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J.K. Whalen · P.R. Warman

Arylsulfatase activity in soil and soil extracts using natural and artificial substrates

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Abstract The arylsulfatase activity of soil and humic arylsulfatase complexes extracted from soil were measured using the substrates *p*-nitrophenyl sulfate and low molecular weight (500–10 000) soil ester sulfate compounds. Soil samples from the Aphorizon of a Podzol from S-amended wheat plots and a Regosol from dykeland hayfield plots were investigated. Soil arylsulfatase activity (assayed with *p*-nitrophenyl sulfate) in the fall was significantly higher than spring samples; however, no seasonal differences were observed when humic-arylsulfatase complexes were assayed with *p*-nitrophenyl sulfate. The discrepancy between arylsulfatase activity in soil and soil extracts was probably due to inhibitors which were found in soil materials. These results appear to support the theory that abiotic arylsulfatase is a relatively stable and persistent component of soil. There was a marked difference in the response by humic–arylsulfatase complexes to the artificial substrate *p*-nitrophenyl sulfate and natural low molecular weight soil substrates. Humic–arylsulfatase complexes hydrolysed 35–80% of added low molecular weight substrates depending on the treatment. The molecular size, concentration, and chemical composition of the low molecular weight ester sulfate compounds affected hydrolysis of the low molecular weight substrates. The response by humic-arylsulfatase complexes to the chromogenic ester sulfate, *p*-nitrophenyl sulfate did not reflect the ability of these complexes to hydrolyse natural soil substrates. In an experiments we examined arylsulfatase activity and soil S status in relation to the total S in plant tissue and grain from wheat plants grown in the Podzol. Tissue S was more strongly associated with soil S than the wheat grain. Hydriodic acid-S, $Ca(H_2PO_4)_2$-extractable sulfate, and hydrolysable ester sulfates in the high molecular weight (>10 000) and low molecular weight (500–10 000) fractions of soil organic matter extracts were strongly positively correlated with tissue S. Arylsulfatase activity in soil and humic-arylsulfatase extracts assayed with *p*-nitrophenyl sulfate were also strongly correlated with tissue S, while humic-arylsulfatase activity assayed with the low molecular weight substrate was negatively correlated with tissue S.

Key words Arylsulfatase activity · Humic-arylsulfatase complex · Soil ester sulfate · Enzyme activity · Plant available sulfur · Plant tissue sulfur

J.K. Whalen
Department of Natural Resource Sciences,
Macdonald Campus of McGill University, 21 111 Lakeshore Road,
Ste Anne de Bellevue,
Quebec, H9X 3X9, Canada

P.R. Warman (✉)
Chemistry and Soil Science Department,
Nova Scotia Agricultural College, P.O. Box 550, Truro,
Nova Scotia, B2N 5E3, Canada

Introduction

Enzymes play an integral role in the decomposition of complex organic molecules and the cycling of nutrients in soil (Kiss et al. 1975; Sinsabaugh 1994). Soil enzymes are classified as either biological or abiotic according to their function and location in soil. Biological enzymes are associated with proliferating microbial, plant, and animal cells, while abiotic enzymes function catalytically outside viable cells. Abiotic enzymes often become bound in stable complexes with clay particles and humic colloids, resulting in stable and persistent enzyme activity (Burns 1986; Speir and Ross 1990).

Abiotic soil arylsulfatase may persist in association with soil humic compounds since arylsulfatase activity has been highly correlated with soil organic matter (Tabatabai and Bremner 1970b; Abramyan and Galstyan 1987; Appiah and Ahenkorah 1989; Speir and Ross 1990). Considerable research has been focused on the association of enzymes with soil humus because abiotic enzymes immobilized in soil organic matter are thought to be important in the biochemical cycling of nutrients in soil (Burns 1983).

Arylsulfatase activity is a measure of the inherent capacity of a soil to catalyze the hydrolysis of ester sulfates.

The assay developed by Tabatabai and Bremner (1970a) to measure total soil arylsulfatase activity is simple and widely used; however, a number of researchers have raised questions as to the suitability of this method (Fitzgerald et al. 1985; Lou and Warman 1992). The high sorptive capacity of many soils often results in the adsorption of both the substrate added to soil and the product cleaved by the enzyme (Houghton and Rose 1976; Pettit et al. 1977). Fitzgerald and Strickland (1987) suggested that the problem of soil sorption can be overcome by conducting the enzyme assays on extracts rather than soil. They successfully detected S mineralization from organic ^{35}S compounds in soil extracts, due to arylsulfatase activity. The traditional assay described by Tabatabai and Bremner (1970a) measures arylsulfatase activity of soil and lysed microorganisms; however, an approach in which the microbial component is removed may be a better indication of truly abiotic enzyme activity.

The use of *p*-nitrophenyl sulfate as a substrate for soil arylsulfatase has also been criticized. Low molecular weight chromogenic (artificial) substrates may not reflect the action of sulfatases in response to their naturally occurring substrates (Dodgson et al. 1982). Fitzgerald et al. (1985) and Jarvis et al. (1987) have suggested that tyrosine *O*-sulfate, an ester sulfate known to occur in soil, might be a more suitable substrate. Whalen and Warman (1996) have identified low molecular weight ester sulfate compounds in soil extracts by measuring SO_4^{2-} hydrolyzed when these extracts are circulated through an immobilized arylsulfatase reactor. These compounds may be the naturally occurring substrate for abiotic arylsulfatase in soil.

The purpose of the present study was to (1) measure abiotic arylsulfatase activity in soil organic matter extracts (humic-arylsulfatase complexes) and compare this activity with total arylsulfatase activity in soil using a chromogenic substrate (*p*-nitrophenyl sulfate); (2) assess the ability of humic-arylsulfatase complexes to hydrolyze a relevant soil substrate (soil ester sulfates); and (3) to relate the soil S status and arylsulfatase activity to the S content of tissue and grain of wheat (*Triticum aestivum* L.) plants.

Materials and methods

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). The properties of these soils have been described previously (Whalen and Warman 1996). 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 control (0 kg N ha^{-1}) were also 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 bags at 4°C until analysis in order to examine the effect of storage on arylsulfatase activity in these soils. 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 for samples from the same plots (Simon-Sylvestre 1965).

Arylsulfatase activity in soil

Arylsulfatase activity in soil was measured using modifications of the arylsulfatase assay of Tabatabai and Bremner (1970a) suggested by Pettit et al. (1977) and Sarathchandra and Perrott (1981). All soil samples were air-dried and sieved (<2 mm), and 1 g soil was pre-incubated for 1 h at 20°C with 0.2 ml toluene to inhibit enzyme activity from microbial proliferation and de novo enzyme synthesis. Next, 4 ml 0.5 *M* NaOAc buffer (pH 5.8) and 1 ml 0.05 *M* *p*-nitrophenyl sulfate were added and the mixture was incubated for 1 h at 37°C. The reaction was terminated by cooling to 0°C in an ice bath, and the samples were centrifuged at 11 000 g for 10 min to collect the supernatant rather than through filtration, for convenience and to prevent product losses. Three milliliters of the supernatant liquid were combined with 2 ml 0.5 *M* NaOH and the absorbance of the yellow product was measured at 400 nm using a Beckman DU-70 spectrophotometer. Three replicates of each soil sample were examined and controls were performed to account for the natural soil color. The *p*-nitrophenol released by soil arylsulfatase enzymes was calculated by refering to a standard calibration curve developed using 10–50 μg *p*-nitrophenol.

Arylsulfatase activity in soil organic matter extracts

Soil organic matter was extracted from pooled soil samples, filtered through a 0.45-μm cellulose acetate filter to remove microbial cells and separated into three molecular weight fractions (<500, 500–10 000, and >10 000) using an Amicon stirred ultrafiltration cell as described by Whalen and Warman (1996). In a preliminary experiment we were unable to detect arylsulfatase activity in the clay fraction generated during the soil organic matter extraction, which by difference confirmed the association between arylsulfatase and soil organic matter.

Arylsulfatase activity was determined in the >10 000 molecular weight fraction of soil organic matter since the molecular weight of this enzyme ranges from 40 000 to 150 000 (Dodgson et al. 1982). Ten millilitres of the >10 000 fraction were placed in dialysis tubing (Spectra/Por 1 molecular porous membrane, MWCO 6000–8000) that had been cleaned with Spectra/Por Sulfide Removal Solutions to remove any S remaining from the manufacturing process. The >10 000 fraction was dialyzed with 200 ml distilled deionized water for 24 h at 25°C in order to remove background sulfate. Next, 3 ml substrate was added and the mixture was dialyzed with 200 ml distilled deionized water for 72 h. The dialysate was collected, and the retentate was dialyzed with water for an additional 24 h to ensure that all sulfate had been retrieved. Sulfate was measured in the dialysates turbidimetrically as $BaSO_4$ at 420 nm using a Beckman DU-70 spectrophotometer (Bardsley and Lancaster 1960).

The substrates used for determination of arylsulfatase activity in the soil organic matter extract were 0.05 *M* *p*-nitrophenyl sulfate in 0.5 *M* NaOAc buffer (pH 7.0) and a natural soil substrate found in the 500–10 000 molecular weight fraction of soil organic matter (pH 7.0). The soil substrate contained a known amount of hydrolyzable ester sulfates, which had been determined using an immobilized arylsulfatase reactor (Whalen and Warman 1996). In preliminary experiments, while adding only the substrates to dialysis tubing, we found no sulfate in the dialysate.

At least three replicates were performed for each arylsulfatase analysis. The results were evaluated statistically by the PROC GLM

function of the Statistical Analysis System (SAS Institute 1990). Significant treatment means were compared using Tukey's test at the 95% confidence level.

Analysis of plants from wheat plots

An experiment was undertaken to assess the influence of soil S and arylsulfatase activity in relation to plant-available S for wheat grown on the Pugwash soil, which had received a variety of S amendments.

Spring wheat (*Triticum aestivum* L. cv. Belvedere) was grown in the Pugwash soil in a randomized complete block design containing four replicates of eight treatments, including K_2SO_4 (100 mg S kg^{-1}), gypsum (100 mg S kg^{-1}), and a control which had not been amended with S. The wheat was planted on May 10, 1993, and May 7, 1994, on soil fertilized with 675 kg ha^{-1} NPK (20:10:10) and 3 MT lime ha^{-1}. Plant tissue was sampled during the growing season by randomly selecting 12 plants from each plot on July 26, 1993 (heading stage), and Aug 9, 1994 (soft dough stage). The plants were cut at ground level, oven-dried at 60°C, and ground to pass through a 1-mm mesh using a Wiley mill. Grain from these plots was collected at harvest (Aug 27, 1993, and Aug 18, 1994), oven-dried, threshed, and ground (<1 mm). Total C, N, and S in grain and plant tissue were determined by combusting 200 mg material at 1350 °C using a LECO CNS-1000 Analyzer.

The means of the soil S analysis of the pooled K_2SO_4, gypsum, and control treatments were compared with the means of wheat grain and tissue S from the corresponding treatments to determine the relationship between soil and plant S. Correlation analysis was done using the PROC CORR function of the Statistical Analysis System (SAS Institute 1990).

Results and discussion

Arylsulfatase assay with soil and humic–arylsulfatase complexes

The arylsulfatase activity of air-dried soil ranged from 29 to 234 µg *p*-nitrophenol g^{-1} soil h^{-1} in the Pugwash soil and from 99 to 228 µg *p*-nitrophenol g^{-1} soil h^{-1} in the Acadia soil. The activity in the Acadia soil was virtually unchanged between fall and spring sampling; however, a dramatic change was observed in arylsulfatase activity in the Pugwash soil between the fall and spring samplings. Cooper (1972) observed a similar trend, which was attributed to seasonal variation in soil conditions.

It seems likely that the low arylsulfatase activity observed in the spring Pugwash samples was due to enzyme inhibition. SO_4^{2-}, S^{2-}, and PO_4^{3-} have been shown to act as non-competitive inhibitors of arylsulfatase activity (Dodgson et al. 1982), while sulfate and phosphate inhibited organic ^{35}S mineralization in organic matter extracts containing arylsulfatase activity (Fitzgerald and Strickland 1987). These inhibitors are removed by plants and microorganisms during the growing season and thus the apparent increase in arylsulfatase activity in the fall samples may have been a function of decreased inhibition. The Acadia soil did not display such profound seasonal differences, probably due to adsorption of the inhibitory anions in this silty clay soil.

It is interesting that when the extracted humic-arylsulfatase complexes were assayed with *p*-nitrophenyl sulfate, no significant seasonal differences were observed nor were

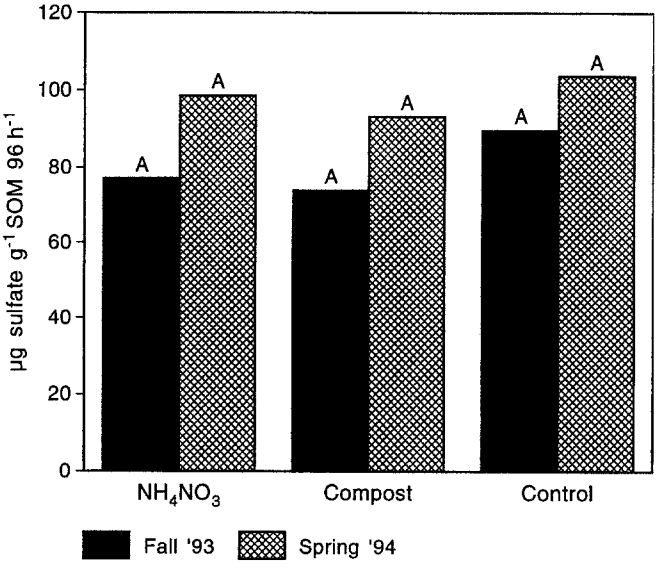

Fig. 1 Humic-arylsulfatase activity in Acadia soil using *p*-nitrophenol sulfate as the substrate. Bars carrying the same *letter* are not significantly different at $P=0.05$. *SOM* soil organic matter

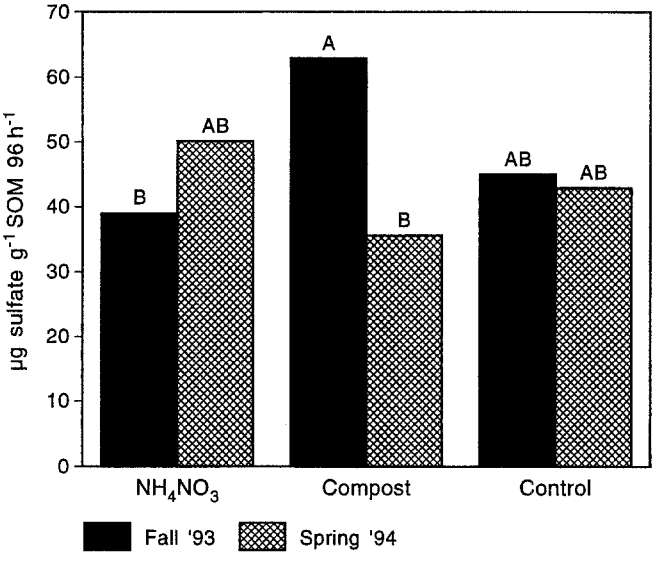

Fig. 2 Humic-arylsulfatase activity in Pugwash soil using *p*-nitrophenol sulfate as the substrate. For further explanations, see Fig. 1

there any differences between treatments in either soil (Fig. 1, 2). The separation of the soil organic matter extracts into different molecular weight fractions probably removed potential inhibitors. The <500 molecular weight fraction has been reported to contain appreciable quantities of sulfate (Whalen and Warman 1996) and likely contained other inhibitory anions. Overall, the Pugwash soil had slightly higher arylsulfatase activity than the Acadia. These results appear to support the theory that soil enzymes are persistent and relatively stable to degradative forces (Burns 1983, 1986). Arylsulfatase activity has been detected in peatland soils more than 6000 years old (Speir

Table 1 Hydrolyzable ester sulfates released by humic-arylsulfatase complexes as a percentage of sulfate released in an immobilized sulfatase reactor. *SOM* soil organic matter

Treatment	SO_4^{2-} from immobilized sulfatase reactor (μg g^{-1} SOM)	SO_4^{2-} from humic-arylsulfatase complexes (μg g^{-1} SOM)		Ratio SO_4^{2-} from arylsulfatase to SO_4^{2-} from immobilized sulfatase (%)	
		Fall 1993	Spring 1994	Fall 1993	Spring 1994
Pugwash soil					
Control	90.3	82.9	74.3	79	56
Gypsum	117.7	52.4	46.4	45	39
K_2SO_4	111.2	71.1	50.2	75	67
Acadia soil					
Control	65.4	45.0	42.9	69	66
Compost	102.1	63.0	35.5	62	35
NH_4NO_3	87.4	38.9	42.9	45	57

and Ross 1990); furthermore, arylsulfatase has been shown to be quite stable to freeze-drying, proteolysis, and irradiation treatments (Tabatabai and Bremner 1970a; Pettit et al. 1977); although temperature extremes (>75°C) rapidly result in complete loss of enzyme activity (Pettit et al. 1977).

Humic-arylsulfatase activity was determined using a soil substrate, the ester sulfates contained in the 500–10 000 fraction of extracts (low molecular weight substrate). The quantity of hydrolyzable ester sulfate in this fraction had been determined previously using an immobilized sulfatase reactor (Whalen and Warman 1996). The sulfate hydrolyzed by humic-arylsulfatase complexes was compared to that hydrolyzed by the immobilized sulfatase reactor (Table 1). In the Acadia soil, the humic-arylsulfatase complexes were able to hydrolyze about 35–70% of the ester sulfates identified by the immobilized sulfatase reactor, while the Pugwash humic-arylsulfatase complexes hydrolyzed about 40–80%, depending on the treatment.

Measurement of humic-arylsulfatase activity with *p*-nitrophenol sulfate does not reflect the ability of this enzmye to hydrolyze naturally occurring soil ester sulfates (low molecular weight substrate; Fig. 3, 4). When an excess of the small chromogenic substrate (*p*-nitrophenyl sulfate) was added to the humic-arylsulfatase complexes, there was no significant difference between treatments in either soil. This substrate was more readily hydrolyzed than the low molecular weight substrate, which was due in part to the forms and molecular sizes of ester sulfates in the low molecular weight substrate extracts. In addition, the concentration of low molecular weight substrate may have been a limiting factor in the ease of hydrolysis of these compounds. Lou and Warman (1994) and Whalen and Warman (1996) showed that the low molecular weight substrate required a longer period of hydrolysis than *p*-nitrophenyl sulfate in the immobilized sulfatase reactor in order to achieve maximum release of the product. The concentration of the humic-arylsulfatase complexes may have also been a limiting factor; the maximum release of product from these complexes was achieved after 96 h of dialysis.

Although the methodology outlined in this paper is more tedious than the traditional arylsulfatase assay, it has a greater potential for detecting mineralization of natural ester sulfate compounds by humic-arylsulfatase complexes.

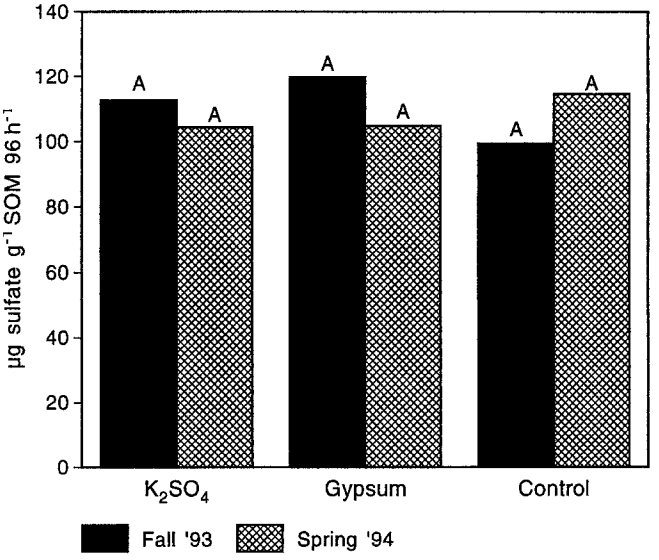

Fig. 3 Humic-arylsulfatase activity in Acadia soil using low molecular weight soil ester sulfates as the substrate. For further explanations, see Fig. 1

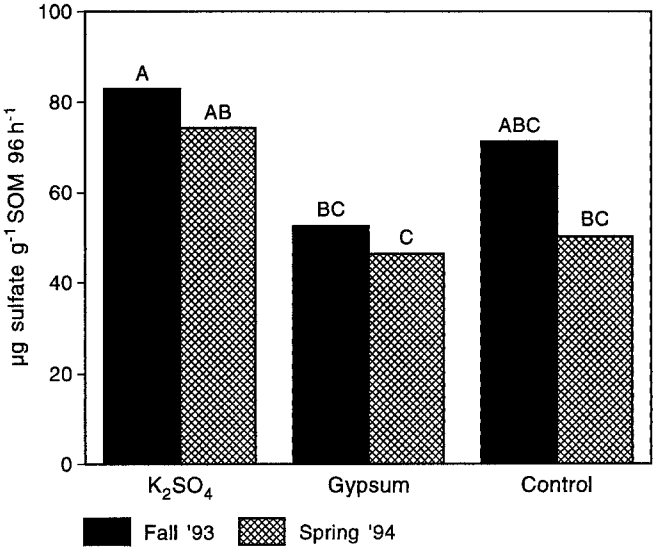

Fig. 4 Humic-arylsulfatase activity in Pugwash soil using low molecular weight soil ester sulfates as the substrate. For further explanations, see Fig. 1

Relationship between soil S and plant-available S

The S status and arylsulfatase activity of the fall soil samples were fairly well correlated to total S in wheat grain or tissue in both years; however, no clear relationships were observed between S in spring soil samples and plant S. The seasonal variation in sulfate concentrations may reflect the fact that S mineralization is primarily microbially mediated (McGill and Cole 1981). Thus, only the correlations between fall soil samples and plant S have been shown (Table 2). Many of the correlation coefficients were not statistically significant because only a few levels of comparison were examined in this preliminary experiment.

Similar trends were observed for both the 1993 and 1994 tissue samples (Table 2). A positive correlation was observed between $Ca(H_2PO_4)_2$-extractable sulfate and tissue S. Plant-available S is derived from water-soluble and adsorbed sulfate fractions, with possibly some contribution from labile organic S fractions (McGill and Cole 1981). $Ca(H_2PO_4)_2$ is known to extract sulfate from all three of these fractions (Alewell 1993), and was highly correlated (r=0.96) with plant-available S for spring wheat at the same stage of development (Warman and Sampson 1994).

Hydriodic acid-reducible sulfate also showed a strong positive correlation with tissue S, although hydrolyzable ester sulfates in the high molecular weight (>10 000) and low molecular weight (500–10 000) fractions were not as strongly associated with tissue S. Hydriodic acid-reducible S is often equated with total soil ester sulfates; however, the procedure releases S from sulfamates, sulfamides, and *S*-sulfocysteine as well as ester sulfates (Freney 1986). The relationship between hydriodic acid-reducible S and tissue S suggests that some other organic S compounds, in addition to ester sulfates, may be more readily mineralized and available for plant uptake than was previously thought.

Arylsulfatase activity in soil and soil extracts assayed with the chromogenic substrate *p*-nitrophenyl sulfate demonstrated a strong positive relationship with tissue S. Humic-arylsulfatase complexes assayed with a natural low molecular weight soil substrate were negatively correlated with tissue S.

Table 2 Pearson correlation coefficients for comparison of plant S and various soil S sources. *HI* hydriodic acid, *LMW* low molecular weight, *HMW* high molecular weight, *NPS* nitrophenol sulfate, *SOM* soil organic matter. * P=0.10, ** P=0.05

Soil S sources	Wheat tissue		Wheat grain	
	1993	1994	1993	1994
HI-reducible S	0.9429	0.9998**	–0.1659	0.6799
$Ca(H_2PO_4)_2$ extractable S	0.8926	0.9940*	–0.2904	0.7679
LMW ester sulfates	0.5581	0.8136	–0.7213	0.9831
HMW ester sulfates	0.6860	0.8975	–0.5987	0.9401
Arylsulfatase activity				
Soil + *p*-NPS	0.9719	0.9928*	–0.0647	0.6018
SOM + *p*-NPS	0.8276	0.9719	–0.4105	0.8436
SOM+LMW substrate	–0.9838	–0.9842	0.0073	–0.5548

The relationships between grains S and soil S sources were not as well defined (Table 2). The data for grain harvested in 1993 demonstrated weak negative correlations with soil S; however, in the 1994 harvest, grain S demonstrated positive correlations with most soil S sources, the exception being a negative correlation with humic-arylsulfatase activity assayed with the natural soil substrate.

Mahler and Maples (1987) found that minimal S accumulation occurs in wheat grain when excess S is supplied to wheat plants. The concentration of S in wheat grain was 2.0–2.2 mg S g^{-1} grain, while tissue S was considerably more variable, ranging from 1.4 mg S g^{-1} tissue in the control plots to 2.6 mg S g^{-1} tissue in S-amended soils.

Obviously, a number of processes occur simultaneously and plant-available S is derived from several sources in soils. More work is needed to elucidate the structure of ester sulfate compounds in soil, particularly low molecular weight hydrolyzable ester sulfates identified using an immobilized arylsulfatase reactor. These compounds are available for hydrolysis by humic-arylsulfatase complexes extracted from soil and have demonstrated linear relationships with S in wheat tissue. Measurement of arylsulfatase activity using humic-arylsulfatase complexes rather than soil not only gives a better indication of how abiotic arylsulfatase enzymes respond to a natural soil substrate, but may also provide insight into the mineralization of hydrolyzable low molecular weight ester sulfate compounds and the subsequent availability of sulfate to growing plants.

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