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MINERALIZATION OF METHIONINE SULPHUR IN SOILS AND FOREST FLOOR LAYERS

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Summary—A1-horizon soils and 01, 02 forest floor layers from a mixed mature hardwood forest rapidly converted methionine-S to readily-available (salt-extractable) and less readily-available (acid- and base-extractable) inorganic sulphate (SO_4^{2-}). It is suggested that this latter conversion represents the incorporation into organic matter of a portion of the SO_4^{2-} released by mineralization. On a dry weight basis, the 02 layer of the forest floor was the most active with respect to both conversions. Moreover, capacities for mineralization and SO_4^{2-} incorporation decreased with increasing sample depth within the mineral horizon. Both conversions were dependent upon temperature and duration of incubation and were absent from samples which had been autoclaved. Sodium azide and the broad-spectrum antibiotic, tetracycline also inhibited each conversion to varying extents depending upon the type of sample incubated with methionine.

INTRODUCTION

Collectively, data obtained with agricultural systems suggest that methionine may represent a poor source of plant-available S. This amino acid is converted primarily to volatile end-products by agricultural soils and soil microorganisms and conversion of methionine-S to SO_4^{2-} is generally negligible (Kallio and Larson, 1955; Frederick *et al.*, 1957; Ruiz-Herrera and Starkey, 1969; Segal and Starkey, 1969; Banwart and Bremner, 1975, 1976). The absence of mineralization capacity noted in these studies is in direct contrast to results obtained using cysteine (Starkey, 1950, 1956; Freney, 1958, 1960). Working with forest soils, Hesse (1957) observed quantitative conversion of methionine-S to SO_4^{2-}. Moreover, the lack of methionine volatilization apparent in this work is supported by determinations for volatile-S carried out by Farwell *et al.* (1979) with other forest soils. These data suggest that forest systems could differ markedly from agricultural systems in the metabolism of this amino acid.

Because the total organic-S content of leaves is comprised of sulpholipid and protein (Anderson, 1975; Harwood and Nicholls, 1979), methionine could represent an important source of SO_4^{2-} for the S′-cycle in hardwood forests especially during periods of deciduous senescence and subsequent proteolysis. We have assessed this possibility by incubating ^{35}S-labelled methionine with forest floor and mineral horizons from a hardwood and pine forest. The use of a ^{35}S label has enabled the determination of both the quantity of SO_4^{2-} generated from methionine as well as the amount of the released anion which is incorporated into organic matter.

MATERIALS AND METHODS

Sample collection

The 01 and 02 components of the litter layer and soils from various genetic horizons were collected during August of 1982 from a mixed-mature hardwood forest located at the Coweeta Hydrologic Laboratory, near Franklin, NC. This watershed (WS 18) has been described in detail by Swank and Douglass (1977). After removal of root material by hand, the samples, contained in sealed bags to prevent moisture loss were maintained below 16°C until assayed. Assays were carried on samples which had not been ground, sieved or dried. In order to gain a watershed perspective on methionine mineralization and subsequent SO_4^{2-} incorporation, three samples from the 01, 02 and 0–5 cm A1 horizon soil (about 20 g wet weight) were taken at random from each of 10 equally spaced plots along a ridge to stream to ridge transect of the watershed (Swank *et al.*, 1984). Samples from each plot were mixed in equal proportions and assayed separately. Mean values for all 10 assays are reported. Samples from plot 5, the mid-point on the transect, were assayed to determine the influence of temperature, sodium azide (2 mmol), tetracycline (0.1 mmol) and autoclaving. To eliminate mixing, samples from this plot were taken horizontally immediately after a pit had been dug so that the influence of sample depth could also be determined. Samples (0–5 cm) from the A1 horizon only were taken from an adjacent watershed (WS 17). This watershed is a 27-yr-old white pine (*Pinus strobus* L.) monoculture. Both watersheds are comprised of soils in the sandy loam Ashe series of the Typic Dystrochrepts classification.

Conversion of methionine-S to SO_4^{2-}

Samples (0.5 g, 01, 02 and 1.0 g soil, wet weight) were incubated with 200 μl of an aqueous solution containing 7.5 nmol of L-methionine (mixture of unlabelled and ^{35}S-labelled amino acid; 4.4×10^{13} Bq mmol^{-1}) for periods and temperatures indicated in the text. To ensure even distribution of the label, 200 μl water was added to each sample after addition of methionine. After incubation, samples were extracted successively with 2 ml vols 1 M Na_2SO_4 in a saturated solution of L-methionine (2.0 mmol, Na_2SO_4) and twice with saturated L-methionine alone (1.8 mmol unlabelled amino acid, total). The samples were then washed with three 200 μl vols each of 1 M NaH_2PO_4, 1 M LiCl and water. The experimental design for extraction and washing of samples was identical to that employed by Houghton and Rose (1976). All fractions were combined to yield a salt extract which was maintained at -20°C until analyzed by electrophoresis.

Incorporation into organic matter of methionine-derived SO_4^{2-}

Salt extraction as described above quantitatively recovered both soluble and adsorbed $^{35}SO_4^{2-}$. ^{35}S remaining in the samples was released by hydrolysis of the residue in 400 μl 6 N HCl for 12 h at 121°C followed by successive extraction with three 200 μl vols water and two 400 μl vols 2 N NaOH. The residue was washed with water as above and the respective washes were combined to yield an acid and a base extract which were maintained at -20°C until analyzed by electrophoresis. ^{35}S which is recovered under these conditions represents that which has been incorporated into organic matter (Strickland and Fitzgerald, 1984). Further treatment of the samples (including reversal of the acid–base sequence) did not increase the amount of ^{35}S released. Routinely, between 85 and 95% of the added ^{35}S was recovered by this salt, acid and base extraction sequence. Since no correlation between total recovery and incubation time was apparent for any determination reported here, it is likely that failure to quantitatively recover added ^{35}S represents a limitation in the procedure rather than a loss of volatile S.

Determination of SO_4^{2-} in salt, acid and base extracts

This anion was separated from methionine and other ^{35}S-labelled metabolites of methionine by electrophoresis of the extracts (5–20 μl) on Whatman No. 1 paper at 250 V for 2 h in a 0.1 M barium acetate–acetic acid buffer, pH 4.5. Under these conditions, SO_4^{2-} characteristically remains at the origin as $BaSO_4$. Each extract was also subjected to electrophoresis as above but in a 0.1 M sodium acetate–acetic acid buffer, pH 4.5. In this buffer, SO_4^{2-} moves away from the origin so that it was possible to determine if other ^{35}S-components in the extracts also remained at the origin. This was not the case for the salt or acid extracts and thus SO_4^{2-} in these extracts was determined from barium acetate electrophoretograms in which a much better defined peak of radioactivity occurs at the origin. Inorganic SO_4^{2-} present in base extracts was determined from sodium acetate electrophoretograms. Radioactive components were located on strips by scanning at low speed in a Packard Radiochromatogram Scanner. Areas of the electrophoretogram corresponding to each peak on the chart paper were cut out and assayed for radioactivity in 10 ml of ScintiVerse. Radioactivity associated with SO_4^{2-} was expressed as a percentage of the total radioactivity of the extract. Typical scans for a salt extract after electrophoresis in each buffer are shown in Fig. 1.

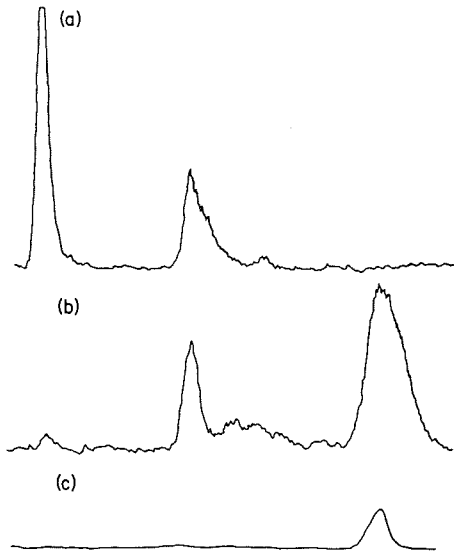

Fig. 1. Electrophoretogram scan of salt extract of an A1-horizon soil following incubation with ^{35}S-methionine: (a) after electrophoresis in barium acetate; (b) after electrophoresis in sodium acetate; (c) electrophoretogram scan of authentic $^{35}SO_4^{2-}$ after electrophoresis in sodium acetate.

Determinations for SO_4^{2-} derived from mineralization (salt-extractable) and for SO_4^{2-} incorporated into organic matter (acid- and base-extractable) were carried out in triplicate. The standard error of the mean was $< \pm 4\%$ in all assays.

RESULTS

Mineralization of methionine-S

Methionine-S was rapidly converted to SO_4^{2-} without a lag period in A1-horizon soil from both the hardwood forest and the white pine plantation (Fig. 2a and b, respectively). Roughly 35% of the total ^{35}S recovered after incubation (28°C for 24 h) was present as salt-extractable SO_4^{2-} in soil from either ecosystem. Little distinction could be made between SO_4^{2-} levels generated between 20° and 28°C, but time-courses for incubation at 5°C (Fig. 2) clearly demonstrate that the process is temperature-dependent. Similar observations were made utilizing 01 and 02 layers from the hardwood forest (Fig. 3; data for 01 layer not shown). Although the 02 component of the forest floor was about 3-fold more active than the underlying soil on a dry weight basis, SO_4^{2-} levels in the salt extract from the former samples represented only about 19% of the total recoverable ^{35}S after incubation for 24 h at 28°C. Owing to the much higher moisture content and lower bulk densities of both the 01 and 02 forest floor

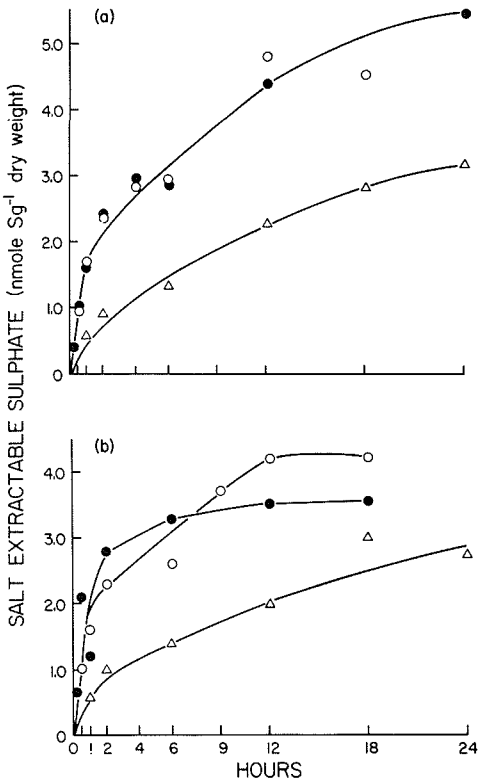

Fig. 2. Influence of temperature on the formation of salt-extractable SO_4^{2-} in A1 horizon soil during incubation with methionine: (a) hardwood forest WS 18; (b) pine plantation WS 17. Samples were incubated at 28°C (●), 20°C (○) and 5°C (△).

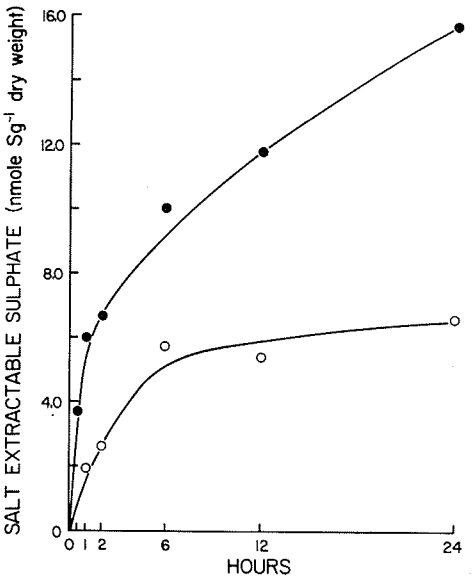

Fig. 3. Influence of temperature on the formation of salt-extractable SO_4^{2-} in the 02 layer from WS 18 during incubation with methionine at 28°C (●) and 5°C (○).

layers relative to the underlying soil, it would appear that the latter is more important for methionine mineralization in hardwood forests.

Incorporation of SO_4^{2-} into organic matter

After salt extraction to release soluble and adsorbed SO_4^{2-}, ^{35}S remaining in the samples was recovered by acid and base extraction. Strickland and Fitzgerald (1984) demonstrated that ^{35}S from organic matter is released under these latter conditions. Electrophoresis of the acid extracts indicated that SO_4^{2-} was a major component suggesting that much of the SO_4^{2-} generated from mineralization was incorporated into organic matter via the formation of covalent ester linkages. Unlike carbon-bonded S, these linkages undergo hydrolysis in acid to release SO_4^{2-} (Fitzgerald *et al.*, 1982). Figure 4 shows that SO_4^{2-} was rapidly incorporated without a detectable lag in A1 horizon samples from both watersheds as well as in the 01 and 02 components of the hardwood forest. As expected for the formation of a covalent linkage, this process was temperature-dependent and incubation at 5°C resulted in roughly a 50% reduction in SO_4^{2-} incorporation in most samples (Fig. 4). On a wet weight basis, the amount of SO_4^{2-} which was incorporated into organic matter corresponded to about 9, 4 and 2.5% of the total ^{35}S recovered from the A1, 02 and 01 samples, respectively.

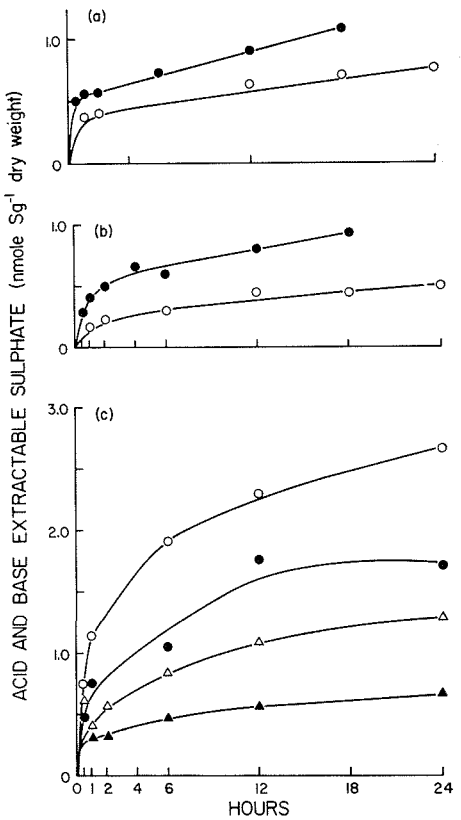

Fig. 4. Influence of temperature on the formation of acid- and base-extractable SO_4^{2-} in soil and forest floor layers during incubation with methionine: (a) A1 horizon WS 18; (b) A1 horizon WS 17; samples incubated at 28°C (●) and 5°C (○); (c) 01 layer WS 18 (●, ▲) and 02 layer WS 18 (○, △) incubated at 28°C (○, ●) and 5°C (△, ▲).

Table 1. Mineralization of sulphur and incorporation of sulphate into organic matter of samples from Watershed 18 incubated with methionine for 12 h at 28°C[a]

Sample	Mean sulphate (nmol g^{-1} dry wt) and SE of the mean	
	Salt-extractable	Acid- and base-extractable
01 Forest floor	1.29 ± 0.32	0.27 ± 0.03
02 Forest floor	10.10 ± 1.92	1.79 ± 0.32
A1-horizon soil	4.06 ± 0.22	0.92 ± 0.04

[a]Samples of each horizon were taken in triplicate from each of 10 permanent plots, mixed in equal proportions, and assayed. The mean of determinations for all 10 plots is reported ($n = 10$).

Influence of spatial variation, sample depth and inhibitors

In order to determine a watershed potential for methionine mineralization and subsequent SO_4^{2-} incorporation, samples were taken from 10 permanent plots established on the hardwood catchment. In all cases, the 02 component of the forest floor exhibited the highest activity for mineralization as well as SO_4^{2-} incorporation. The A1 horizon soil was about 4-fold more active than the 01 forest floor component (Table 1, dry weight comparison only). As expected from their heterogenous nature, activities of the forest floor layers exhibited the greatest degree of variability with respect to both processes; whereas, activities characteristic of the soil remained remarkably constant over the entire transect (Table 1).

The ability of soil to mineralize methionine and incorporate SO_4^{2-} decreased with sample depth at incubation temperatures of 5° or 28°C (Table 2). It is of interest that samples taken to a depth of 40 cm still retained more than 75% of the activity exhibited by the A1 horizon, but activity decreased substantially in samples taken at greater depths. Indeed, samples taken between 120–180 cm were almost inactive at both incubation temperatures (94 and 91% reduction at 28°C in salt and acid–base extracts, respectively; Table 2). Sterilization of A1 horizon and forest floor samples by autoclaving resulted in similar decreases in both activities (Table 3). Apart from activity associated with methionine mineralization by the 01 layer in which a decrease of only 69% was observed, both activities were abolished in other samples by treatment with sodium azide. Incubation in the presence of tetracycline also inhibited both processes to extents which were dependent upon the type of sample analyzed. Tetracycline-sensitive bacteria appear to be primarily responsible for methionine mineralization in the 02 and A1 horizons since activities in these samples were inhibited by 79 and 90%, respectively. In contrast, SO_4^{2-} incorporation by only the A1 horizon samples was subject to significant (72%) inhibition by this antibiotic. There is evidence (Strickland and Fitzgerald, 1984) that fungi which are sensitive to the antibiotic, candicidin are primarily responsible for mediating SO_4^{2-} incorporation in the 01 and 02 forest floor components.

Table 2. Influence of soil depth on sulphur mineralization and sulphate incorporation in samples from WS 18 incubated with methionine for 12 h at 28°C or 5°C

Depth (cm)	Horizon	Sulphate (nmol g^{-1} dry wt)			
		Salt-extractable		Acid- and base-extractable	
		5°C	28°C	5°C	28°C
0–5	A1	3.69	5.75	0.67	1.13
8–15	Ap	3.41	5.13	1.00	0.80
15–40	B	1.59	4.62	0.38	0.87
40–100	B + C	0.44	2.28	0.12	0.48
120–180	C	0.15	0.32	0.04	0.10

Table 3. Influence of autoclaving and inhibitors on mineralization and sulphate incorporation in samples from WS 18 incubated with methionine for 48 h at 28°C

Treatment	Sample	Sulphate (nmol g^{-1} dry wt)	
		Salt-extractable	Acid- and base-extractable
None	01	1.84	1.35
	02	13.22	2.15
	A1	4.37	1.27
Autoclaving	01	0.28	0.11
	02	0.19	0.12
	A1	0.06	0.07
Sodium azide	01	0.57	0.06
	02	0.45	0.13
	A1	0.19	0.07
Tetracycline	01	0.72	1.27
	02	2.77	1.52
	A1	0.45	0.35

DISCUSSION

The plant sulpholipid and protein-S comprise the majority of the S complement of leaves (Harwood and Nicholls, 1979). Although the S-containing amino acid concentration in soil organic matter is substantial (Freney *et al.*, 1972), the lipid and these amino acids generally occur in trace amounts in soil and soil solution, respectively (Chae and Tabatabai, 1981; Paul and Schmidt, 1961; Fitzgerald *et al.*, 1982). These constituents of leaf-S may thus be rapidly metabolized after deciduous senescence.

As with the study of Strickland and Fitzgerald (1983) of the ability of forest soil to mineralize S in the sulpholipid, the use of a ^{35}S label has enabled us to determine the readily-available (salt-extractable) and less readily-available (acid- and base-extractable) amounts of SO_4^{2-} released during mineralization of methionine. Results of both studies indicate that conversion to SO_4^{2-} was almost complete after 24 h and that a portion of the SO_4^{2-} derived from mineralization was recovered only by acid and base extraction. Studies with $^{35}SO_4^{2-}$ suggest that S from organic-S is released under these conditions of extraction (Fitzgerald *et al.*, 1982, 1983). Thus, some of the SO_4^{2-} resulting from mineralization was incorporated into organic matter. Although this S is not readily available on the basis of extraction properties, results of studies with A1-horizon soils from the same hardwood forest suggest that recently-formed organic-S can serve as a source of SO_4^{2-} (Strickland *et al.*, 1984). Moreover, organic-S formed from SO_4^{2-} has been isolated from the 02 layer of this forest under conditions which minimize loss of S-linkage groups. Incubation of this fraction with parent material or with the underlying A1-horizon results in the release of SO_4^{2-} by a process which appears to be regulated by energy rather than SO_4^{2-} availability (Strickland and Fitzgerald, 1984). The re-utilization of S from organic matter does not depend upon the activity of soil microflora *per se* but appears to be due to the activity of pre-formed, extracellular depolymerase and sulphohydrolase enzymes (Dodgson *et al.*, 1982). Once conversion of methionine-S to SO_4^{2-} has occurred, it is likely that this S will be conserved for plant uptake since inorganic forms of S do not tend to be converted to volatile S (Bremner and Steele, 1978).

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