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Sulfur cycling in grassland and parkland soils*†

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Abstract. A conceptual diagram of the S cycle in grassland soils is presented as a framework for discussing S cycling process studies. Changes in the mineralization of S and in the redistribution of ^{35}S-labeled sulfate among soil organic matter fractions were investigated during incubation of cropped and uncropped soils.

Little mineralization or net immobilization of sulfur occurred in closed system incubations where the soils were left undisturbed throughout the incubations. Significantly more S was mineralized in open system incubations where the soils were leached periodically. Net mineralization was significantly greater in cropped soils compared with uncropped soils. The distribution of ^{35}S was significantly affected by the addition of various substrates (sulfate, cellulose or a combination of both) and by the presence of plants. Under conditions of high solution sulfate, the majority of ^{35}S incorporated was observed in the HI-reducible S fraction. When the solution sulfate concentrations were lower, there was a reduction in the proportion of ^{35}S incorporated into the HI-reducible S fraction. The results of these experiment will be discussed in relation to the hypotheses presented by McGill and Cole (1981) and the conceptual diagram of the S cycle in grassland soils.

Introduction

The arable soils of the prairie provinces of western Canada are diverse having developed under grassland and forest ecosystems. The native grassland soils are generally nutrient rich, however, cultivation of these soils by traditional practices has resulted in a significant decline in their natural fertility (Tiessen et al., 1982; Bettany et al., 1980). Reports of S deficiencies on the cultivated soils of the prairies are becoming more frequent and refer not only to former Parkland or Gray Wooded (Luvisolic) soils first reported to be S-deficient soils in western Canada by Newton (1936) but also to the transitional and grassland Chernozemic soils (Bettany et al., 1983). This increasing occurrence of S deficiencies in the former grassland and parkland soils has emphasized

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the need to obtain an understanding of the processes responsible for the supply of S to plants. In these soils, where the majority of S is in an organic form, the biological processes that control the cycling of S are of particular importance.

Inputs of S to the cultivated grassland soils are minimal and are mainly limited to asmospheric deposition and fertilizer. Detailed information on inputs and outputs of S to former grassland areas have been presented elsewhere (Freney and Williams, 1983; Bettany and Stewart, 1983). The forms of S in the plant-soil system and the relative distribution of the various forms will be discussed. This paper will review the S cycle within grassland soils using a conceptual mode (Bettany and Stewart, 1983; Maynard, 1983) as a framework. Special attention will be given the immobilization-mineralization process of S in soils.

Forms of S in the soil-plant system

1. Inorganic S

In most well drained soils the dominant form of inorganic S is sulfate with a negligible percentage of compounds of lower oxidation state present (Freney, 1958). Sulfate, in the soil solution is most commonly found in association with Ca, Mg, Na, and K cations. The amount of water soluble sulfate varies greatly both within the soil profile itself and among soil types (Freney and Williams, 1983). Sulfate can also be retained in soils with pH's below 6.6 that contain appreciable amounts of hydrous oxides of Fe and Al or clay colloids with Al or Fe as major components (Metson, 1979). Sulfate adsorption in soils involves a ligand exchange with Fe or Al oxides as free oxides or on the surface of mineral particles (Harward and Reisenauer, 1966; Hingston et al., 1972). However adsorbed sulfate was not measurable in significant quantities in the neutral or calcareous soils of the Canadaian prairies (Bettany et al., 1974).

In the neutral or calcareous grassland soils that contain negligible amounts of adsorbed S, inorganic sulfates are extracted in a 0.01M $CaCl_2$ solution (1 to 5 soil to solution ratio) (Hamm et al., 1973). Sulfur in the extract can be determined by several methods. One of the most common methods involves the reduction of sulfate to hydrogen sulfide followed by a colorimetric determination (Johnson and Nishita, 1952). Both inorganic sulfate and organic sulfate (ester sulfates) are reduced. The S determined by this method is referred to as $CaCl_2$-extractable SO_4^{2-}–S. Extracts of weak neutral salts such as $CaCl_2$ do not contain significant levels of organic sulfates (Walker and Doornenbal, 1972) therefore the extraction of soils with $CaCl_2$ and the determination of S by the reduction method provides a suitable measure of inorganic S in grassland soils.

2. Organic S

Current analytical techniques permit only a broad fractionation of organic S compounds in plant-soil systems. Two major groups have been identified: one in which S is directly bonded to carbon (C-bonded S) and another in which S is linked to carbon through an O- or N- atom (organic sulfates). The C-bonded S group would include free or combined amino acids, cellular metabolites such as glutathione and S amino acid intermediates such as homocysteine and cysteic acid (sulfonic acid). Other reduced C-bonded S species of biological importance include co-factors such as co-enzyme A, antibiotics, vitamins and iron sulfur protein (Roy and Trudinger, 1970). The organic sulfates include the ester sulfates and other related sulfoconjugates. The true ester sulfate group has the general formula $ROSO_3$ and contains a C–O–S bond. Related to the true ester sulfates are compounds containing N–O–S linkages such as glucosinolates and N–S linkages typified by sulfamates.

The ester sulfate fraction is often referred to as the hydriodic acid (HI) reducible S fraction, which is the fraction of organic S reduced directly to H_2S by a mixture of hydriodic acid, formic acid and hyphosphorus acid (Fitzgerald, 1976; Freney, 1961). This fraction also includes inorganic sulfate. However, in this paper HI-reducible S refers to the organic sulfates only. The $CaCl_2$-extractable SO_4^{2-}–S has been subtracted. Carbon bonded S is calculated as total S minus HI-reducible S and $CaCl_2$-extractable SO_4^{2-}–S.

Sulfur in plants

Plants contain approximately as much S as P, with an average S content of 0.25% (dry wt. basis). Of the wide variety of S-containing compounds found in plants, only a few have been found essential for normal cell function. These include the S amino acids, glutathione, and co-factors such as thiamine, vitamin B, biotin, ferredoxin, lipoic acid, co-enzyme A and the sulfolipids of chloroplasts. Compounds whose function in plants is unknown include glucosinolates, choline sulfate, penicillin and several other organic compounds (Thompson et al., 1970). A variable portion of S in plants may be inorganic sulfate.

The S amino acids account for approximately 90% of the S found in plants (Blair, 1979). The majority of the S amino acids are contained in protein and the average N/S ratio of protein is 14 for gramineous plants 17.5 for legumes (Dijkshoorn and Van Wijk, 1967). When excess S is available, however, sulfate may accumulate in plant tissue. In a recent study using rapeseed (Maynard et al. 1983b), the proportion of the total S in a HI-reducible S form (including inorganic sulfates) varied from less than 10% under extreme S deficiency to greater than 60% when the rapeseed was fertilized (50 kg S/ha). Similar observations have been observed in forest systems (Johnson, 1984).

There are little published data on the composition of the HI-reducible S fraction in plants. It is assumed that inorganic sulfate is the main component of the HI-reducible S fraction in most plant species. Glucosinolates can make up a significant portion of the HI-reducible S fraction in plants of the Cruciferae family. There are little published data on the forms of ester sulfates in plants other than glucosinolates. Choline sulfate has been identified in the roots and leaves of barley, corn, and sunflower (Nissen and Benson, 1961). Sulfated polysaccharides have not been idetified in higher plants (Schiff and Hodson, 1973).

Sulfur in soils

Carbon bonded S accounts for between 40 and 70% of the total soil S in grassland soils and consists mainly of the S amino acids, protein S, and sulfonic acids (sulfonates). Cystine (cysteine) and methionine S accounted for an average of 26% of the total soil S (equivalent to 46% of the C-bonded S) for two Podzolic soils of Australia (Freney et al., 1972) and 11 to 15% of the total soil S (equivalent to 19 to 31% of the C-bonded S) for several Scottish soils (Scott, 1981). Similar observations were found for a variety of Canadian soils including several grassland soils (Kowalenko and Lowe, 1975; Lowe and Delong, 1963). Thus, a substantial proportion of the C-bonded S in soils may be contained in sulfonic acids other than the S amino acids.

Attempts to identify C-bonded S compounds other than amino acids have concentrated on the soil sulfolipids. Soil lipid S denotes any S associated with lipid components including sulfolipids of plant and microbial origin. The amount of lipid S found in 27 forest and agricultural soils accounted for only 0.5 to 3.5% of the total soil S (Chae and Lowe, 1980). Values for the well-drained grassland surface soils were generally lowest, accounting for $< 1\%$ of the total soil S. The largest proportion of lipid S was found in the organic horizons associated with the forest soils.

The HI-reducible S fraction accounted for between 30 and 60% of the total S in grassland soils (Freney et al., 1971; Bettany et al., 1973). In contrast, ester sulfates averaged only 18% of the total soil S in all horizons of two forest soils (David et al., 1983; Mitchell et al., 1984). The compounds that make up the HI-reducible S fraction are largely unknown, and although aryl-sulfates (tyrosine-O-sulfate), alkysulfates (choline-o-sulfate) and sulfated polysaccharides have been suggested as major components of this fraction, qualitative and quantitative data are lacking. Lowe (1968) described and extraction of sulfated polysaccharides from the surface horizons of chernozemic and podzolic soils and found they accounted for $< 2\%$ of the total soil S. Indirect evidence, suggesting a portion of the HI-reducible S fraction may be associated with carbohydrates, was obtained by analysis of two chernozemic soils of Saskatchewan (Anderson et al., 1981). The soils

were fractionated into particle sizes after ultrasonic dispersion. A concentration of HI-reducible S and carbohydrates was found in the fine clay fraction (80% of the total HI-reducible S and 64% of the carbohydrate). In a study on the fate of exogenously supplied sulfate to a forest soil, an unknown polysaccharide compound containing sulfate ester linkages was identified as a soil metabolite (Fitzgerald et al., 1982). Other soil ester sulfates possibly produced included choline or tyramine-o-sulfate (Fitzgerald et al., 1982).

Sulfur transformations in soil-plant system

The conceptual flow diagram describes the main forms of S in soil, the main pathways of transformation and sets the boundaries to the cycle in the grassland soil-plant system under study (Figure 1). Atmospheric and groundwater processes are not documented except for the net inflow and outflow of various S compounds at the soil-air and soil-water interface. In Figure 1, both soil inorganic and organic S are divided into labile and stable forms. The diagram is a concept of hypothesis of the known types of transformations that take place in grassland soils and does not attempt to show the quantities or dynamics involved.

The mineralization of organic S represents the dominant source of S in most aerated grassland surface soils as organic S amounts for 95% of the total S (Bettany et al., 1973; Freney and Williams, 1983). The mechanisms involved in the mineralization of organic S are largely unknown. Breakdown of organic substrate is mediated by microbial populations and thus the transfer of organic S from the various organic pools (labil, clay-protected, and

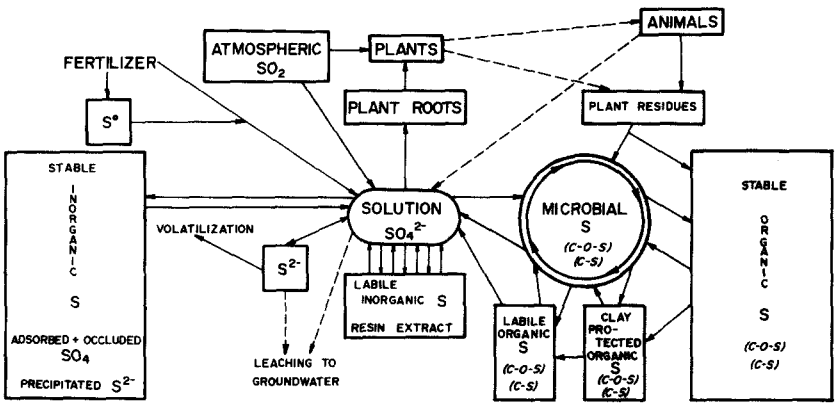

Figure 1. A conceptual diagram of the main forms and transformations of sulfur in the soil-plant system (Bettany and Stewart, 1983; Maynard, 1983). Both soil inorganic and organic S are divided into labile and stable forms. C–O–S refers to ester sulfates and C–S refers to carbon bonded S.

stable organic S) to inorganic S would be via an intermediate microbial S pool or directly by exoenzymes excreted by microoganisms. The excess amounts of S are released to the soil 'available pool' as inorganic sulfate and the remaining S would be incorporated into the microbial S pool and recycled into the various soil organic fractions.

A recent review of nutrient cycling (McGill and Cole, 1981) proposed that the mechanisms stabilizing organic C, N, S, and P in soils are not necessarily common to all four elements. The authors suggested that the mechanisms and pathways of S mineralization are specific to the form of organic S. Carbon bonded S would be mineralized as a result of C oxidation to provide energy (biological mineralization). The need for C not S would result in the mineralization of S. Ester sulfates would be hydrolysized by extracellular or periplasmic sulfohydrolases (produced by soil microorganisms and plant roots) controlled by the end-product supply (biochemical mineralization). The S associated with the ester sulfates would be mineralized in response to a need for S not C. The net mineralization of S would be the result of the combination of the two mechanisms.

Separate controls may also regulate the stabilization or immobilization of the various organic S forms. Sulfate ester production may be a mechanism used by soil microorganisms to store S without altering the pH of their surroundings (McGill and Cole, 1981; Fitzgerald, 1978). Two soil fungi were found capable of storing excess S in an HI-reducible S form (Table 1, Saggar et al., 1981). It was not possible to determine if the HI-reducible S was

Table 1. Sulfur concentrations of bacteria and fungi grown at three sulfur concentrations (Saggar et al., 1981a).

Treatment[a]	*A. globiformis*	*P. cepacia*	*F. solani*	*T. harzianum*
		Total S (mg S kg^{-1} o.d. mass)		
Low-S	928	1108	1013	852
Medium-S	1123	1341	2318	1261
High-S	1355	1339	2772	2034
(LSD .01)	47	36	108	93
		HI-reducible S (mg S kg^{-1} o.d. mass)		
Low-S	96	141	116	51
Medium-S	119	136	565	216
High-S	98	163	1159	752
(LSD .01)	NS	NS	83	27
		Proportion of total S as HI-reducible S (%)		
Low-S	10	13	12	6.0
Medium-S	11	10	24	17
High-S	7.2	12	42	37

[a]Low-S, medium-S, and high-S represent 1, 4, and 16 ug S ml^{-1} of culture medium, respectively.

inorganic S or organic ester sulfates, however, previous observations found that choline sulfate accumulated in higher fungi when S was in adequate supply (McGuire and Marzluf, 1974; Scott and Spencer, 1968; Spencer et al.,

1968). Carbon bonded S would be immobilized according to the microbial need for C (McGill and Cole, 1981).

The conceptual model of McGill and Cole (1981) proposed that C (and N) are stabilized together and mineralized by microbes to provide energy (biological mineralization) and introduced the concept of biochemical mineralization as separate from biological mineralization and controlled by the need for S rather than for C. The concept of stabilization (immobilization) of ester forms independently of, but not necessarily separate from, C-bonded forms was also presented. The following section deals with the results of several short-term process studies aimed at understanding the processes of mineralization, immobilization, and redistribution of S in grassland soils in relation to the concepts put forward by McGill and Cole (1981). Several manipulations have been carried out to alter both the supply of inorganic S and the microbial demand for S.

Process studies

The first experiment compared the net amount of S and N mineralized in three soils using two incubation techniques (Maynard et al., 1983a). The open incubation technique involved periodic leaching of extractable sulfate and nitrate from the soils, similar to the method of Stanford and Smith (1972). Leaching the soils periodically during incubation may be similar to the removal of N and S by plants and represent leaching processes of these elements under moist field conditions (Tabatabai and Al-Khafaji, 1980). The closed incubation technique involved the incubation of soils for a set period and the extractable sulfate and nitrate were measured before and after the incubation. The soil was kept moist but otherwise was left undisturbed during the incubation period. The closed system may demonstrate the trends of S and N mineralization in fallow systems in semi-arid grassland soils where the soil concentrations of N and S are higher.

Considerably more S was found to mineralize in the open system compared to the closed system (Table 2, Maynard et al., 1983a). The total amount of N mineralized, however, was not significantly different (Table 2). The N results suggest that the closed incubation provided adequate environmental conditions for microbial activity (i.e., mineralization) and the differences in the net S released between the incubation procedures support the concept of biochemical mineralization proposed by McGill and Cole (1981).

There was an adequate supply of soluble S in the closed system and the net S mineralized would have been mainly the result of mineralization of C-bonded S material. Mineralization of the ester sulfates would have been partially repressed by the S present in solution. In the open system, however, there would have been a demand for available S by the microbial population since periodic leaching removed S leaving low soil solution concentrations of

Table 2. Amounts of SO_4^{2-}–S and NO_3^-–N mineralized during 17 weeks incubation at 20 °C (Maynard et al., 1983a).

Soil	Open-system incubation		Closed-system incubation	
	SO_4^{2-}–S[a]	NO_3^-–N[ns]	SO_4^{2-}–S[a]	NO_3^-–N[ns]
	mg kg^{-1} soil			
Waitville[b] Orthic Gray Luvisol (Typic Cryoboralf)	11.5	34.4	−0.7	32.7
Loon River Orthic Gray Luvisol (Typic Cryoboralf)	11.8	57.8	1.3	53.8
Indian Head Black Chernozem (Udic Haploborall)	11.3	32.6	1.4	34.8

[a]Significant difference at $P \leq 0.01$ between incubation methods.
[ns]No significant difference at $P \leq 0.01$ between incubation methods.
[b]Canadian classification (U.S. classification).

S. Enzymatic hydrolysis of ester sulfates would have occurred because of a need for S, resulting in a greater net mineralization of organic S.

The second set of experiments were designed to simulate the addition of plant residues to the soil system by the treatment of the soil with cellulose. The object of the study was to alter the microbial demand for energy and S by the addition of C, C plus S, and S. The incubations were carried out by the closed incubation technique and included the following treatments:

(1) Control – no additions except carrier-free $H_2\ ^{35}SO_4$
(2) Sulfate – 15 mg $^{35}SO_4$ kg^{-1} soil
(3) Cellulose (+ S) – 1500 mg C plus 15 mg $^{35}SO_4$ kg^{-1} soil
(4) Cellulose (− S) – 1500 mg C kg^{-1} soil plus carrier-free $H_2\ ^{35}SO_4$.

Details of the experimental design are presented elsewhere (Maynard et al., 1984).

Cellulose additions decreased solution sulfate concentrations, as expected from previous studies (Saggar et al., 1981b; Maynard 1983a), and little net change occurred in solution sulfate concentrations in incubated soils which did not receive cellulose (Table 3). The addition of glucose to a forest soil (Strickland and Fitzgerald, 1984) was also found to decrease the availability of sulfate and increase the formation of organic S (increased immobilization).

Changes in the ^{35}S concentration in the $CaCl_2$-extractable SO_4^{2-}–S fraction, however, clearly indicate that both mineralization and immobilization processes were occurring simultaneously. The ^{35}S in the solution sulfate pool, of the control treatment, was shown to decrease with little net change in the total $CaCl_2$-extractable SO_4^{2-}–S, indicating ^{35}S was transformed from the

Table 3. The $CaCl_2$-extractable SO_4^{2-}–S and the mg ^{35}S remaining in the $CaCl_2$-extractable SO_4^{2-}–S fraction in a closed incubation (Maynard 1983).

Soil	$CaCl_2$-extractable SO_4^{2-}–S		
	Start of Incubation	Day 48	End of Incubation (88 Days)
		mg S kg^{-1} soil	
Waitville			
Control	7.9 (7.9)[a]	10.0 (3.9)	8.4 (3.2)
Sulfate	23.4 (23.4)	18.9 (15.9)	18.7 (14.5)
Cellulose (+ S)	23.4 (23.4)	16.2 (13.0)	14.0 (10.6)
Cellulose (– S)	7.9 (7.9)	5.5 (1.7)	3.4 (0.6)
Loon River			
Control	9.6 (9.6)	11.2 (5.3)	9.2 (3.5)
Sulfate	23.8 (23.8)	21.1 (14.9)	19.9 (13.1)
Cellulose (+ S)	23.8 (23.8)	17.2 (12.2)	11.6 (7.4)
Cellulose (– S)	9.6 (9.6)	6.8 (1.9)	5.3 (0.8)
Indian Head			
Control	5.5 (5.5)	7.4 (3.5)	6.9 (3.0)
Sulfate	19.7 (19.7)	19.7 (15.6)	21.4 (15.7)
Cellulose (+ S)	19.7 (19.7)	13.6 (11.0)	12.8 (8.4)
Cellulose (– S)	5.5 (5.5)	3.5 (0.1)	3.7 (0.1)

[a]Values in parentheses represent the mg ^{35}S kg^{-1} soil remaining in the $CaCl_2$-extractable SO_4^{2-}–S fraction.

solution sulfate into the organic S fractions, while unlabelled organic S was released into the soluble sulfate poole (Table 3).

The distribution of labelled ^{35}S was significantly affected by the addition of various substrates (sulfate, cellulose, or both) (Table 4). Incorporation was least in the control treatments and highest in the cellulose (+ S) treatments. Most of the sulfate was incorporated during the intial 48 days of the incubation. Labelled ^{35}S was recovered in both HI-reducible S and C-bonded S forms in the organic matter (Table 4). In the control and sulfate only treatments the majority of ^{35}S incorporated was found in the HI-reducible S fraction. Cellulose additions increased the total amount of ^{35}S incorporated into the soil organic matter compared to the sulfate and control treatments and decreased the proportion of ^{35}S incorporated into the HI-reducible S fraction (Table 4). When cellulose was added without S a smaller proportion of ^{35}S was incorporated into the HI-reducible S fraction than when cellulose was added with S.

The results of the distribution of ^{35}S in the soil organic matter support the hypothesis of McGill and Cole (1981) and Fitzgerald (1978) that sulfate ester production may be a mechanism used by soil microoganisms (particularly fungi) to store S when solution sulfate is adequate. When the solution sulfate content was high, the fungal population would have a higher HI-reducible S content. When microbial growth was stimulated by the addition of extra C, a demand for S was created and the soil solution concentration decreased.

Table 4. The distribution of ^{35}S between HI-reducible S (HI–S) and C-bonded S (C–S) fractions of soil organic matter in uncropped incubated soils[a] (Maynard et al., 1984)

	After 48 days incubation				After 88 days incubation			
	HI–S		C–S[b]		HI–S		C–S[b]	
	Total	^{35}S	Total	^{35}S	Total	^{35}S	Total	^{35}S
	mg S kg^{-1} soil							
Waitville								
Control	71	3.7	107	0.3	79	4.3	115	0.5
Sulfate	92	5.5	89	2.0	82	7.6	111	1.4
Cellulose (+ S)	78	4.3	104	6.0	86	8.4	88	4.3
Cellulose (— S)	84	2.2	117	4.0	91	3.4	101	3.8
Loon River								
Control	103	2.1	122	2.3	104	3.9	113	2.1
Sulfate	100	3.7	125	5.1	100	4.5	128	6.1
Cellulose (+ S)	101	4.7	124	6.9	109	7.8	122	8.6
Cellulose (— S)	103	2.6	136	5.1	110	3.4	132	5.5
Indian Head								
Control	184	1.3	155	0.7	152	1.6	163	0.9
Sulfate	168	3.0	143	1.2	142	2.0	162	2.0
Cellulose (+ S)	156	2.1	157	6.7	160	6.5	143	4.7
Cellulose (— S)	160	1.8	152	3.6	148	1.7	170	3.1

[a]Based on the initial specific activity of the $CaCl_2$-extractable SO_4^{2-}–S.
[b]C–S = total S — HI–S and $CaCl_2$-extractable SO_4^{2-}–S. Samples extracted prior to reduction with HI.
[c]*** Significant at $P \leq 0.001$

Significance of F values[c] (^{35}S data):

	Waitville			Loon River			Indian Head		
	Treat.	Frac.	TxF	Treat.	Frac.	TxF	Treat.	Frac.	TxF
Day 48	***	***	***	***	***	***	***	***	***
Day 88	***	***	***	***	NS	NS	***	NS	NS

Table 5. Net mineralization or immobilization[a] of S in cropped and uncropped soils during 40 days incubation and two harvests (Maynard et al., 1984).

Soil	Cropped[b]	Uncropped
	mg S kg^{-1} soil	
Waitville		
Control	6.8 ± 1.0[c]	−1.6 ± 0.4
Sulfate	9.6 ± 2.8	−0.2 ± 0.5
Cellulose (+ S)	3.7 ± 0.7	−2.2 ± 0.2
Cellulose (− S)	6.5 ± 0.4	−2.1 ± 0.6
Loon River		
Control	9.3 ± 1.5	−1.9 ± 0.4
Sulfate	6.9 ± 3.5	−1.1 ± 1.0
Cellulose (+ S)	8.6 ± 0.6	−5.7 ± 0.5
Cellulose (− S)	7.8 ± 0.3	−1.5 ± 0.2
Indian Head		
Control	2.8 ± 1.3	−0.7 ± 0.1
Sulfate	8.1 ± 1.8	1.7 ± 0.1
Cellulose (+ S)	7.7 ± 0.8	−0.7 ± 0.2
Cellulose (− S)	6.0 ± 1.2	0.0 ± 0.3

[a]Negative values refer to net immobilization.
[b]Significant difference at $P \leq 0.001$ between cropped and uncropped soils. No significant difference at $P \leq 0.01$ among treatments.
[c]Standard error (n = 3).

This would result in a reduction in the percentage HI-reducible S in an increased fungal population (Maynard et al., 1984). The distribution of ^{35}S found in the soils incubated with the various treatments agrees with this explanation.

The final experiment involved the addition of growing plants to the incubating soils. The results of the earlier experiments suggested (Tables 2 and 4), solution sulfate concentrations may influence the net S mineralized and the incoporation and final form of S in organic matter. Plant growth was used to decrease the soil solution SO_4^{2-}–S and increase microbial demand. This study is described in detail elsewhere (Maynard et al., 1984). Sulfur deficient rapeseed plants were placed on the soil 48 days after the various amendments were added. The same four treatments discussed above were used.

Net mineralization was significantly greater in the cropped than uncropped soils of all treatments (Table 5, Maynard et al., 1984). This difference in S mineralization supports the concept of increased S mineralization due to an increased demand for S (McGill and Cole, 1981). The plant demand for S lowered the concentration of sulfate in soil solution (Maynard et al., 1984) which could have resulted in an increased production of sulfohydrolyases by plant roots and rhizosphere microorganisms.

There was significantly less ^{35}S incorporated into the HI-reducible S fraction than into the C-bonded S fraction in cropped soils (Table 6). Less than 47% of the incorporated ^{35}S was in the HI-reducible S fraction of the soil

Table 6. The distribution of labeled ^{35}S between the HI-reducible S (HI-S) and C-bonded S (C–S) fractions of the soil organic matter in cropped incubated soils (Maynard et al., 1984).

Soil	After first harvest		After second harvest	
	HI–S	C–S	HI–S	C–S
	mg ^{35}S kg^{-1} soil			
Waitville				
Control	0.9	2.6	1.0	2.5
Sulfate	1.2	5.6	2.5	6.1
Cellulose (+ S)	3.8	8.2	4.2	8.1
Cellulose (– S)	2.3	4.7	2.5	3.9
Loon River				
Control	1.4	3.0	1.6	2.6
Sulfate	2.4	6.5	1.8	7.1
Cellulose (+ S)	3.1	8.9	3.5	9.2
Cellulose (– S)	2.1	5.5	2.1	4.8
Indian Head				
Control	1.5	1.4	1.4	1.6
Sulfate	0.8	5.3	1.8	6.0
Cellulose (+ S)	2.4	8.9	3.9	8.0
Cellulose (– S)	1.5	3.7	1.6	4.0

Significant of F values[a]:

	Waitville			Loon River			Indian Head		
	Treat.	Frac.	TxF	Treat.	Frac.	TxF	Treat.	Frac.	TxF
After 1st Harvest	***	***	NS	***	***	NS	***	***	***
After 2nd Harvest	***	***	**	***	***	***	***	***	***

[a]***Significant at $P \leq 0.001$
**Significant at $P \leq 0.01$

organic matter at the end of the incubation. In the cropped soils the quantity of solution sulfate was rapidly lowered and enzymatic hydrolysis of ester sulfates would be increased. However, due to the competition from plants a smaller portion of the solution sulfate was incorporated into the soil organic matter. The distribution of ^{35}S in the soil organic matter at the end of the incubation is further evidence that the formation of ester sulfates is independent of C-bonded S and may be related to the supply of S available to the soil microorganisms.

Conclusions

These studies have provided further support to the hypothesis originally suggested by McGill and Cole (1981); that S may be mineralized by two related but independent processes. Carbon bonded S would mineralize as a result of C oxidation to provide energy and HI-reducible S (ester sulfates) would be hydrolysized in response to a need for S not C. The results obtained in this series of incubations suggest an important controlling role of soil

solution sulfate concentration not only on rates of mineralization but also on the distribution of recently immobilized exogeneous sulfate (Maynard et al., 1984). When S was in adequate supply or in excess, the proportion of added ^{35}S incorporated into the HI-reducible S fraction was higher than the distribution of indigeneous HI-reducible S in the total soil. Only in the treatments where there was a demand for S (e.g. Cellulose [–S], cropped soils) did the proportion of incorporated ^{35}S in the HI-reducible S fraction approximate the distribution of ^{32}S in this form in the total soil. However, these soils have developed over centuries and are the net result of a host of environmental and plant factors, whereas the incubations described in this paper reflect processes in the grassland S cycle affected by specific amendments over the short-term. More work is needed to document the role of soil solution sulfate on the mineralization processes and the immobilization and distribution of S under field conditions.

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