Availability of carbon-bonded sulfur for mineralization in forest soils[1]

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The capacities of soil from hardwood, clear-cut, and pine forests of the Coweeta basin to mineralize, adsorb, and incorporate into organic matter carbon-bonded sulfur in the form of L-methionine was investigated. These soils adsorbed and incorporated between 40 and 66% of this amino acid within a 0.5-h incubation period, but much of the immobilized sulfur was mineralized after 48 h incubation. An additional hardwood forest (watershed 18) was chosen for further study of the incorporation process in both litter and mineral horizons. The O2 forest floor layer exhibited the highest levels of activity in samples taken along a transect of this watershed. Incorporation of methionine into the organic matter of these samples was complete within about 12 h of incubation and was inhibited by pretreatment of the samples with sodium azide; a general inhibitor of cell respiration. The capacities for methionine incorporation determined *in vitro* complement observations of the high levels of carbon bonded sulfur found *in situ* in forest litter and soil.

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Les auteurs ont étudié les capacités de minéralisation, d'adsorption et d'incorporation à la matière organique du soufre organique sous forme de L-méthionine dans le sol de forêts feuillues, de forêts de pin et d'aires de coupe du bassin Coweeta. Ces sols ont adsorbé et incorporé de 40 à 66% de cet acide aminé en moins de 0,5 h d'incubation, mais une partie importante du soufre immobilisé était minéralisé après 48 h d'incubation. Une forêt feuillue additionnelle (bassin 18) fut choisie pour une étude complémentaire portant sur les processus d'incorporation du soufre dans la couverture morte et les horizons minéraux. Parmi les échantillons prélevés le long d'un transect dans ce bassin, c'est l'horizon O2 de la couverture morte qui a montré les plus hauts niveaux d'activité. L'incorporation de la méthionine dans la matière organique de ces échantillons était complète en moins de 12 h d'incubation et elle était inhibée par prétraitement des échantillons à l'azide de sodium, un inhibiteur général de la respiration cellulaire. La capacité d'incorporation de la méthionine mesurée *in vitro* complémente les observations relatives aux niveaux élevés de soufre organique trouvés *in situ* dans la couverture morte et le sol forestier.
[Traduit par le journal]

Introduction

In addition to sulfate inputs from precipitation and throughfall (Johnson et al. 1980), mineralization of sulfur-containing organics released during decomposition may represent an equally important source of this anion for hardwood forests, especially during deciduous senescence. Carbon-bonded sulfur represents a major component of hardwood foliage (Mitchell et al. 1984) and consists mainly of sulfolipid and protein comprised of the sulfur-containing amino acids, cysteine, and methionine (Harwood and Nicholls 1979). Inputs of these components to forest soils during leaf protein decomposition are referred to here as arising from exogenous sources, to distinguish these forms of organic sulfur from those formed endogenously in soil from sulfate (Fitzgerald et al. 1982; Fitzgerald et al. 1983).

Preliminary work (Strickland and Fitzgerald 1983) demonstrated that A-horizon forest soil rapidly mineralized sulfur added as sulfolipid. Effort and the expense associated with the preparation of the sulfolipid bearing a ^{35}S label precludes a detailed study of the metabolism of this substance. However, methionine is an alternative substance for determining the mineralization potential of leaf protein decomposition products because of its commercial availability and relative stability to degradation by irradiation. This latter property enables the use of a ^{35}S label to determine the true biological fate of this amino acid. Assays of forest floor and soil from an undisturbed hardwood forest in the Coweeta basin of western North Carolina indicate that ^{35}S-labelled methionine was also mineralized to completion within a 24-h period (Fitzgerald and Andrew 1984). However, unlike results obtained with the sulfolipid and soil from watersheds in the same region (Strickland and Fitzgerald 1983), only about 35% of the added sulfur was mineralized. These results suggest that factors other than existing microbial flora may determine the extent to which methionine sulfur is mineralized in forest ecosystems.

In addition to further work on the capacity of soil from managed forests of the Coweeta basin to mineralize methionine sulfur, we also report aspects of the metabolism of methionine which govern the availability of this amino acid for mineralization. These metabolic fates include adsorption and incorporation of the amino acid into the organic matter of litter and mineral horizons.

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Materials and methods

Site description and sampling

Five experimental watersheds located at the Coweeta Hydrologic Laboratory, near Franklin, NC, were sampled. These catchments consist of two undisturbed, mixed mature hardwood forests (WS 2 and 18), a 27-year-old white pine (*Pinus strobus* L.) plantation (WS 17), and two clear-cut hardwood forests (WS 7 and 48). The latter differed in the age of regrowth following cutting (1 year, WS 48; 3 years, WS 7 as of sampling date). Watersheds 17 and 18 are comprised of soil in the sandy loam Ashe series, whereas soil from WS 2 and WS 48 are in the sandy loam Chandler series. These soils are members of the Typic Dystrochrepts. Soil from WS 7 is in the fine-loamy Tusquitee series and belongs to the Humic Hapludult classification. Additional descriptions of the Coweeta basin, WS 17, WS 18 (Johnson and Swank 1973) and of the clear-cut forests (Swank and Douglass 1977) are available.

One random soil sample (15 g wet weight, 5 cm in depth) was taken in September 1981 from the A1 horizon of all watersheds except WS 18. Litter and soil were collected during August 1982 along a ridge to stream to ridge transect of WS 18 (Swank et al. 1984). In this instance, three random samples from the O1, O2, and A1 horizon (all 20 g wet weight) were taken from each of 10 permanent plots. Each plot is characterized by contrasting vegetation. For example, the upper slope position (plot 2) has a mixed chestnut – red oak overstory with a laurel, dogwood, azalae understory, whereas plot 9 (cove–stream edge position) possesses a thick Rhododendron understory with sparse overstory vegetation. The litter layers along the transect during August extend to depths of about 3 and 2 cm, respectively, whereas the A1 horizon extends to a depth of about 5 cm. Samples of the O1, O2, and A1 horizons from each plot were mixed in equal proportions and assayed separately. Mean values for all 10 assays made on each component are reported. To determine the influence of depth within the mineral horizon, plots 2, 9 (upper slope and cove positions, respectively), and 5 (midpoint on the transect) were sampled in September and August 1982, respectively. For plots 2 and 9, samples were taken vertically with an auger, whereas samples from plot 5 were taken horizontally from a pit. All samples were kept in sealed bags at about 15°C, and root material was removed by hand prior to analysis. Assays were made on fresh, field moist samples without prior grinding, sieving, or drying.

Laboratory incubations and sample extraction

Unless otherwise indicated, samples (0.5 g O1, O2, and 1.0 g soil, wet weight) were incubated with 7.5 nmol ^{35}S-labelled methionine (4.4×10^{13} Bq mmol^{-1}) for 12 h at 28°C. To ensure even distribution of the label, 200 μL water was added to each sample after addition of methionine. After incubation, samples were extracted twice with 2-mL volumes of water, 1 *M* Na_2SO_4 in a saturated solution of methionine, and saturated methionine alone. The samples were then washed three times each with 200-μL volumes of 1 *M* NaH_2PO_4, LiCl, and water. The remaining ^{35}S was recovered by hydrolysis of the samples in 400 μL 6 *N* HCl for 12 h at 121°C, followed by successive extraction three times each with 400 μL water, 2 *N* NaOH, and water. Unless otherwise indicated, salt–methionine and associated water washes were combined and analyzed as such. After addition of the respective water washes, acid and base extracts were analyzed separately. Except in work designed to determine adsorbed levels of added methionine, the initial water wash was deleted from this procedure so that the metabolism of this amino acid could be terminated more rapidly after incubation. The experimental design for sample extraction was identical to that utilized by Houghton and Rose (1976).

Efficacy of extraction procedure

Preliminary work confirmed the following: (*i*) that the initial water wash yielded complete recovery of soluble ^{35}S; (*ii*) that the Na_2SO_4–methionine extraction (2 mmol SO_4^{2-} – 1.8 mmol amino acid) prevented further incorporation of ^{35}S-labelled sulfate or ^{35}S-labelled methionine by isotope dilution; and (*iii*) that this step together with the PO_4^{3-}, LiCl extraction (0.6 mmol PO_4^{3-}) completely recovered adsorbed ^{35}S-labelled sulfate and ^{35}S-labelled methionine. Moreover, increased extraction with acid or base failed to recover additional ^{35}S. Generally between 85 and 95% of the added ^{35}S was recovered by the methionine–salt, acid and base extraction procedure.

TABLE 1. Incorporation of methionine and its oxidation product into organic matter of surface horizons of watershed 18

Horizon	Mean acid–base extractable and SE of the mean[a] (nmol S·g dry weight^{-1})	
	Methionine	Oxidation product
O1	11.14±1.24	0.57±0.09
O2	20.23±1.96	1.12±0.11
A1	1.84±0.15	0.45±0.05

[a] $n = 10$.

Analysis of extracts for ^{35}S-labelled sulfate, methionine, and methionine oxidation products

Except for sulfate present in base extracts, these components were separated by electrophoresis of extracts (5–20 μL) on Whatman No. 1 paper at 250 V for 2 h in 0.1 *M* barium acetate – acetic acid buffer, pH 4.5. To separate sulfate, base extracts were subjected to electrophoresis as above but in a 0.1 *M* sodium acetate – acetic acid buffer, pH 4.5. After separation, radioactive components were located on dried paper strips by scanning at slow speed in a Packard Radiochromatogram Scanner (see Fitzgerald and Andrew (1984) for typical scans). Areas on the paper strip corresponding to each peak on the chart paper were cut out and quantitated in 10 mL of scintillation fluid (ScintiVerse, Beckman LS 9000 scintillation counter). Radioactivity associated with each component was expressed as a percentage of the total radioactivity of the extract. To enable comparison of samples of differing dry weight, the amount of each component (nanomole) was calculated from the amount of ^{35}S added, and these were standardized to a value for a 1 g dry weight. Determined dry weights for litter and soil were <0.2 and <0.8 g, respectively. The standard error of the mean for each determination was <±4% with $n = 3$.

Results

Metabolism of methionine in litter and soil from watershed 18

Electrophoresis of extracts revealed the presence of three major radioactive components resulting from the incubation of methionine with litter or soil samples. These were identified as sulfate (Fitzgerald and Andrew 1984), nonmetabolized methionine and as a component with electrophoretic mobility identical to the performic acid oxidation product of methionine (Moore 1963). All components were detected in salt as well as in acid–base extracts of the samples after incubation. Table 1 shows the levels of ^{35}S-labelled methionine and its oxidation product recovered from acid–base extracts of litter and soil from WS 18. The mineralization of methionine sulfur by these samples was reported previously (Fitzgerald and Andrew 1984). In view of treatment of the samples with unlabelled methionine prior to acid extraction, it is unlikely that these ^{35}S-labelled components were simply adsorbed to organic matter extracted with acid and base. These latter treatments hydrolyze peptide linkages, and it is more likely that methionine was initially incorporated into organic matter via peptide bond formation during incubation and then released by hydrolysis. The observation that prior treatment of samples with sodium azide resulted in a >95% decrease in acid–base extractable methionine supports this interpretation. A similar reduction in the level of the oxidation product was observed in azide treated samples, but because this oxidation can occur abiotically (Freney et al. 1972), it is not known if some of the methionine was oxidized and then incorporated into organic

TABLE 2. Influence of sample depth on methionine S mineralization and on methionine incorporation into organic matter in litter and mineral horizons from watershed 18

Plot	Horizon and sample depth (cm)	Sulfate formed (nmol S·g dry weight^{-1})		Methionine (nmol S·g dry weight^{-1})
		Salt extractable	Acid–base extractable	Acid–base extractable
2	O1	1.74	0.14	5.59
	O2	10.03	1.28	11.02
	A1, 0–5	4.47	0.90	2.76
	B, 15–25	3.89	1.01	1.67
	C, 95–100	1.88	0.44	1.11
9	O1	1.78	0.35	10.90
	O2	11.61	3.80	24.79
	A1, 0–5	2.71	0.79	2.21
	B, 15–25	4.58	0.92	0.88
	B–C, 30–50	3.12	0.64	0.63
	C, 75–90	1.61	0.31	0.45

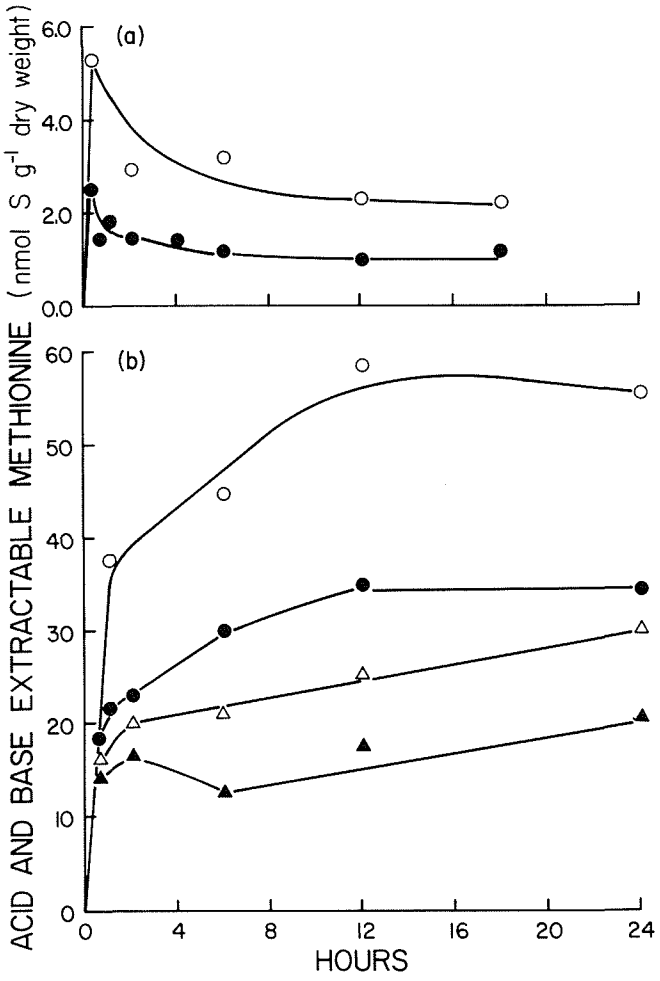

FIG. 1. Incorporation of methionine into litter and soil organic matter of watershed 18. (*a*) A1 horizon soil extracted after incubation with methionine at 28°C (○) and 5°C (●). (*b*) O1 layer (closed symbols) and O2 layer (open symbols) extracted after incubation with methionine at 28°C (circles) and at 5°C (triangles).

matter or whether oxidation of some of the methionine occurred during release from organic matter. The incorporation of ^{35}S-labelled methionine into the organic matter of litter and soil from WS 18 was temperature dependent and occurred without a lag reaching completion within 12 and 0.5 h in these components, respectively (Fig. 1). Levels of incorporated methionine in soil samples decreased thereafter suggesting that this amino acid can be released for mineralization after incorporation into soil organic matter. On a dry weight basis, the O2 layer exhibited the greatest activity for mineralization (Table 2 and Fitzgerald and Andrew 1984) and for incorporation of methionine into organic matter (Table 1). The O1 component was usually 5- to 10-fold more active in this latter respect than the A1 horizon (Tables 1 and 2), and the capacity to incorporate methionine decreased with depth within the mineral horizon (Tables 2 and 3). As expected, the A1 horizon mineralized larger quantities of methionine than the O1 layer, and this capacity again was found to decrease with the depth of soil samples taken from plots 2 and 9 (Table 2). Table 2 shows that some of the sulfate from mineralization could only be recovered by acid–base extraction and was therefore likely incorporated into organic matter during incubation, an observation made previously when sulfate was added directly to litter and soil samples from this watershed (Swank et al. 1984).

TABLE 3. Influence of temperature and mineral horizon depth on the ability of samples from Watershed 18, plot 5 to incorporate methionine into organic matter

Depth (cm)	Horizon	Acid and base extractable methionine (nmol S·g dry weight^{-1})	
		5°C	28°C
0–5	A1	1.98	3.08
8–15	Ap	1.42	2.83
15–40	B	1.50	1.50
40–80	B + C	1.03	1.22
80–125	C	0.60	1.25
125–180	C	0.28	0.54

Fate of methionine in soil from other watersheds

To determine if soil from other forests can incorporate methionine into organic matter, samples from another hardwood control (WS 2) and from three managed watersheds were investigated. In view of the lack of variability in the capacity to incorporate methionine evident for samples taken from WS 18 (8.2% error, n = 10, Table 1), one random soil sample from these additional watersheds was considered sufficient to make this assessment. Table 4 shows the water soluble, salt extractable, and acid–base extractable levels of ^{35}S-labelled sulfate

TABLE 4. Distribution of sulfur in fractions obtained from sequential extraction after incubation with ^{35}S-labelled methionine of A1 horizon soils collected from the Coweeta basin

Fraction	Watershed No.	S (nmol · g dry weight^{-1}) as: sulfate 0.5 h	sulfate 48 h	methionine 0.5 h	methionine 48 h	S (% of total recovered)[a] as: sulfate 0.5 h	sulfate 48 h	methionine 0.5 h	methionine 48 h
Water	2	0.03	0.15	2.32	0.01	0.2	1.4	21.9	0.1
	48	0.01	0.01	0.75	0.01	0.1	0.1	7.1	0.1
	7	0.03	0.50	2.59	0.34	0.3	5.4	24.5	3.7
	17	0.03	0.07	1.61	0.04	0.2	0.6	15.2	0.3
Salt	2	0.42	4.84	2.78	1.02	3.9	45.6	26.4	9.6
	48	0.35	5.06	2.16	0.69	3.3	47.0	20.5	6.4
	7	0.77	4.33	1.73	0.26	7.3	46.5	16.4	2.8
	17	0.65	4.96	0.82	0.64	6.1	43.7	7.7	5.6
Acid	2	0.38	1.05	2.53	1.49	3.6	9.9	24.0	14.1
	48	0.43	0.81	4.41	1.61	4.1	7.5	41.7	15.0
	7	0.45	0.94	2.16	1.16	4.3	10.1	20.5	12.4
	17	0.45	0.91	4.76	1.74	4.3	8.0	45.1	15.3
Base	2	0.06	0.34	0.36	0.45	0.6	3.2	3.4	4.3
	48	0.06	0.40	0.38	0.92	0.6	3.7	3.6	8.5
	7	0.09	0.23	0.31	0.31	0.8	2.5	2.9	3.4
	17	0.06	0.60	0.65	1.31	0.6	5.3	6.1	11.5

[a]Not corrected for dry weight.

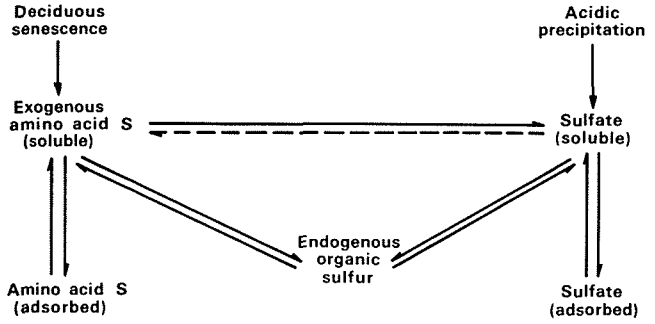

FIG. 2. Inorganic and organic sulfur transformations in forest soils of the Coweeta basin. Major and minor conversions are indicated by solid and broken arrows, respectively.

and methionine after incubation of A1 horizon samples with this amino acid for 0.5 and 48 h. Apart from the pine forest soil (WS 17), which had little tendency to adsorb methionine, soil from these watersheds exhibited a large capacity for adsorption and incorporation of added methionine especially after 0.5 h of incubation. Incubation for 48 h resulted in substantial decreases in both adsorption and incorporation, and these reductions were associated with increased levels of sulfate in all fractions, especially in the salt extracts. Methionine, which was initially immobilized either by adsorption or incorporation, was therefore capable of being remobilized for mineralization after prolonged incubation. Despite limited sampling, the capacity of soil to incorporate and subsequently remobilize methionine was not restricted to watershed 18. Of interest was the observation that the sample from the pine forest soil was capable of incorporating about 45% of the added methionine after 0.5 h and of mineralizing a similar amount of this amino acid after 48 h. Soil from this watershed (WS 17) also exhibited a large capacity for mineralization of plant sulfolipid sulfur (Strickland and Fitzgerald 1983). Watershed 17 was originally a hardwood forest which was clear-cut and subsequently planted to pine (Johnson and Swank 1973). Collectively, these results suggest that this forest soil has retained the capacity for mineralization and incorporation of methionine into organic matter irrespective of past management practices.

Discussion

Although few studies of the metabolic fate of methionine in soil have been conducted, the data obtained in the current work agree with the existing data that little of this amino acid occurs in soil solution (Putnam and Schmidt 1959; Paul and Schmidt 1961; Grov 1963; Grov and Alvsaker 1963). This suggests that methionine released from decaying plant matter is rapidly metabolized in soil. Agricultural soils convert substantial amounts of added methionine to volatile forms of sulfur (Frederick et al. 1957; Banwart and Bremner 1975), whereas soils and litter from forest ecosystems convert methionine S primarily to sulfate. Significant volatilization of amino acid S did not occur in these latter samples (Hesse 1957; Fitzgerald and Andrew 1984).

An alternative fate of methionine, apart from adsorption, is that some of this amino acid released during decomposition can be incorporated directly into soil and litter organic matter. This possibility is supported by results of laboratory incubations carried out in the current work. These observations also complement those made by Freney et al. (1972) with agricultural soils. Although the level of soluble methionine was insignificant, Freney et al. (1972) found that the amino acid and its oxidation products comprised a substantial proportion of the total sulfur of soil organic matter. The potential that soils may have for incorporating exogenous methionine in laboratory assays may account for the high levels of this amino acid which must be incorporated into organic matter *in situ*. Although organic sulfur from forest soil has not been analyzed for amino acid content, this possibility may nevertheless apply particularly to hardwood forest soils since these receive at least periodic inputs of carbon-bonded sulfur from leaf protein decomposition, throughfall, and litter. Mitchell et al. (1984) attributed the carbon bonded sulfur in throughfall to canopy leaching and M. B. David (unpublished data) has calculated that leaf litter from northern hardwood forests contributes an

annual sulfur flux of 0.6 g m^{-2} of which 90% constitutes carbon-bonded sulfur. Unlike agricultural soils (see e.g., Bettany and Stewart 1982; Freney and Williams 1983), most (74%) of the total sulfur of coniferous and hardwood forest soil consists of carbon-bonded sulfur rather than ester sulfate (David et al. 1982). Similar results for both the O2 and A1 horizons were obtained for Coweeta watershed 18 (T. C. Strickland, unpublished data). A substantial proportion of the carbon-bonded sulfur content of soil is known to comprise the S-containing amino acids (Freney et al. 1975), and incorporation of these acids into organic matter may again account for the high levels of carbon-bonded sulfur in forest soil. The incorporation process may function *in situ* to conserve amino acid S for subsequent mineralization provided that rerelease of these amino acids from organic matter can occur. Data obtained from time course incubations in the current work indicate that release of incorporated methionine does occur at least *in vitro*. The interconversions of this amino acid and organic matter may thus be similar to those established previously for sulfate and soil from the same hardwood forest (Strickland et al. 1984; Swank et al. 1984). These transformations for sulfate together with those for methionine are summarized in Fig. 2.

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