

Atmospheric SO₂ Emissions Since the Late 1800s Change Organic Sulfur Forms in Humic Substance Extracts of Soils

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Atmospheric SO₂ emissions in the UK and globally increased 6- and 20-fold, respectively, from the mid-1800s to the 1960s resulting in increased S deposition, acid rain, and concurrent acidification of terrestrial and aquatic ecosystems. Structural analyses using synchrotron-based X-ray near-edge spectroscopy (XANES) on humic substance extracts of archived samples from the Rothamsted Park Grass Experiment reveal a significant ($R^2 = -0.58$; $P < 0.05$; $N = 7$) shift in soil organic sulfur (S) forms, from reduced to more oxidized organic S between 1876 and 1981, even though soil total S contents remained relatively constant. Over the last 30 years, a decrease in emissions and consequent S deposition has again corresponded with a change of organic S structures of humic extracts—reverting in the direction of their early industrial composition. However, the observed reversal lagged behind reductions in emissions by 19 years, which was computed using cross correlations between time series data ($R^2 = 0.66$; $P = 0.0024$; $N = 11$). Presently, the ratio of oxidized-to-reduced organic S in humic substance extracts is nearly double that of early industrial times at identical SO₂ emission loads. The significant and persistent structural changes of organic S in humic substances as a response to SO₂ emissions and S deposition may have effects on recuperation of soils and surface waters from acidification.

Introduction

Large industrial and domestic SO₂ emissions have drastically changed global S fluxes during the past 150 years (1, 2). The effects of anthropogenic SO₂ emissions on inorganic S cycles in terrestrial ecosystems include acid rain, resultant forest decline, and surface water acidification (3, 4). It is now clear that S deposition and concurrent acidification not only affect inorganic soil properties (3, 5), but also organic S pools in soils (6–13). More than 95% of total S occurring in unpolluted soil is typically in the organic form and therefore largely controls the dynamics and leaching of inorganic sulfate (2). Therefore, changes in S cycling in soil organic matter have

important implications for ecosystem functions of forests and grasslands with respect to acidification of surface waters.

Over the past 30 years anthropogenic emissions of SO₂ have been significantly reduced in many European and North American countries (15, 16). Yet inorganic S cycles have not fully recovered and inorganic sulfate is still being released from forested watersheds (17–19) together with large amounts of base cations, causing acidification of surface waters (20–24). In some cases, increasing acidification was reported even long after emissions had been reduced (25). Wherever recovery from SO₂ emissions is found, it lags behind the emission reductions (23) and is not reflected in a biological recovery comparable to the observed reductions of chemical acidification (26). Additionally, there is evidence that most of the atmospherically derived sulfate found in streams is microbially cycled (27–29) and that mineralization of soil organic S is largely responsible for the delayed reduction in stream sulfate (6, 30–34). The question remains whether the reason for the continued mineralization of organic S despite the significant reductions in atmospheric deposition is simply due to the buildup and subsequent mineralization of organic S or whether this is more related to structural changes in organic S.

One of the reasons for the lack of conclusive data on organic S forms under long-term atmospheric S deposition in general has been the limitations of the available wet-chemical fractionation procedures such as the HI method (35). In this method, the organic S forms are distinguished by different operationally defined fractions, where some of them are directly determined while others are calculated as arithmetic differences between analytically determined fractions. Thus, error propagation for the calculated S fractions may result in considerable inaccuracy (36). In addition, this chemical reduction technique can not be used to quantify intermediate oxidation states of organic S (37). Novel analytical technologies such as sulfur K-edge X-ray absorption near-edge spectroscopy (XANES) allow intermediate oxidation states to be quantified and do not require sequential extractions and afford new perspectives on structural composition of organic S in soils (38, 39).

In this study, we investigate the effects of anthropogenic SO₂ emissions and subsequent S deposition on structural composition of organic S in humic substance extracts over the past 130 years, using sulfur K-edge XANES spectroscopy of archived soil samples from the Rothamsted Park Grass Experiment. To our knowledge, such a long-term field study on organic S forms under changing SO₂ emissions and deposition have not been reported, and information about the effects of such emissions on organic S dynamics specifically for grassland ecosystems is largely missing.

Materials and Methods

Experimental Sites. The Rothamsted Park Grass Experiment is located in a semirural environment in Hertfordshire, England, approximately 40 km north of London (longitude 0° 21' W, latitude 51° 49' N, elevation 128 m above sea level). Annual rainfall ranged from 540 to 905 mm over the last 140 years, with a mean of 650 mm. The soil is a moderately well drained Aquic Paleudalf with a flinty silty clay loam topsoil on clay-with-flints overlying chalk parent material. The topsoil to 0.23 m contains approximately 25% clay, 57% silt, and 18% sand. The experimental site had been under permanent grass for several centuries before the experiment began in 1856. The original objective of the experiment was to test the effects of fertilizer on the yields of herbage. Herbage is cut and removed twice yearly: in June and between

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TABLE 1. Characteristics of Unfertilized Grassland Soils at the Rothamsted Park Grass Experiment since 1876^a

year	BD (Mg m ⁻³)	pH (CaCl ₂)	organic C (mg g ⁻¹)	total N (mg g ⁻¹)	total S (mg kg ⁻¹)	extractable S (mg kg ⁻¹)
1876	1.06	5.2	31.8 (1.4) ^b	2.62 (0.1)	386 (9)	36.3 (5.2)
1906	nd	nd	26.6	2.41	334	29.8
1923	nd	4.9	32.4	2.78	353	34.1
1959	nd	nd	31.5	2.85	346	55.9
1966	1.03	4.6	28.2	2.47	333	30.6
1976	nd	nd	31.0	2.76	363	31.5
1981	nd	4.5	28.5	2.55	333	24.9
1984	1.05	nd	30.4	2.70	366	31.8
1991	nd	4.2	24.8	2.24	337	22.4
1996	nd	nd	35.4 (1.1)	3.07 (0.1)	393 (3)	9.6 (0.6)
2002	nd	4.4	32.6	2.59	332	16.6

^a Soil C, N, and S concentrations are from ref (44) and soil pH is from ref (42); BD = bulk density; nd = not determined.
^b Standard error (N = 4).

September and November. Details of the experiment have been described elsewhere (40). In our study we used the soil samples from the unlimed control plot, which has never received any fertilizer or lime treatments. The plot sizes were initially 74.5 by 13.3 m, which were later changed to 18.8 by 13.3 m. The treatments were unreplicated because the experiment was initiated before the advent of modern statistics.

Soil Sampling and Analysis. Soil samples from 1876, 1906, 1923, 1959, 1966, 1976, 1981, 1984, 1991, 1996, and 2002 were subsampled from the Rothamsted sample archive (Table 1). Soils were taken from the top 0.23 m depth, bulked into one sample for the whole plot, air-dried, ground to pass a 2 mm sieve, and stored in sealed glass jars. Over the storage period of 130 years, changes in properties relevant to the present study cannot be excluded. Care was taken that the soil was not contaminated during storage. Oxidation of organic sulfur may have occurred over time and remains unverifiable. Such oxidation would, however, rather decrease the trends shown in this study and the conclusions drawn remain valid. In 1876 and 1906, three subsamples were collected with a square metal box (30 × 30 cm). In later years, between 15 and 20 cores were taken with an auger (2.5 cm diameter). Soil pH and extractable S were measured on <2 mm soil samples, whereas for total C, N, and S soils were further ground to <0.25 mm. Soil pH was determined with a glass electrode in a suspension of 1:2.5 soil: CaCl₂ (0.01 M). Total C and N were analyzed using a LECO CNS-2000 elemental analyzer (LECO, St. Joseph, MI). Soils were digested with a mixture of nitric and perchloric acids (41), and the concentration of S in the digests was determined using inductively coupled plasma atomic emission spectrometry (ICP-AES; Fisons ARL Accuris, Ecublens, Switzerland). Soils were extracted with 0.016 M potassium phosphate in a 1:5 (w/v) ratio, and the extractable S was determined using ICP-AES.

Organic S forms were analyzed by X-ray spectroscopy which required separation of organic S from the remainder of the soil which also contains inorganic S forms. Therefore, organic matter was extracted from the soils with a 0.1 M NaOH and 0.4 M NaF mixture at a soil-to-extraction solution ratio of 1:5 (w/v) under N₂ environment and the combined extracts were dialyzed (Spectra/Por Membrane, MWCO, 12 000–14 000 Da; Spectrum Laboratories, CA) and called hereafter humic substances (37). Solid-state characterization of S oxidation states in these humic substance extracts was carried out using S K-edge X-ray absorption near-edge structure (XANES) spectroscopy at beamline X-19A of the National Synchrotron Light Source (NSLS), at Brookhaven National Laboratory. Scans ranging from 150 eV below to 300 eV above the absorption edge of S at 2472 eV (calibrated with elemental S) were collected with a step size of 0.2 eV. Each XANES spectrum was composed of an average of three scans. A monochrome consisting of a double crystal Si (111)

TABLE 2. Relative Abundance (% of Total Extracted Organic S) of Organic Sulfur Forms in Unfertilized Grassland Soils As Affected by the Deposition of Anthropogenic Sulfur Emissions at the Rothamsted Park Grass Experiment Since 1876

year	Gaussian curve				
	G1 thiols and sulfides (0.14–0.38) ^a	G2 thiophenes (0.80–0.96)	G3 sulfoxides (1.25–1.82)	G4 sulfonates (5.00)	G5 ester sulfates (6.00)
1876	19.0	7.8	17.1	25.0	31.2
1906	15.1	3.8	9.0	22.6	49.5
1923	15.3	4.8	16.1	28.0	35.8
1959	16.9	5.2	12.5	27.7	37.8
1966	12.0	6.4	17.2	27.9	36.6
1976	15.0	4.1	13.8	27.6	39.5
1981	6.0	8.1	17.4	22.7	45.9
1984	9.3	5.0	18.0	23.7	44.0
1991	6.0	6.6	20.8	19.9	46.7
1996	14.4 (0.3) ^b	3.6 (0.3)	14.0 (1.8)	28.1 (0.4)	39.9 (1.2)
2002	13.2	6.7	17.9	27.3	34.9

^a Electronic oxidation states in eV. ^b Standard error (N = 4).

with an entrance slit of 0.5 mm and a minimum energy resolution of 2×10^{-4} (0.5 eV) at the S K-edge was used. The spectra were recorded in fluorescence mode using a passivated implanted planar silicon (PIPS) detector (Canberra Industries, CT). The beam path from the incident ion chamber to the sample chamber was purged with He gas. The samples were pressed into a 0.5 mm deep acrylic holder and covered with 2.5 μm thick Mylar film (Complex Industries, NY). Deconvolution of XANES spectra for each sample into pseudocomponents was done using the nonlinear least-squares fitting routine solver of MS Excel (39). The XANES spectra were fitted using a series of Gaussian curves (G1, G2, G3, G4, and G5) that represent the s→p transitions (white line) and arctangent step functions (AT1 and AT2) of the transitions of ejected photoelectrons to the continuum (step height or background) (37, 39). These Gaussian curves correspond to oxidation states and organic S forms (Table 2).

Statistical analyses of the effects of time and SO₂ emissions (used as a surrogate for deposition which follows a similar trend (42) and matches dynamics of herbage S isotope contents shown in Figure 1a) on organic S forms in humic substance extracts were done by linear regressions and *t*-tests (Statistica 5.1, Tulsa, OK). Time series analyses were performed using cross correlations between emissions and organic S forms in soil humic substances (43). The lag time was determined by linear regression using the best fit in yearly intervals. The time-series included 11 soil samples

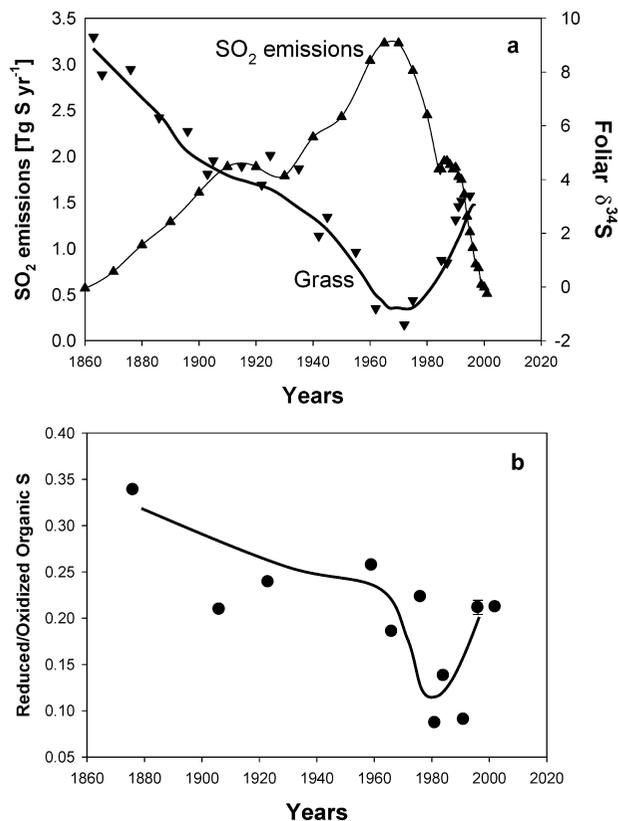


FIGURE 1. Annual emissions of SO_2 in the UK, $\delta^{34}\text{S}$ isotope values (from ref 44) for foliar S of the forage grass (a) and ratio of reduced-to-oxidized organic sulfur forms (organic sulfides to sulfonates + ester-sulfates, $G1/(G4 + G5)$ from Table 2) of humic substance extracts from grassland soils ($N = 4$ for 1996, mean and standard deviation) (b) as affected by anthropogenic sulfur emissions at the Rothamsted Park Grass experiment since 1876. Curves fitted to emission data using cubic splines and to $\delta^{34}\text{S}$ using distance weighted least-squares.

taken from the same plot at 0.5–3 decade intervals. Although more samples at different time points would be desirable, this sample set is probably the best of its type in the world in terms of the duration and records of the experiment. The satisfactory fit of model and data allowed for an appropriate representation of the long-term dynamics of organic S forms in soil humic substances. Additionally, the samples used in the present study are part of a larger data set for which the observed dynamics hold as seen from the stable S isotopic values (44). In 1876 and 1996, 3 or 4 subsamples were taken from different locations within the same plot and analyzed separately for basic chemical characteristics of S, in 1996 additionally for organic S forms in humic substance extracts using XANES. The results showed a variability of less than 10% for samples taken at different locations within the studied field (Tables 1 and 2).

Results and Discussion

Anthropogenic SO_2 Emissions. Anthropogenic SO_2 emissions have increased in the UK since the early 19th century up until the 1970s (Figure 1a). Wet deposition of sulfate measured at the experimental site follows the general temporal pattern as that of the national SO_2 emissions (42). Furthermore, Zhao et al. (44) showed that the S stable isotopic composition of the herbage from this experimental plot mirrored the SO_2 emission pattern between 1866 and 1996, suggesting that emission data can be used as a surrogate for S deposition in our study.

The increase in emissions and deposition over the 140-year period was accompanied by a trend in increased soil

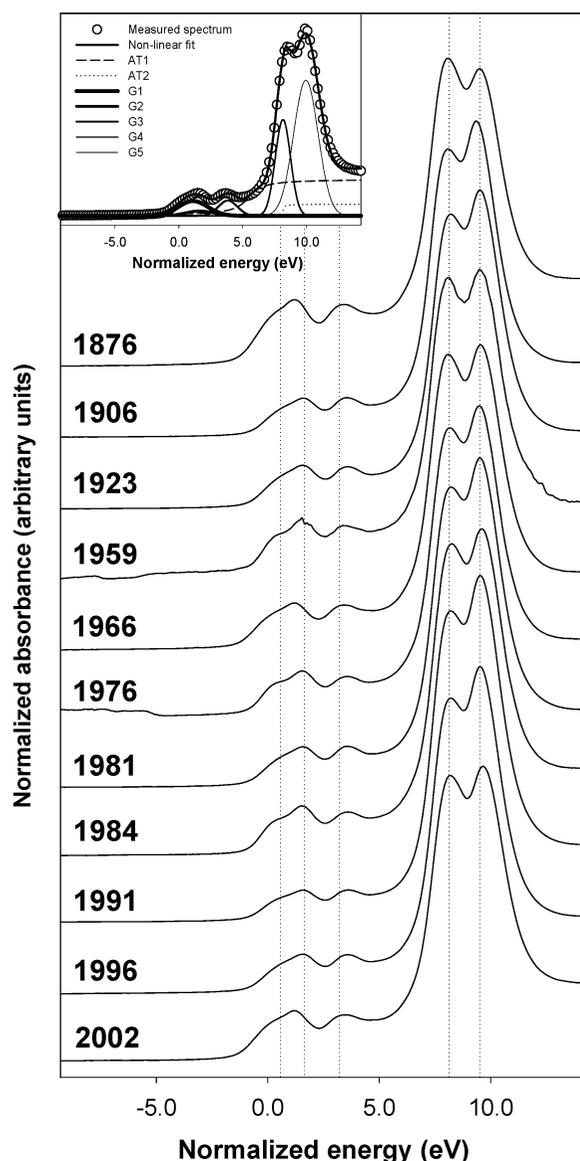


FIGURE 2. Near-edge X-ray absorption fine structure (NEXAFS) spectra of humic substance extracts from grassland soils at the Rothamsted Park Grass experiment since 1876. Dotted lines indicate approximate location of energy transitions for the different organic S forms (G1 to G5 from left to right, see Table 2).

acidification and a weaker trend in higher extractable soil S (Table 1 and Blake et al. (42)). The increase in N deposition has been much smaller than S deposition since 1850 (42), and thus would constitute a smaller proportion of the total proton input to soil than S. This acidification led to a depletion of exchangeable Ca and Mg and an 8-fold increase in exchangeable Al (42). Similar evidence of acidification and depletion of base cations have been linked directly to atmospheric S emissions and estimated deposition for example in west-central Alberta (7) or at Hubbard Brook in New Hampshire (20). An increase in pH as a response to the decreasing emissions and deposition since the 1970s remains speculative from the data in Table 1. Modeling by Sverdrup et al. (45) using a dynamic biogeochemical model suggested that a decrease of total proton input to 17% of the 1995 values is required to maintain the soils at their present pH.

Changes in Organic S Forms. The proportion of reduced organic S compounds in the humic substance extracts decreased from 1876 to 1991, while the proportion of oxidized organic S increased (Figure 2; Table 2). The ratio of reduced-

to-oxidized organic S (Figure 1b) was significantly greater before 1921 than between 1966 and 1996 ($P < 0.05$; $N = 3$) and followed a linear decrease pattern with a slope of -0.02 yr^{-1} from early industrial times to the peak of SO_2 emissions in the late 1960s ($R^2 = 0.58$; $P < 0.05$; $N = 7$). This effect was a result of both a net increase of ester-sulfates (G5) and a net decrease of reduced carbon-bonded S (amino acid-S, G1; Table 2) as total S contents did not change with time (Table 1).

We propose to explain these shifts from more reduced to more oxidized organic S forms of the humic substances by the increase in S emissions and deposition (the slight lag in shifts compared to peak emissions is discussed below). Biochemical mineralization of ester-sulfates can be significantly reduced under conditions of inorganic sulfate inputs (46); the activity of aryl-sulfatase was found to be much lower under high inorganic sulfate inputs (47). At the Park Grass site, water-extractable inorganic sulfate peaked in 1959 and decreased thereafter. A decrease of enzymatic ester-sulfate hydrolysis has commonly been explained by a negative feedback from the amount of inorganic sulfate present in soil on the release of inorganic sulfate from ester-sulfates by exoenzymes (48). Therefore, the increase in the proportions of ester-sulfates may not have been caused directly by a transformation of inorganic sulfate into ester-sulfate but by a decrease in ester-sulfate mineralization. On the contrary, sulfate from atmospheric deposition was most likely incorporated biologically into carbon-bonded S as shown for several forested soils in Germany (6, 11, 13). However, incorporation of inorganic sulfate into the ester-sulfate pool by microorganisms has been reported in short-term laboratory incubations (8, 9). This incorporation is most likely an intermediate step in microbial S immobilization (49) and may have contributed to an increase in ester-sulfates over short periods of time. Yet, this process appears to be unlikely to have a dominant effect over long periods of time, as microorganisms may not need S storage (48) under conditions of prolonged inorganic sulfate deposition. It can only be speculated whether also a greater transformation of carbon-bonded S to more oxidized organic S forms played a role in the accumulation of oxidized organic S forms, especially under conditions of increased N deposition (14). In summary, it is possible that the mechanism for the increase in ester-sulfates through SO_2 emissions includes either one or all of the above processes, since the microbially mediated pathways between organic S pools are still poorly understood (49). The pathways may additionally depend on the S balance determined by the amount of S deposition and S output. For example Mayer et al. (13) showed in a lysimeter experiment that S retention in soil organic matter was significantly affected by the amount of S applied through irrigation, although stable isotope results from 13 different catchments in the Czech Republic suggested similar pathways irrespective of historic deposition (28). Also the pool into which inorganic sulfate is incorporated may depend on the amount of sulfur added. Using wet-chemical fractionation with HI, Ghani et al. (50) report a greater incorporation of S into ester-sulfate with decreasing inorganic sulfate additions. An additional factor may be differences between ecosystems. Forest soils may contain lower proportions of ester-sulfate than grassland soils, comparing XANES data for example from three grassland soils in South Africa (35% in humic substance extracts (37)) with two forest soils in Germany (15–17% in total soil including inorganic sulfate (36)), with the caveat that the amount of data available is still scarce.

In this respect it is noteworthy that the total S contents did not change over the observation period (Table 1). This may be explained by, first, the continued biological mineralization of carbon-bonded S driven by microbial demands for energy (48). For example, Zhao et al. (51) found a better

correlation between S mineralization with reduced organic S forms (aminoacids, G1 and G2 in Table 2) determined by K-edge XANES than with ester-sulfate of humic substances extracts from a range of soils, explaining observed plant uptake of S released from carbon-bonded organic S forms (52). This biological release of inorganic sulfate would not be decreased by an abundance of inorganic sulfate in the soil solution. Second, the annual removal of herbage or more likely sulfate leaching (53) may have offset the input of S by deposition. Following the experimental evidence from other studies discussed above, this lack of total S increase in soil may explain the relative increase of ester-sulfate as a net effect of atmospheric S deposition over long periods of time.

Organic S Forms and Emission Reductions. Since 1972, following reductions in SO_2 emissions and consequent reductions in S deposition (42), stable S isotope values in the herbage from the Rothamsted Park Grass Experiment showed a lagged though substantial recovery toward those of the early soil source (Figure 1a). The XANES analyses also suggest that the organic S forms in humic substances extracts changed in the direction of values observed before the increase in UK emissions and S deposition with a greater proportion of reduced organic S forms (Figure 1b). The soil organic S forms, however, did not change as rapidly as the deposition reductions and the ratio of reduced-to-oxidized S started to revert only after 1991. Cross correlations between time series suggested a lag time of 19 years ($R^2 = 0.66$; $P = 0.0024$; $N = 11$) between changes in emissions and soil organic S forms of humic substances extracts (Figure 3a; a simple correlation between emissions and organic S forms was not significant with $R^2 = 0.05$). This may suggest that structural changes of organic S forms in humic substance extracts were delayed in comparison to reductions in SO_2 emissions (Figure 3b) and deposition. Today, at SO_2 emission loads identical to those of early industrial times, we find nearly double the amount of oxidized organic S compounds than reduced organic S in humic substance extracts at the Park Grass site.

A shift from more reduced organic S forms, such as amino acids, to more oxidized organic S forms, as a consequence of increased SO_2 emissions and S deposition, could result in a greater biochemical release of inorganic sulfate from ester-sulfate and therefore continued leaching of inorganic sulfate once emissions are reduced (14). The continued yet possibly attenuated biochemical release of inorganic sulfate despite reductions in S deposition may occur because the production of inorganic sulfate from ester-sulfates by exoenzymes is under feedback control of inorganic sulfate in soil (46, 48) as pointed out above, and therefore increases with low inorganic sulfate concentrations. Such altered dynamics could explain the observed importance of mineralization for sulfate export from polluted forest ecosystems (31). Isotopic data suggests that 40% of drainage sulfate in two watersheds in Central Europe is microbially recycled S originating from S deposition (54), with a value of more than 60% reported from a watershed in Vermont (29), and of close to 100% below the organic surface layer at a site in Sweden (33).

The delayed reversal of organic S forms could also explain the continued acidification of surface waters through sulfate leaching in spite of the significant reductions in atmospheric SO_2 emissions and S deposition. This biochemical release of inorganic sulfate from the readily mineralizable form of ester-sulfate is in addition to any potential buildup of organic S as a consequence of the long-term deposition of SO_2 emissions as observed in a watershed in Sweden (34). The continued biochemical release of inorganic sulfate from organic S has to be kept in mind with respect to the ever increasing anthropogenic SO_2 emissions in large parts of the world such as Asia (55, 56). Since the present study is restricted to one long-term experiment in the UK, observed changes in organic S forms in humic substance extracts with increasing

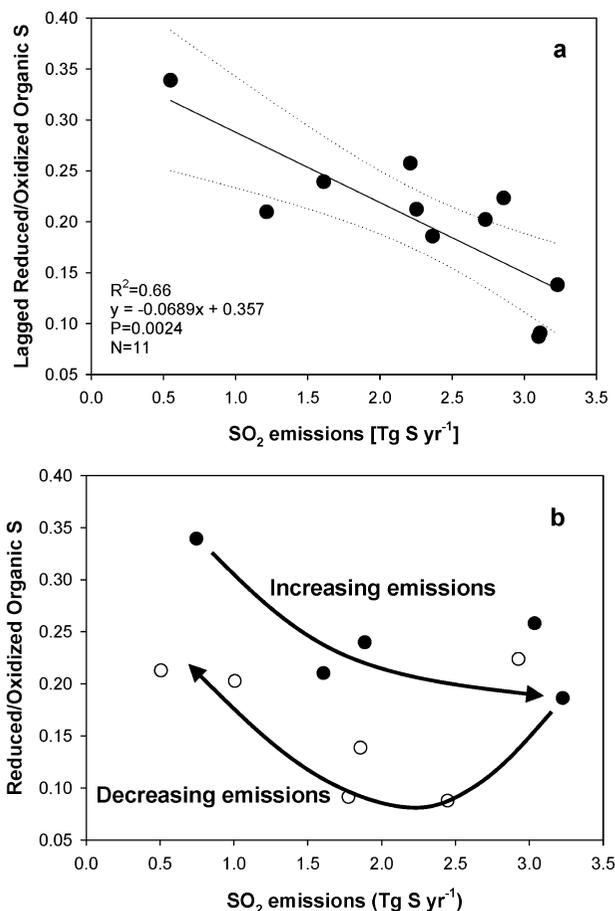


FIGURE 3. Relationship between total annual emissions of SO₂ in the UK and the ratio of reduced-to-oxidized organic sulfur forms (organic sulfides to sulfonates + ester-sulfates, G1/(G4 + G5) from Table 2) of humic substance extracts from grassland soils as affected by anthropogenic sulfur emissions at the Rothamsted Park Grass experiment since 1876. (a) Transformed data using cross correlation with a time lag of 19 years, dotted lines denote 95% confidence interval; (b) original data, filled symbols denote increasing, and open symbols denote decreasing SO₂ emissions.

and decreasing SO₂ emissions and deposition should be verified at other locations using S K-edge XANES. The conclusions drawn here justify an increased scrutiny in predicting the effects of pollution reductions on water quality.

This example of a soil ecosystem response to environmental change (here, SO₂ emissions) demonstrates that induced changes, such as the observed shift to more oxidized organic S, can proceed much more rapidly than their reversal after the environmental stress has stopped (in the case of reductions of atmospheric emissions and deposition). An environmental consequence of such a lag time is that ecosystems may recuperate much slower than predicted. This knowledge should be taken into account in streamwater prediction models as well as in environmental policy.

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