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Examination of ester sulfates in Podzolic and Regosolic soils using an immobilized arylsulfatase reactor

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Abstract The enzyme kinetics of an immobilized arylsulfatase reactor were examined. We found that the optimum operating conditions for the reactor were pH 7.0 and 25°C, using *p*-nitrophenyl sulfate in acetate buffer. The Michaelis constant (K_m) of immobilized arylsulfatase was 5.29 m*M*, compared with a K_m of 2.18 m*M* for soluble arylsulfatase from the same source (*Helix pomatia*). Since arylsulfatase hydrolyzes organic ester sulfate linkages, the immobilized arylsulfatase reactor was used to examine ester sulfate compounds in two soils subjected to different fertility management schemes. Soil samples were obtained from the Ap horizons of a Podzol from S-amended wheat plots and a Regosol from dykeland hayfield plots which had received additions of NH_4NO_3 and compost. The distribution of S in these soils was examined in the fall of 1993 and the spring of 1994. Soil organic matter was extracted and separated into three molecular weight fractions (<500, 500–10 000, >10 000). There was no difference in the ester sulfate content for the >10 000 fraction of control and S-amended Podzol soils; however, the S-amended samples had significantly higher quantities of hydrolysable ester sulfates than controls for the 500–10 000 range, indicating that S amendments resulted in the incorporation of ester sulfate into this lower molecular weight fraction. Both control and NH_4NO_3 treatments to the Regosol showed significantly higher quantities of hydrolysable ester sulfates in the >10 000 fraction, while compost-amended plots showed no difference between the >10 000 and 500–10 000 fractions due to suspected microbial degradation of high molecular weight organic S compounds in the compost. Since there was no significant effect of sampling time, this study indicated that naturally occurring low molecular weight ester sulfate compounds accumulate in soil and persist during storage. Hydrolysable ester sulfates constituted 35–55% of the hydriodic acid-reducible S in these different soils and probably represent an important and easily mineralizable portion of total ester sulfates.

Key words Ester sulfates · Soil organic matter · Immobilized arylsulfatase · Enzyme activity

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Introduction

The advantages of immobilizing an enzyme to a solid support have been well documented (Weetall 1975). The ability to reuse an enzyme system in continuous processing is both practical and economical. Arylsufatase (EC 3.1.6.1) has been immobilized to controlled porosity glass beads by Lou and Warman (1994). Since an enzmye bound to a solid support rarely behaves in the same way as the soluble form of that enzyme, it is important to investigate the properties of this immobilized enzyme system using soil organic matter.

Arylsulfatase is responsible for the hydrolysis of organic ester sulfate linkages in the presence of water (Dodgson et al. 1982). In soil, as much as 95% of the total S may be in organic forms. Ester sulfates represent 30–75% of total organic S and are thought to be more easily mineralized than other forms of organic S (McGill and Cole 1981; Germida et al. 1992). The ability to measure the quantity of potentially mineralizable ester sulfates is of considerable importance, particularly in agricultural soils where inorganic sulfate is required for the normal growth and function of plants (Tabatabai 1982; Freney 1986; Warman and Sampson 1994).

Lou and Warman (1992a, b) have shown that soil ester sulfate compounds are available for hydrolysis by soluble arylsulfatase. An immobilized arylsulfatase reactor (Lou and Warman 1994) was developed to further examine the

hydrolysis of ester sulfates from soil organic matter extracts. S amendments (gypsum) induced the formation of a labile ester sulfate fraction which was more readily hydrolyzed by immobilized arylsulfatase than the ester sulfates in untreated soil.

The use of an immobilized arylsulfatase reactor may be more realistic than the traditional measurements of hydriodic acid-reducible S, which is often equated with total ester sulfates in soil. The measurement of hydriodic acid-reducible S uses a mixture of strong acids which cleave S from ester sulfates, sulfamates, sulfamides, and the second S of S-sulfocysteine (Freney 1986), all of which may result in an overestimate of ester sulfate compounds in soil. Further investigation of the labile ester sulfate fraction may be useful in predicting the potential for mineralization of these compounds and subsequent uptake of sulfate by plants.

The objectives of this work were to (1) report the enzyme kinetics of an immobilized arylsulfatase reactor using the chromogenic substrate *p*-nitrophenyl sulfate and to compare the enzyme activity of immobilized and soluble arylsulfatase from the same source (*Helix pomatia*); (2) hydrolyze different molecular weight fractions of soil organic matter extracts using the immobilized arylsulfatase reactor in order to determine the molecular size of hydrolysable ester sulfate compounds in two soils with different morphological properties and management histories.

Materials and methods

Immobilized arylsulfatase reactor

Arylsulfatase (EC 3.1.6.1, type H-5 from *Helix pomatia*, purchased from Sigma Chemical Co., St. Louis, Missouri, USA) was immobilized according to established procedures (Robinson et al. 1971; Masoom et al. 1990; Lou and Warman 1994). Controlled-porosity glass beads (pore size 700 Å, 80–120 mesh, Sigma Chemical Co.) were boiled in 5% HNO_3 to clean the carrier surfaces, activated with 3-aminopropyltriethoxsilane in acetone (2% solution), and treated with 2.5% glutaraldehyde in 0.05 *M* sodium acetate buffer (pH 7.0) for 1 h at 20°C (Weetall 1975).

Five hundred milligrams of activated glass beads were treated with 100 mg of arylsulfatase dissolved in 10 ml 0.05 *M* sodium acetate buffer (pH 7.0) overnight at 4°C. After the immobilization reaction was complete, the beads were washed with an excess of acetate buffer. The final product was violet in color.

The covalent coupling of arylsulfatase to glass beads resulted in 88.5 mg of protein immobilized per gram of beads, which was determined by total N analysis using a Tecator–Kjeldahl method (Bremner and Mulvaney 1982).

Approximately 200 mg of the immobilized arylsulfatase was placed in a glass column (18 cm×0.3 cm) encased in a water jacket. The column was plugged with glass wool at both ends. When not in use, the reactor was stored at 4°C in 0.05 *M* acetate buffer, pH 7.0.

Arylsulfatase assay

Potassium *p*-nitrophenyl sulfate was purchased from Sigma Chemical Co. to be used as the substrate for kinetic studies of the immobilized arylsulfatase reactor. Three milliliters of substrate solution (*p*-nitrophenyl sulfate in 0.05 *M* sodium acetate buffer) was added to the reactor, which had already been equilibrated with acetate buffer. The substrate was recirculated in this upflow reactor at a flow rate of 0.5 ml min^{-1} by a Masterflex Quick Load peristalic pump equipped with a 7020-40 pump head for up to 1 h.

Determination of *p*-nitrophenol released by the enzyme was measured colorimetrically at 400 nm with a LKB Novaspec spectrophotometer equipped with a flow-through cell. The spectrophotometer was attached to a BBC Goerz Metrawatt strip chart recorder for continuous monitoring of the reactor. The quantity of *p*-nitrophenol was calculated by referring to a calibration curve produced from a series of standards (10—50 μg ml^{-1} *p*-nitrophenol in 0.05 *M* NaOAc, pH 7.0).

The temperature optimum for the arylsulfatase reactor was determined. Reactor temperatures of 4, 15, 25, 37, and 45°C were examined using 5 m*M* *p*-nitrophenyl sulfate solutions. The reaction temperature was maintained by the water jacket surrounding the reactor, which was attached to a constant-temperature water bath.

The pH optimum of the immobilized arylsulfatase was determined with 5 m*M* *p*-nitrophenyl sulfate in 0.05 *M* acetate buffer adjusted to 10 pH values in the range of 5.4–9.4.

Enzyme activity was determined for the arylsulfatase reactor using a range of substrate concentrations (1–7 m*M* *p*-nitrophenyl sulfate in 0.05 *M* acetate buffer, pH 7.0) at 25°C for 30 min (based on the results of the pH and temperature optimum for the reactor).

The kinetics of soluble arylsulfatase from *Helix pomatia* were measured in order to compare the activity of soluble and immobilized arylsulfatase. Fifteen milligrams of soluble arylsulfatase was incubated with 3 ml of substrate (1–5 m*M* *p*-nitrophenyl sulfate) and 22 ml of acetate buffer (pH 7.0) at 25°C for 30 min. This quantity of soluble enzyme contained an equivalent amount of protein as the immobilized reactor (on the basis of total N analysis).

The Michaelis–Menten kinetics were calculated using the Lineweaver–Burke transformation where:

$$1/V = [S] + K_m/V_{max}[S] \quad (1)$$

$$= K_m/V_{max} \times 1/[S] + 1/V_{max} \quad (2)$$

A plot of $1/V$ versus $1/[S]$ gives a straight line where the intercept equals $1/V_{max}$ and the slope is K_m/V_{max}. Values reported for enzyme kinetics are the means of triplicate determinations.

Soil samples

Soil samples were obtained from the Ap horizon (0–15 cm) of two sites in the fall of 1993 and the spring of 1994. Samples were taken from an Orthic Humo-Ferric Podzol (Pugwash sandy loam) and a Gleyed Regosol (Acadia silty clay). Some properties of these soils are shown in Table 1. The Pugwash soil samples were from established wheat plots with a 4-year history of S amendments. The treatments examined were K_2SO_4 (100 mg S kg^{-1}), gypsum (100 mg S kg^{-1}), and control (0 mg S kg^{-1}). Equal quantities of three replicates from each treatment were combined in order to reduce sampling variance and provide a representative sample of each treatment. The Acadia soil samples were from dykeland hayfield plots which had received a variety of organic and conventional fertilizers. Plots amended with composted chicken manure (113 kg N ha^{-1}), NH_4NO_3 (113 kg N ha^{-1}), and a control (0 kg N ha^{-1}) were studied, and four replicates were pooled in order to create a composite sample of each treatment.

The fall soil samples were air-dried and sieved (<2 mm), while the spring samples were stored field-moist in sealed polyethylene

Table 1 Properties of the soils used in this experiment

	Pugwash	Acadia
Total C (%)	2.07	1.55
Total N (%)	0.162	0.136
Total S (%)	0.024	0.022
pH (H_2O)	6.05	5.95
Sand (%)	53	10
Silt (%)	35	44
Clay (%)	12	46

bags at 4°C until analysis in order to examine the effect of storage on soil ester sulfate content. It has been reported that air-dried and moist soils show similar patterns of S mineralization (Williams 1967); however, mineralization of sulfate under field conditions is quite variable, with high sulfate concentrations in summer samples, decreasing to lower values in the winter and spring (Simon-Sylvestre 1965).

Soil organic matter fractionation

Soil organic matter was extracted from the pooled samples following the procedure of Lou and Warman (1992a), 8 g dry soil was extracted with 30 g Chelex 100 chelating resin (purchased from Bio-Rad Laboratories, Mississauga, Ontaria, Canada) in 100 ml distilled deionized water by shaking for 16 h. The suspension was centrifuged at 16000 g, filtered through Whatman 42, and recentrifuged at 11000 g to remove clay particles. Next, the extracts were filtered through a 0.45-μm cellulose acetate filter to remove microbial cells and finally separated into molecular weight fractions of >10000, 500–10000, and <500, using an Amicon stirred ultrafiltration cell equipped with YM10 (molecular weight 10000) and YC05 (molecular weight 500) Diaflo ultrafilter membranes.

Three milliliters of the soil organic matter fractions were adjusted to pH 7.0 and added to the immobilized arylsulfatase reactor (pre-equilibrated with 0.05 *M* NaOAc, pH 7.0). The optimal reaction time was determined by circulating the organic matter fractions at a flow rate of 0.5 ml min^{-1} for 30, 60, 90, 120, and 300 min at 25°C. The hydrolyzed fractions were collected and made up to 10 ml with 0.05 *M* NaOAc buffer. Controls containing 3 ml of organic matter fraction and 7 ml 0.05 *M* NaOAc buffer (pH 7.0) were incubated at 25°C for the same length of time. The amount of sulfate cleaved by immobilized arylsulfatase was determined turbidimetrically as $BaSO_4$ (Bardsley and Lancaster 1960) at 420 nm, using a Beckman DU-70 spectrophotometer. Three replicates of each fraction were examined.

Analytical methods

The total C, N, and S content of the pooled soil samples was determined by combusting 200 mg soil at 1350°C using a Leco CNS-1000 Analyzer. Total organic sulfate was measured as hydriodic acid-reducible sulfate by digesting 0.3 g soil with 4 ml hydriodic acid-reducing mixture using a modified Johnson and Nishita apparatus and measuring the amount of H_2S trapped as methylene blue at 670 nm (Tabatabai 1982). Inorganic sulfate was extracted from 5 g soil with 25 ml 8 m*M* $Ca(H_2PO_4)_2 \cdot H_2O$ solution containing 500 ppm P and analyzed tubidimetrically as $BaSO_4$ (Bardsley and Lancaster 1960). Total ester sulfates in soil were assumed to be the difference between total organic sulfates and $Ca(H_2PO_4)_2$-extractable inorganic sulfate. Inorganic sulfate was also measured in the <500 molecular weight fraction of the organic matter extracts. Soil pH was measured after 15 min using a 1:1 (v/v) soil:solution ratio. At least three replicates were performed for each analysis. The results were evaluated statistically by analysis of variance using an SAS procedure (SAS Institute 1990). Significant treatments means were compared using Tukey's test at the 95% confidence level.

Results and discussion

Kinetics of immobilized arylsulfatase

The optimum temperature for the immobilized arylsulfatase reactor was found to be 25°C. In many instances, immobilization changes the temperature stability of an enzyme (Weetall 1975). Since enzymes are proteins, they are subject to denaturation at high temperatures, and so the operation of the reactor at a relatively low temperature is useful for maintaining the biological activity of the enzyme and prolonging its effective life-span. Although *p*-nitrophenol production occurred for up to 1 h after the reaction was initiated, a 30-min reaction time was considered sufficient for the observation of released product from the enzyme reactor.

Table 2 Kinetic constants of arylsulfatase from *Helix pomatia*. The Michaelis constant (K_m) is expressed as m*M* of *p*-nitrophenyl sulfate and the maximum reaction velocity (V_{max}) as m*M* *p*-nitrophenol released per 30 min. Results are the means of three determinations ± SD

	$K_m \pm$ SD (m*M*)	$V_{max} \pm$ SD
Immobilized arylsulfatase	5.29±0.23	2.95±0.17
Soluble arylsulfatase	2.18±0.14	4.35±0.09

The optimum pH for reactor operations with *p*-nitrophenyl sulfate was found to be pH 7.0, in accord with results reported by Lou and Warman (1994). The pH optimum for this enzyme appears to be changed upon immobilization; Dodgson and Powell (1959) found the pH optimum for soluble arylsulfatase from *Helix pomatia* was 6.0 with 0.5 m*M* *p*-nitrophenyl sulfate, whereas Weetall (1971) reported the pH optimum for immobilized arylsulfatase from an unnamed source as pH 9.0–9.5.

Michaelis–Menten kinetics were calculated using a Lineweaver–Burke plot of arylsulfatase activity for immobilized and soluble arylsulfatase from *Helix pomatia*. The immobilized arylsulfatase had a K_m value of 5.29 m*M*, compared with a K_m of 2.18 m*M* for soluble arylsulfatase (Table 2). Dodgson and Powell (1959) found their soluble arylsulfatase had a K_m value of 1.9 m*M* upon assay with 0.5 m*M* *p*-nitrophenyl sulfate. A larger K_m value for an immobilized enzyme indicates poorer binding affinity between the substrate and the immobilized enzyme than the soluble enzyme. The binding affinity of arylsulfatase for *p*-nitrophenyl sulfate may have decreased upon immobilization due to negative attraction for the substrate by the solid support (Klibanov 1983). Similarly, the maximum reaction velocity (V_{max}) was lower for immobilized arylsulfatase than soluble arylsulfatase (Table 2). The reaction velocity of the immobilized enzyme may be retarded by steric hindrances which affect the ability of the enzyme to undergo the conformational changes required to release the products from the active site. The ability of one of the products of this reaction, sulfate, to inhibit arylsulfatase activity is well known (Dodgson et al. 1982), and in this case the inhibition is irreversible.

A comparison between equivalent amounts of immobilized and soluble enzyme (on the basis of total N analysis) showed that the activity of immobilized arylsulfatase was 50% of soluble arylsulfatase under the same reaction conditions. This is not uncommon; Weetall (1975) noted that only 30–50% of enzyme activity is retained upon immobilization.

S status of Pugwash (Podzolic) and Acadia (Regosolic) soils

The total S content of the Pugwash and Acadia soils was similar but the distribution of S in these soils was quite different (Tables 3, 4). In the Pugwash soil, there was a significant increase in hydriodic acid-reducible S in S-amended treatments from fall to spring sampling, although there was no difference for the control (Table 3). Correspondingly, there was a significant decrease in the $Ca(H_2PO_4)_2$-extractable S for the S-amended treatments. Since there was no significant difference in the total S content of fall and spring samples, it appears that an overall net immobilization of S from inorganic to organic forms occurred.

In the Acadia soil, there was no significant difference in the hydriodic acid-reducible S content of three treatments over time; however, it is interesting to note the significant decrease in the $Ca(H_2PO_4)_2$-extractable S for all treatments from fall to spring (Table 4). Perhaps this marshland soil underwent a reduction of inorganic sulfate (SO_4^{2-}) to sulfides (S^{2-}), which may be followed by reaction with either Fe and Mn, or oxidation to organic S as sulfoxides (R-SO-R; Hoyle 1988).

The quantity of inorganic S in the <500 molecular weight fraction of the soil organic matter, extracts was measured turbidimetrically (Bardsley and Lancaster 1960). This method did not precipitate sulfate from unhydrolyzed *p*-nitrophenyl sulfate solutions, so it seems unlikely that the measurement included any organic ester sulfates present in this fraction. It is interesting to compare the quantities of inorganic sulfate in the <500 fraction of organic matter extracts with the quantities of $Ca(H_2PO_4)_2$-extractable sulfate in soils (Tables 3, 4). $Ca(H_2PO_4)_2$-extractable sulfate includes water-soluble, adsorbed sulfates, and possibly some organic sulfates (Alewell 1993), whereas the <500 fraction contains only water-soluble sulfates.

The quantity of water-soluble sulfate was substantially less than that of $Ca(H_2PO_4)_2$-extractable sulfate in both soils. In the Pugwash soil, only the K_2SO_4 treatment showed a significant decrease in water-soluble sulfate between the fall and spring samples. There was no significant difference between the S-amended treatments and the control at either sampling time. In the Acadia soil, there was no difference in water-soluble sulfate over time for any of the treatments, although the NH_4NO_3 treatment had significantly higher water-soluble sulfate than the control at both fall and spring sampling times. This may have been due to the readily available N from the NH_4NO_3 which promoted microbial degradation or organic S forms. Ghani et al. (1992) found that the addition of N increased S mineralization in soils incubated for 14 weeks at 30°C.

Table 3 Distribution of S in Pugwash soil and in the <500 molecular weight soil organic matter fraction (unhydrolyzed). *HI* hydriodic acid. Means in a column followed by the same letter are not statistically different at $P=0.05$

Treatment	Soil			Inorganic S in organic matter fraction (μg g^{-1})
	Total S (%)	HI-reducible S (μg g^{-1})	$Ca(H_2PO_4)_2$-extractable S (μg g^{-1})	
Fall 1993				
Control	0.023a	47.7a	24.4a	10.4ab
Gypsum	0.026a	57.6a	55.3b	9.6bc
K_2SO_4	0.028a	56.4a	47.7b	14.5a
Spring 1994				
Control	0.025a	47.4a	11.3a	6.1bcd
Gypsum	0.026a	94.6b	12.8a	5.3cd
K_2SO_4	0.027a	71.0b	22.7a	4.0d

Table 4 Distribution of S in Acadia soil and in the <500 molecular weight soil organic matter fraction (unhydrolyzed). For further explanations, see Table 3

Treatment	Soil			Inorganic S in organic matter fraction (μg g^{-1})
	Total S (%)	HI-reducible S (μg g^{-1})	$Ca(H_2PO_4)_2$-extractable S (μg g^{-1})	
Fall 1993				
Control	0.026a	41.9a	41.1a	10.4bc
Compost	0.029a	32.7a	37.7a	10.0bc
NH_4NO_3	0.025a	42.0a	29.6a	15.4a
Spring 1994				
Control	0.023a	44.4a	11.7b	6.6c
Compost	0.027a	43.0a	17.1b	10.8abc
NH_4NO_3	0.029a	49.4a	15.0b	14.0ab

Hydrolysis of ester sulfate in soil organic matter fractions using the immobilized arylsulfatase reactor

The optimum reaction time for the soil organic matter fractions was 120 min. The concentration of ester sulfates in the extracts was lower than in the standard *p*-nitrophenyl sulfate solutions and so the longer reaction time was used for all soil organic matter analysis.

It was difficult to examine ester sulfates in the <500 fraction of the organic matter extracts because this fraction contained a large amount of inorganic sulfate, which was found to be inhibitory to the immobilized arylsulfatase reactor. Attempts were made to precipitate the excess sulfate with Ba^{2+} but this was not entirely satisfactory. According to the molecular size of this fraction, it could contain ester sulfates bound to 1–3 phenolic groups; however, ester sulfates in this fraction are probably small and highly labile.

The 500–10 000 and >10 000 molecular weight organic matter fractions of the two soils were hydrolyzed by the immobilized arylsulfatase reactor. Statistical analysis showed that sampling time had no effect. This implies that either (1) hydrolyzable ester sulfates are fairly stable (minimal turnover through mineralization during processing and storage of the fall samples) or (2) the proportion of these compounds in soil is relatively constant. Pooled means were reported for the sampling time and compari-

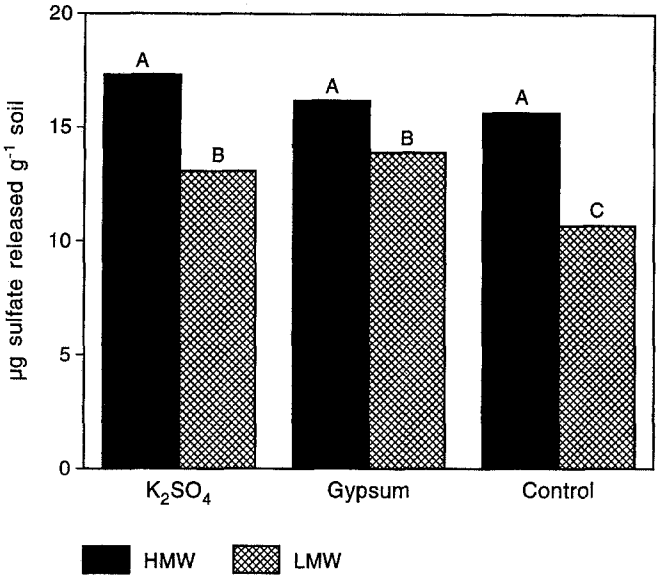

Fig. 1 Ester sulfates hydrolyzed in Pugwash soil organic matter extracts by immobilized sulfatase reactor. *HMW* >10 000 molecular weight, *LMW* 500–10 000 molecular weight. *Bars* carrying the same *letter* are not significantly different at P=0.05

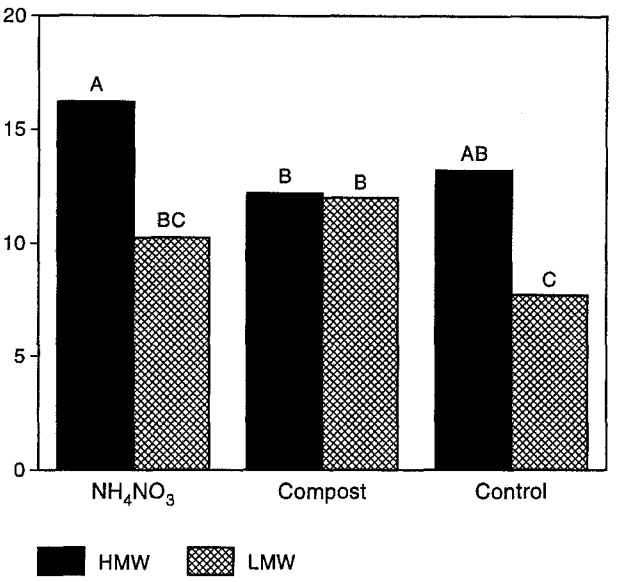

Fig. 2 Ester sulfates hydrolyzed in Acadia soil organic matter extracts by immobilized sulfatase reactor. For further explanations, see Fig. 1

sons were made for the two molecular weight fractions of each treatment.

In the Pugwash soil, there were significantly higher levels of hydrolyzable ester sulfates in the >10 000 molecular weight fraction than in the 500–10 000 fraction (Fig. 1); however, there was no difference between treatments in the quantity of hydrolysable ester sulfates for the >10 000 fraction. S amendments produced significantly higher quantities of hydrolyzable ester sulfates than controls in the 500–10 000 fraction. This corresponds with the results of other researchers, who have found that the addition of S amendments in arable and pasture soils (Keer et al. 1990) and forest soils (Vannier and Guillet 1994) results in a large proportion of hydriodic acid-reducible S compounds, referred to as ester sulfates, in the fulvic acid fraction (low molecular weight); however, it contradicts the supposition of Houghton and Rose (1976) that naturally occurring low molecular weight sulfate esters do not accumulate in soil. It appears that the most recently incorporated ester sulfate compounds are found in the 500–10 000 molecular weight fraction. This may be a very important pool in terms of S cycling and plant availability. Freney et al. (1975) found that the majority of $^{35}SO_4^{2-}$ added to two soils was transformed into hydriodic acid-reducible S (ester sulfates). Plants grown on these soils derived all their radiolabelled ^{35}S from this fraction.

Distribution of the hydrolysable ester sulfates in the Acadia soil was less distinct (Fig. 2). The NH_4NO_3 and control treatments had a significantly higher quantity of hydrolysable ester sulfate in the >10 000 molecular weight fraction than the 500–10 000 fraction, while the compost treatment displayed no difference between the two fractions. Microbial degradation of organic S compounds in the compost treatment, which contained about 0.4% S, would have resulted in less hydrolysable ester sulfate in the >10 000 molecular weight fraction than the NH_4NO_3 and control treatments.

Hydrolysable ester sulfates comprised 35–55% of the total hydriodic acid-reducible S for treatments in the Pugwash soil and 45–55% for treatments in the Acadia soil. The measurement of hydriodic acid-reducible S employs a mixture of strong acids which cleave S from ester sulfates (-C-O-S), sulfamates (-C-N-S), sulfamides (-C-N-O-S), and the second S* of S-sulfocysteine (-C-S-S*) (Freney 1986). Hydriodic acid-reducible S is often equated with total soil ester sulfates; however, this is likely an overestimate of the quantity of these compounds in soil.

Although soil organic S is expected to participate in humus formation, McGill and Cole (1981) considered it unlikely that ester sulfates would become covalently incorporated into humic materials and suggested a weak association with the humic component. Hydrolysable ester sulfates are likely limited to ester groups on the external surfaces of humic polymers (Lou and Warman 1994). The measurement of ester sulfates using the immobilized arylsulfatase reactor may be a more natural representation of the availability of these compounds for hydrolysis in soil.

Even though ester sulfates are considered to be the most easily mineralizable form of organic S in soil, hydriodic acid-reducible S has not been well correlated to plant-available S (Freney et al. 1975; Tabatabai 1982; Germida et al. 1992). It seems likely that only a portion of the hydriodic acid-reducible S is potentially available for mineralization and release as inorganic sulfate. An understanding of the mineralization of organic ester sulfates to inorganic sulfate in soils is of great interest, particularly in S-deficient soils. The hydrolysable ester sulfates identified in the present work may be a substrate for endogenous

soil arylsulfatase enzymes. Further research is being carried out to examine this hypothesis.

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