrast, microbial immobilization accounted for 49% of the ^{35}S recovered in the surface horizons, suggesting that biological retention was important within these horizons. However, it is important to place our results in the context of long-term ecosystem-level S dynamics (MacDonald *et al.* 1991, 1992; Pregitzer *et al.* 1992). Our results, in combination with information on leaf chemistry and ion leaching, suggest that the SO_4^{2-}-S retention processes we studied function as limited or temporary S sinks. This appears to be especially true at sites C and D, where annual leaching losses of SO_4^{2-}-S equalled or exceeded atmospheric inputs (MacDonald *et al.* 1992). Similar conclusions were reached regarding northern hardwood forests in the eastern United States by David and Mitchell (1987) and Schindler and Mitchell (1987), who suggested that both biochemical incorporation and abiotic adsorption had only limited ability to incorporate large quantities of S for extended periods of time.

Sulfate retention: adsorption versus microbial immobilization

The relative importance of adsorption and microbial immobilization as S retention mechanisms has been frequently discussed (Schindler *et al.* 1986; Stanko and Fitzgerald 1990). The potential for adsorption was greater than the potential for the microbial immobilization of SO_4^{2-} in hardwood and conifer forests of the southeastern United States (Fitzgerald *et al.* 1988; Watwood *et al.* 1988). However, Autry *et al.* (1990) suggested that microbial immobilization may represent the primary mechanism for S retention in these forest soils.

The soils we studied were Spodosols, which in the Great Lakes region have low to moderate potential to adsorb additional SO_4^{2-}-S in upper B horizons (MacDonald and Hart 1990; MacDonald *et al.* 1991). Spodosols in the northeastern United States may have higher capacities to adsorb SO_4^{2-}-S, which are related to their higher Al and Fe concentrations (Johnson and Todd 1983; Fuller *et al.* 1985). In our study, adsorption was the dominant ^{35}S-retention mechanism in the upper B horizon, but microbial immobilization was also important and accounted for 25% of the total ^{35}S recovered in the B horizon. In the A+E horizon, however, microbial immobilization was the dominant S retention mechanism, sequestering $^{35}SO_4^{2-}$ in quantities equivalent to those adsorbed in the upper B horizon. Total B horizon thicknesses were much greater than combined A+E horizon thicknesses (Table 1), so that S-retention mechanisms operating in B horizons may be proportionally greater in magnitude. Sulfate adsorption appears to be an immediate retention mechanism because of the rapid attainment of anion exchange equilibria (Schindler *et al.* 1986), whereas microbial immobilization is governed by physiological processes and should operate over a relatively longer period of time (Autry *et al.* 1990). For example, Strickland *et al.* (1986) found adsorption to be an immediate sink for $^{35}SO_4^{2-}$; however, much of the adsorbed isotope eventually entered organic pools via microbial activity.

Microbial dynamics

Organic matter inputs from above- and below-ground plant litter production are the primary determinants of substrate availability within terrestrial ecosystems (McGill and Cole 1981). Carbon is the driving mechanism for rates of heterotrophic metabolism and the subsequent cycling of plant nutrients within terrestrial ecosystems (Gray and Williams 1971; Van Veen *et al.* 1985). In addition, temperature and moisture availability directly regulate the rate of biochemical reactions and hence S transformations within the soil. The most rapid rates of microbial S immobilization were expected to occur during early spring and fall, when C and moisture availability are high and soil temperatures do not greatly restrict microbial metabolism. In a related study, Strickland *et al.* (1987) found that greater quantities of $^{35}SO_4^{2-}$-S were immobilized during spring as compared with other portions of the year.

In contrast, we found that microbial immobilization did not display a high degree of seasonal variation. Possibly, an 8-d

laboratory incubation was not long enough to reflect the influence that substrate availability, moisture availability, and soil temperature may have on microbial S transformation. Altering soil incubation time from 2 to 7 d can increase immobilization in some soils (Strickland *et al.* 1986); it follows then that altering incubation time from 8 d to a longer period might result in a similar immobilization increase. Alternatively, our sampling dates may not have adequately spanned the seasonal range of soil temperature and moisture availability. Finally, while no statistical differences existed, biological differences may have been masked by the variability surrounding pool means for each sampling date. Immobilization of $^{35}SO_4^{2-}$-S was relatively high in May compared with the July and October sampling dates (Table 3), possibly reflecting seasonal patterns of plant litter addition to the soil. Carbon availability should be high in early spring because of the presence of decomposing leaf litter from the previous autumn. Microbial immobilization may differ seasonally because of the influence of plant litter additions, soil temperature, and soil moisture availability, but we found only small temporal changes in microbial activity and S transformations.

For most of the year, soil microorganisms remain relatively inactive because of substrate limitation (Babiuk and Paul 1970). Moreover, microbial biomass has been estimated to comprise only 1 to 3% of the soil organic S (Saggar *et al.* 1981; Strick and Nakas 1984; Chapman 1987; Paul and Clark 1989). Although a small percentage of the total S is contained in microbial cells at any given time, this small amount cycles rapidly and could become significant over time. For example, 3 to 8% of total S can be found in a rapidly cycling S pool that contributes significantly to soil S dynamics (Smith and Paul 1990). Furthermore, microbial biomass S probably turns over on an annual basis because microbial cells are composed of C, N, and S in a relatively narrow stoichiometric ratio, and microbial C and N contents are thought to turn over annually (Babiuk and Paul 1970; Gray and Williams 1971). Swank *et al.* (1984) suggested that A and Bw horizons each provided a potential S flux of 11 $kg \cdot ha^{-1} \cdot year^{-1}$ in southern hardwood forests. The dynamics of this labile pool in the northern forests we studied requires further investigation.

SO_4^{2-} deposition: long-term versus short-term sinks

There has been much debate over whether S retention in forest soils is primarily a function of adsorption or immobilization. Our data suggest that both SO_4^{2-} adsorption and microbial S immobilization are important as limited or temporary S sinks. It is difficult to make long-term statements based upon short-term process-level work. However, our work in combination with other work done on these same sites enables us to look at S cycling in these systems on a broad scale. In the forest systems we studied, SO_4^{2-} assimilation by the overstory represents only a temporary S retention mechanism. Trees on these sites assimilated large quantities of SO_4^{2-}-S, but the S cycled back to the soil each autumn (Pregitzer *et al.* 1992). Soil adsorption also appears to be only a limited, short-term sink for SO_4^{2-}. Our data indicate that adsorbed S pools (Table 2) equal only a small fraction of the total S deposition at these sites (assuming rates from 3 to 12 kg SO_4^{2-}-$S \cdot ha^{-1} \cdot year^{-1}$). Furthermore, MacDonald *et al.* (1991) found that total adsorbed S to a depth of 75 cm was generally less than 20 $kg \cdot ha^{-1}$ for these sites. This value corresponds to approximately 4% of the total SO_4^{2-}-S deposited over a 40-year period, given a rate of 12 $kg \cdot ha^{-1} \cdot year^{-1}$.

Microbial immobilization also appears to be only a temporary S sink at current S deposition levels in these forests. Our data indicate that microbial S pools (Table 2) equal only a small fraction of the total S deposition at these sites. Because microbial biomass turns over on an annual time frame, it is unlikely that microbial S immobilization would be a long-term sink; mineralized S released would approximate the quantities of SO_4^{2-}-S assimilated into microbial cells. Total organic S to a depth of 75 cm (summed for A, E, and B horizons) at these sites ranged from 390 to 640 kg S/ha (MacDonald *et al.* 1991). Assuming 10 000 – 12 000 years have elapsed since deglaciation (Flint 1971), the net annual rate of organic S accumulation in the upper solum of these soils ranges from 0.03 to 0.06 kg $S \cdot ha^{-1} \cdot year^{-1}$. Soils undoubtedly can and will accumulate additional organic S over pedogenic time. However, the rate at which organic S accumulation takes place appears insignificant when compared with total annual wet plus dry atmospheric deposition of 5 to 10 kg $S \cdot ha^{-1} \cdot year^{-1}$. Thus, over the long term and especially in the face of elevated atmospheric SO_4^{2-}-S input, neither microbial immobilization nor adsorption appears to have the ability to prevent SO_4^{2-}-S leaching in these northern hardwood forest ecosystems.

In summary, although some forests accumulate S from acidic precipitation (Johnson *et al.* 1982), others, such as these, experience a net output of S (Schindler and Mitchell 1987). In our sites, SO_4^{2-}-S losses approximately equalled or exceeded rates of atmospheric input at sites with moderately elevated SO_4^{2-}-S deposition (MacDonald *et al.* 1992), suggesting that under current deposition rates the immediate capacity of these forest ecosystems to sequester significant quantities of S has been saturated. Because of the relatively large sustained SO_4^{2-}-S inputs these northern hardwood forests receive, overstory uptake, adsorption, and microbial immobilization no longer seem to function as a net sink for SO_4^{2-}-S.

Conclusions

Most studies of SO_4^{2-}-S adsorption and microbial immobilization have been conducted on a point-specific basis. Our study differed in that we investigated seasonal variation in these processes in spring, summer, and autumn, as well as on a regional, ecosystem basis. Our results suggest that adsorption and immobilization of ^{35}S did not exhibit significant variation among sampling dates. Moreover, the quantity of ^{35}S immobilized in the surface soil horizons was equivalent to the amount of S adsorbed in an equal thickness of B horizon, and this pattern was consistent throughout the growing season. In the B horizon, microbial immobilization made additional contributions to total SO_4^{2-}-S retention, even though adsorption was the predominant S retention mechanism in that horizon. At sites with moderately elevated deposition, ecosystem SO_4^{2-}-S outputs equalled or exceeded atmospheric additions, suggesting that neither adsorption nor microbial immobilization represents a significant sink for SO_4^{2-}-S in these northern hardwood ecosystems.

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