Speciation and Long- and Short-term Molecular-level Dynamics of Soil Organic Sulfur Studied by X-ray Absorption Near-Edge Structure Spectroscopy

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We investigated speciation, oxidative state changes, and long- and short-term molecular-level dynamics of organic S after 365 d of aerobic incubation with and without the addition of sugarcane residue using XANES spectroscopy. Soil samples were collected from the upper 15 cm of undisturbed grasslands since 1880, from undisturbed grasslands since 1931, and from cultivated fields since 1880 in the western United States. We found three distinct groups of organosulfur compounds in these grassland-derived soils: (i) strongly reduced (S^0 to S^{1+}) organic S that encompasses thiols, monosulfides, disulfides, polysulfides, and thiophenes; (ii) organic S in intermediate oxidation (S^{2+} to S^{5+}) states, which include sulfoxides and sulfonates; and (iii) strongly oxidized (S^{6+}) organic S, which comprises ester–SO_4–S. The first two groups represent S directly linked to C and accounted for 80% of the total organic S detected by XANES from the undisturbed soils. Aerobic incubation without the addition of sugarcane residue led to a 21% decline in organanosulfur compounds directly linked to C and to up to an 82% increase inorganic S directly bonded to O. Among the C-bonded S compounds, low-valence thiols, sulfides, thiophenic S, and intermediate-valence sulfoxide S seem to be highly susceptible to microbial attack and may represent the most reactive components of organic S pool in these grassland soils. Sulfonate S exhibited a much lower short-term reactivity. The incorporation of sugarcane residue resulted in an increase in organosulfur compounds directly bonded to C at the early stage of incubation. However, similar to soils incubated without residue addition, the proportion of organic S directly linked to C continued to decline with increasing duration of aerobic incubation, whereas the proportion of organic S directly bonded to O showed a steady rise.

Sulfur is essential for the development of all forms of life, and its cycling provides a key to understanding changes in the biosphere. It plays a critical role in the synthesis of amino acids, chlorophyll, a variety of enzyme cofactors (biotin, coenzyme A, coenzyme M, thiamine, and lipoic acid), and secondary S compounds (allins, glucosinolates, and phytochelatins). Sulfur is also vital in many redox processes as a building block for iron-S centers and as the redox-active component of disulfide bonds (Kertesz, 1999, 2007; Solomon et al., 2005; Eriksen, 2009). Electronic expansions into *d*-orbitals allow S to assume several valencies and exhibit by far the greatest range in oxidation states (2^- to 6^+) of the most geochemically abundant elements in Earth's crust (Fleet, 2005). This provides S the ability to catenate (i.e., to form multiple S-S linkages) and thereby form mixed oxidation state species, permitting this element to exist in variety of organic and inorganic compounds and adding complexity to its speciation dynamics in the soil environment (Vairavamurthy, 1998; Fleet, 2005; Solomon et al., 2009). Inorganic S occurs in reduced (elemental S [S^0], thiosulfates [$S_2O_3^{2-}$], and sulfite [SO_3^{2-}]) and oxidized (sulfate [SO_4^{2-}]) states (Gerardi, 2006). However, in a freely draining oxic soil environment, inorganic S occurs primarily as SO_4^{2-}, whereas the reduced inorganic S forms are often transitory (although some reduced inorganic S forms, such as pyrites, were shown to persist in soils over an extended period of time even after the soils were oxidized [Prietzel et al., 2009]), and their concentration is usually negligible (Kowalenko, 1993; Saggar et al., 1998; Solomon et al., 2005). Thus, the major proportion of total soil S (up to 98%) in most temperate and tropical soils is present in organic S forms (Biederbeck, 1978; Stanko-Golden and Fitzgerald, 1991; Janzen and Ellert, 1998; Solomon et al., 2001, 2003, 2005). Enhanced emission control measures and the increasing use of high-analysis, low–S-containing fertilizers have led to dramatic reductions in the deposition of S in the soil environment in recent years (Zhao et al., 1996; Blair, 2002). These changes have made plants and soil biota increasingly dependent on soil organic S for the supply of this critical nutrient element (Kertesz and Mirleau, 2004).

Soil organic S is a heterogeneous mixture of organic molecules representing substrates released from living plants and animals

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Abbreviations: I-S, sulfur in intermediate oxidation state; O-S, strongly oxidized sulfur; R-S, strongly reduced sulfur; XANES, X-ray absorption near-edge structure.

(enzymes, surface-active proteins, chelating compounds) as well as plant and animal residues transformed by microbial degradation ranging in size and complexity from simple monomers to complex biopolymers (Zhao et al., 1996; Solomon et al., 2009). It is a major component of the global biogeochemical S cycle, acting as source and sink of various soil S species in different ecosystems across the globe. Its composition, geochemical environment, and dynamics often influence S oxidation state and speciation, which in turn affect its bioavailability and metal pollutant mobility within the soil environment (Dhamala and Mitchell, 1995; Martínez et al., 2002; Schroth et al., 2007; Solomon et al., 2003, 2009). Until recently, the chemical complexity and variation along decomposition and the size continuum of soil organic S created significant analytical problems for routine biochemical characterization techniques, largely limiting our knowledge of the short- and long-term S cycle in terrestrial ecosystems and the precise form and chemical characteristics of the various organic S functionalities involved in this cycle (Xia et al., 1998; Prietzel et al., 2003; Solomon et al., 2003, 2005; Zhao et al., 2006; Lehmann et al., 2008). Furthermore, Saggar et al. (1998) and Solomon et al. (2003) indicated that, although a number of soil organic S speciation studies involving differential reduction techniques have observed qualitative and quantitative changes due to anthropogenic interventions (Freney et al., 1975; McLaren and Swift, 1977; David et al., 1982; Ghani et al., 1991; Solomon et al., 2001), no consistent trends regarding the interchange between different organic S forms are apparent. There is conflicting evidence on the globally dominant labile form of the organic S pool, which can be considered as a major source of mineralizable S in the soil environment. Hence, there is a widespread interest in understanding the amount and form of the different organic S species, their short- and long-term molecular-level transformations, and the various factors controlling their reactivity in environmental and geochemical systems. This knowledge is vital to effectively describe S fluxes from various pools and, as such, the transfer of S between the soil environment and other reservoirs in the global S cycle (Janzen and Ellert, 1998; Schroth et al., 2007; Solomon et al., 2009).

With the advent of novel noninvasive synchrotron-based S K-edge X-ray absorption near-edge structure (XANES) spectroscopy, it is possible to circumvent the limitations of degradative wet-chemical reduction techniques and directly determine the various organic S moieties and their oxidation states in a variety of environmental and geochemical samples based on the energy position of the whiteline resulting from the $s \rightarrow p$ electron transition properties unique to individual S species (Waldo et al., 1991; Vairavamurthy et al., 1993; Morra et al., 1997; Xia et al., 1998; Hundal et al., 2000; Martínez et al., 2002; Prietzel et al., 2003; Schroth et al., 2007; Solomon et al., 2003, 2009). Solomon et al. (2009) and Scherer (2009) indicated that the ability to effectively speciate organic S provides a clear understanding of the biogeochemical transformations occurring within the soil system and is critical to identify the more active organic S forms and the specific reactions related to these S species to obtain a mechanistic understanding of factors determining the overall mobility of this element in the soil environment. Only a limited number of studies have been conducted using S XANES spectroscopy to evaluate the relative reactivity and long-term dynamics of the various soil organic S functionalities in natural and managed ecosystems during decomposition processes (Jokic et al., 2003; Prietzel et al., 2003; Solomon et al., 2003, 2009), and detailed molecular-level understanding about the link between the various S moieties and the underlying organic S mobilization processes and the short-term biogeochemical cycling of S in agroecosystems is lacking (Zhao et al., 2006; Schroth et al., 2007).

In the present study, we investigated the speciation, long- and short-term molecular-level biogeochemical dynamics, oxidative state, and accompanying functional group chemistry changes of the various organic S moieties after aerobic incubation experiments with and without fresh organic residue additions in soils from undisturbed grassland ecosystems and from fields that were under various levels of disturbance for up to 112 yr located at one of the oldest long-term agroecosystem experiments in western United States using S K-edge XANES spectroscopy.

Materials and Methods

Study Site

This study was conducted using samples collected from the Columbia Basin Agricultural Research Center near Pendleton, OR. The research center is home to some of the oldest replicated long-term agroecosystem experiments in western United States, with experiments dating back to 1931 (Machado et al., 2006). The center is located at 46°15′ N and 119°58′ W in the Columbia Plateau physiographic province between the Cascades and the Rocky Mountains. It is 438 m above sea level and has a mean annual temperature of 10°C and mean annual precipitation of 406 mm. The topography of the site is nearly level, with slopes ranging from 0 to 4%. The soils are derived from loess overlying cobbly caliche and basalt rock. They are well drained, dark gray in color, and silty loam in texture (18% clay, 70% silt, and 12% sand) and are classified as mesic Typic Haploxeroll (Soil Survey Staff, 1999). Cultivation in this area started in the 1880s and continued for about 50 yr until the research center was established (Rasmussen et al., 1998). The research center includes nearby virgin grassland sites that have not been cultivated since 1880 and are dominated by *Pseudoroegneria spicata* (Pursh) Á. Löve and *Festuca idahoensis* Elmer species. We collected composite soil samples (composed of four subsamples) from the upper 15 cm of three subsites: (i) undisturbed grassland fields since 1880, (ii) grasslands that have been under various levels of disturbance from about 1880 to 1930 but remained undisturbed since 1931 (referred to in this study as undisturbed grasslands since 1931), and (iii) from fields under various levels of cultivation from about 1880 to 1931 and since 1931 intensively used for winter wheat (*Triticum aestivum* L.) cultivation with an alternating fallow cycle (referred to here as cultivated since 1880). The cultivated fields received no fertilizers, and crop residues were not burned on these fields. The undisturbed grasslands fields since 1880 were used as an ecological reference site from which baseline data about the compositional chemistry of soil organic S forms were generated to evaluate changes that occurred due to human intervention in the managed ecosystems. Rasmussen et al. (1998) and Boone et al. (1999) indicated that undisturbed reference sites could serve as "time capsules" that can provide an excellent opportunity to investigate temporal changes in functional group chemistry and other soil attributes in the environment after land use and other ecosystem changes,

particularly as new analytical capabilities such as XANES spectroscopy techniques develop. All soil samples were air-dried and sieved to <2 mm size.

Incubation Experiment and Chemical Analysis

This study was part of an incubation experiment where the fate and dynamics of organic C, N, and S in long-term agroecosystem experiments were evaluated using a unique approach involving the addition of triple-labeled (^{13}C, ^{15}N, and ^{34}S) sugarcane residue (*Saccharum officinarum* L.; a C_4 plant with 44% C, 1.04% N, and 0.79% S) in soils predominantly under C_3 grass vegetation followed by aerobic incubation of the soils under controlled laboratory conditions (Knoth, 2004).

A total of 100 g (94 g in the case of soils that received sugarcane residue) of soil from undisturbed grasslands since 1880, undisturbed grasslands since 1931, and cultivated fields since 1880 were added to 0.95-L, wide-mouth, airtight Mason jars and incubated without or with the addition of 6 g of oven-dried sugarcane leaf residue (ground to pass a 2-mm sieve) under a constant temperature of 30°C for 3, 15, 90, 180, and 365 days. The jars were arranged in a randomized complete-block design with three replicates. Soil moisture content was determined using a ceramic high-pressure plate apparatus from the subsamples of the soils used in each jar as described by Topp et al. (1993) and Moebius et al. (2007). The water content in each jar was adjusted to 55% of field capacity (determined to be 30–40% w/w) before the incubation started (Kimetu et al., 2009; Liang et al., 2010). Because this study is part of an incubation experiment where the fate of organic C is studied using soda lime (Mallinckrodt Baker, Paris, Kentucky; highest absorption capacity 26%) as a CO_2 trap to capture the CO_2 evolved from the organic C mineralization, the airtight Mason jars were opened after the Days 3 and 15 of the experiment. At the latter stage, the jars were opened every 15 days to aerate the samples and to remove the CO_2 traps. This could lead to evaporation of water and sample drying. To avoid this, each jar was weighed, and the moisture content was adjusted gravimetrically to the original weight by adding water with a syringe (Henke Sass Wolf GMBH, Tuttlingen, Germany) to maintain the water content in each jar at 55% of field capacity throughout the incubation experiment. At the end of each incubation interval, designated jars containing the incubated soil samples were removed from the incubation chamber, and subsamples were air-dried and stored for analysis. Due to limitations in the availability of beamtime, we combined the three replicates from each incubation interval together and prepared one representative sample for each agroecosystem for S K-edge XANES measurements. Total organic C, N, and S concentrations were determined by an Elementar Vario C/H/N/S analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). Cation exchange capacity was determined with 1 mol L^{-1} NH_4OAc (pH 7.0; soil/solution ratio [w/v] 1:10) according to Hendershot et al. (1993), and pH-KCl was determined in 1:2.5 soil/solution (w/v) suspension using a glass electrode. Selected soil chemical characteristics are shown in Table 1.

X-ray Absorption Near-edge Structure Spectroscopy

Solid-state characterization of soil organic S oxidation states from undisturbed grassland fields since 1880, undisturbed grasslands since 1931, and continuously cultivated fields since 1931 was conducted using S K-edge XANES spectroscopy at X-19A beamline of the National Synchrotron Light Source, Brookhaven National Laboratory. The soil samples were extracted three times with a mixture of 0.1 mol L^{-1} NaOH and 0.4 mol L^{-1} NaF solutions in a 1:5 soil/solution ratio (w/v) under N_2 environment. The pH of the extraction solution was maintained at 12.4 as suggested by Hutchison et al. (2001) to prevent oxidation of reduced organic S compounds. The extraction procedure followed the outline of Schnitzer (1982) as modified by Sumann et al. (1998). The F^- ion was introduced to dissolve silicate impurities and reduce the influence of paramagnetic metals on XANES spectra (Solomon et al., 2003, 2009). Solomon et al. (2003) showed that the experimental S XANES spectra measured from matrix containing dissolve silicate impurities and paramagnetic metals show a large background, deflated signal, and an upward curving baseline compared with the spectra obtained from samples where such dissolved silicate impurities and paramagnetic metals impurities were removed. These features make uniform data analysis (background correction and normalization of the data) difficult using standard programs and hindered reliable data analysis and quantitative comparison of the results. The extracts were filtered twice through a 0.2-µm pore-size membrane filter (Pall Gelman Laboratory, Ann Arbor, MI) to remove fine clay that may interfere with XANES measurements (Solomon et al., 2003), transferred into dialysis tubes (MWCO 12,000–14,000 Da; Spectrum Laboratories, Gardena, CA), dialyzed against deionized water to eliminate soluble salts, and lyophilized using a freeze dryer (Kinetics Thermal Systems, Stone Ridge, NY).

The S XANES measurements were conducted under standard operating conditions (i.e., with 2.584 GeV electron beam energy and a beam current of 120–250 mA). The X-ray energy was calibrated to the K-edge of elemental S at 2472 eV, and scans ranging from 150 eV below to 300 eV above the absorption edge of S were collected with a step size of 0.2 eV. We used a monochromator consisting of double-crystal Si (111) with an entrance slit of 0.5 mm and an energy resolution ($\Delta E/E$) of 2×10^{-4} at the S K-edge. The monochromator was detuned to 70% for harmonic rejection at the S K edge to reduce fluorescence induced by high-order harmonics (Xia et al., 1998). The spectra were recorded at room temperature in fluorescence mode using a passivated implanted planar silicon detector (Canberra Industries, Meriden, CT) posi-

Table 1. Selected chemical properties of surface soil horizons (0–15 cm) sampled from the long-term agroecosystem experiments at the Columbia Basin Agricultural Research Center near Pendleton, Oregon.

Land use	pH CaCl2	CEC†	TOC	Total N	Total S	C/N	C/S	N/S
		cmol kg^{-1} soil	g kg^{-1} soil	— mg kg^{-1} soil —				
Undisturbed grassland since 1880	6.1	35.0	25.2	1.8	244	14.4	103	7.2
Undisturbed grassland since 1931	6.6	22.8	21.1	2.1	246	10.0	86	8.6
Continuous cultivation since 1931	5.7	16.8	11.8	1.1	168	10.0	70	7.0

† CEC, cation exchange capacity; TOC, total organic carbon.

tioned 90° to the incident beam. 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-m-thick acrylic holder and covered with 2.5-μm-thick Mylar film (Chemplex Industries, Palm City, FL). Background correction, normalization, and deconvolution of XANES spectra for each sample into pseudocomponents were done using the nonlinear least squares fitting routine solver supplied by MS-Excel according to Xia et al. (1998), Martínez et al. (2002), Solomon et al. (2003), and Einsiedl et al. (2007). The linear part of the spectral baseline was removed, and the spectrum was normalized before fitting to avoid spectral dependence on the total organic S content; therefore, spectral properties were indicative of changes in S chemistry. The XANES spectra were fitted using a series of Gaussian peaks (G1, G2, G3, G4, and G5) that represent the whitelines ($1s \rightarrow 3p$ photoelectron transition peaks). The first and the second arctangent functions represent the transition of ejected photoelectrons to the continuum for the unoxidized S (whitelines located between 0 and 5 eV above the elemental S K-edge) and for the oxidized S forms (whitelines located between 5 and 12 eV above the elemental S K-edge), respectively. Additional justifications and assumptions made for the fitting procedure and other details related to data collection are described by Xia et al. (1998), Martínez et al. (2002), Einsiedl et al. (2007) and Solomon et al. (2003, 2009). The energy positions (eV) of the Gaussian curves were used to identify the oxidation states of S present in the samples, and the percentages of S functionalities present at each oxidation state in a samples were determined independently from the area under the respective Gaussian peak relative to the total area under the five Gaussian peaks after correcting for the change in absorption cross-section with increasing oxidation state (Xia et al., 1998). Because XANES reflects the distribution of electrons in the valence shell of S atoms in their actual bonding environment, the difference between electronic and formal oxidation states can be substantial, especially for reduced S species in complex organic materials, depending on whether S is bonded to S, H, C, or metals (Xia et al., 1998; Martínez et al., 2002). Due to the higher electronegativity of O, the difference is not significant for higher-valence ($\geq 4^+$) S species or for S atoms bound to multiple O atoms. Therefore, we reported the electronic oxidation states rather than formal oxidation states because they reflect the actual electron density in the valence shell of S. Integer values were used to report the electronic oxidation states of the high-valence S species ($\geq 4^+$), and noninteger values were used for the low-valence (4^+) S compounds.

Results and Discussion

Characterization of Organic Sulfur Functionalities of Grassland Soils

The baseline corrected and normalized experimental S K-edge XANES spectra of soils collected from undisturbed grassland fields since 1880, undisturbed grassland fields since 1931, and fields cultivated since 1880 were characterized by the presence of five prominent absorption edge- and post-edge bands in the energy ranges of 2470 to 2476, 2476 to 2478, 2478 to 2482, 2482 to 2487, and 2491 to 2509 eV (Fig. 1–3). These peak regions were ascribed to transitions of the S 1*s* core electrons to the lowest unoccupied antibonding states of the S atom and possibly to the various post-edge features generally attributed to multiple scattering resonances (Fleet 2005; Fleet et al., 2005). These features were consistent throughout our experimental XANES spectra, indicating the presence of numerous oxidation states and thus allowing us to deconvolute and fit the experimental spectra using a series of Gaussian curves to quantitatively determine the relative proportions of the various organic S functionalities in the soils under investigation. The relative energy position, predicted electronic oxidation states, and structure of representative organic S compounds accompanying these oxidations are summarized in Table 2. Based on these results, we differentiated the various oxidation states identified by our experimental S XANES spectra, fingerprinted the discrete organic S functionalities found in these grassland-derived soils, and classified them into three distinct groups: (i) organic S functionalities present in strongly reduced (S^0 to S^{1+}) oxidation states, which include thiols, monosulfides, disulfides, polysulfides, and thiophenes; (ii) organic S in intermediate (S^{2+} to S^{5+}) oxidation states that include sulfoxides and sulfonates; and (iii) organic S present in strongly (S^{6+}) oxidized state, which represent ester–SO_4–S sulfur. The first two groups of organic S compounds (organic S in strongly reduced and intermediate oxidation states) represent S directly linked to C in C–S or C–S–O linkages, as in the case of S-containing amino acids and sulfonates, respectively, and are commonly referred to in the conventional classification systems as C-bonded S, whereas S in highly oxidized state denotes organic S compounds where S is linked to C mostly through O atoms in the form of C–O–S linkage, as in the case of true ester sulfates (C–OSO_3–C). Small proportions of compounds containing N–O–S linkages, such as glucosinolates and N–S linkages typified by sulfamates, might be related to these organosulfur forms (Maynard et al., 1984; Saggar et al., 1998).

Molecular-level Organic Sulfur Composition in Undisturbed Grassland Soils

The relative abundance of S species resolved by deconvolution of the experimental XANES spectra indicate that S directly linked to C in C–S or C–S–O linkages was the dominant form of organic S in the soils collected from the undisturbed grassland ecosystems of western United States, representing up to 80% of the total organic S in the surface layers of the undisturbed soils since 1880 (Fig. 4). Our results concur with the results obtained using the conventional wet-chemical reactivity (Bettany et al., 1980) and spectroscopy-based investigations of soil organic S composition (Zhao et al., 2006, Lehmann et al., 2008; Solomon et al., 2009), which indicated the presence of large proportions of C-bonded S (60–84%) in variety of undisturbed temperate soils. Solomon et al. (2009) stated that the dominance of organosulfur compounds directly bound to C in the surface layers of grassland soils could be attributed partly to the fact that this organic S fraction originates from deposition of biological material such as plant exudates, root and leaf litter, animal residue, and microbial metabolites that make up the bulk of soil organic matter input to the surface layers of these undisturbed ecosystems and partly through biological immobilization of inorganic sulfate (Kertesz et al., 2007). Our results provide further evidence that the majority of the total soil organic S directly linked to C in these undisturbed grassland soils constituted predominantly of organosulfur compounds in the most reduced (41%) and intermediate oxidation (39%) states (Fig. 4). Organic S directly linked to C in

the most reduced oxidation states was almost entirely composed of sulfide (C–S_n–C) and thiol (C–SH)-containing S moieties (88%), whereas thiophenic S accounted only for 12% of the organic S directly bonded to C in the most reduced oxidation states. Sulfonates (C–SO_3–C) were the most dominant forms of organosulfur compounds, constituting 82% of the total organic S detected by S K-edge XANES spectroscopy in the intermediate oxidation states, whereas sulfoxide (C–O–S–C) S accounted only for a small fraction (18%) of organic S in the intermediate oxidation states in these undisturbed grassland ecosystems.

The likely explanation for the dominance of organic S directly linked to C in the most reduced oxidation states by sulfides and thiols was the ability of plants and microorganisms to use SO_4^{2-} as a primary source of S and to reduce it to sulfides, its lowest oxidation state, through a reductive assimilation pathway (Leustek, 2002; Solomon et al., 2009). This process is assimilative because sulfides are exclusively used for the synthesis of highly reduced organic S compounds, such as cysteine (HS–CH_2–CH(NH_2)–COOH), cystine (S–CH_2–CH(NH_2)–COOH)$_2$, and methionine (CH_3–S–(CH_2)$_2$–CH(NH_2)–COOH), and other metabolites. In higher plants, this process takes place mostly in the chloroplasts of green leaves, where the enzymes for assimilatory SO_4^{2-} reduction are mainly localized. This results in the localization of S-containing amino acids in the leaf protein (Autry and Fitzgerald, 1990; Solomon et al., 2009), perhaps explaining the higher concentration of these organic S moieties in the surface horizons of the investigated undisturbed grassland soils. Similarly, sulfonate S linkages are found in the sulfoquinovose component of the plant sulfolipids, which are the primary constituent of leaves and photosynthetic tissues and are increasingly recognized as a major component of the biological S cycle in the soil environment (Harwood and Nicholls, 1979; Kertesz et al., 2007). Sulfocarbohydrates possessing the sulfonate linkage are also widely distributed in green plants and to a lesser extent in bacterial membranes and spores in soils (Fitzgerald, 1976). Stanko-Golden et al. (1994) indicated that grassland vegetation typically has shallower root systems and tends to concentrate plant material in the surface layers of undisturbed grassland soils. Substantial amounts of these organic S compounds enter the soil system through leaf litterfall after senescence and could lead to the accumulation of sulfonate S in the mineral layers of these grassland-derived soils. These results agree with the findings by Maynard et al. (1984), Autry et al. (1990), and Stanko-Golden et al. (1994), who stated that a significant fraction of the intrinsic organic S mainly comprised of amino acids and sulfolipids in mineral horizons of native grassland soils may originate from direct accumulation of litter and foliage in the organic horizons and through subsequent translocation of this organic S fraction into the mineral horizons. Biogenic sulfonates could also be derived from oxidation products of cysteine (Kertesz, 1999), a

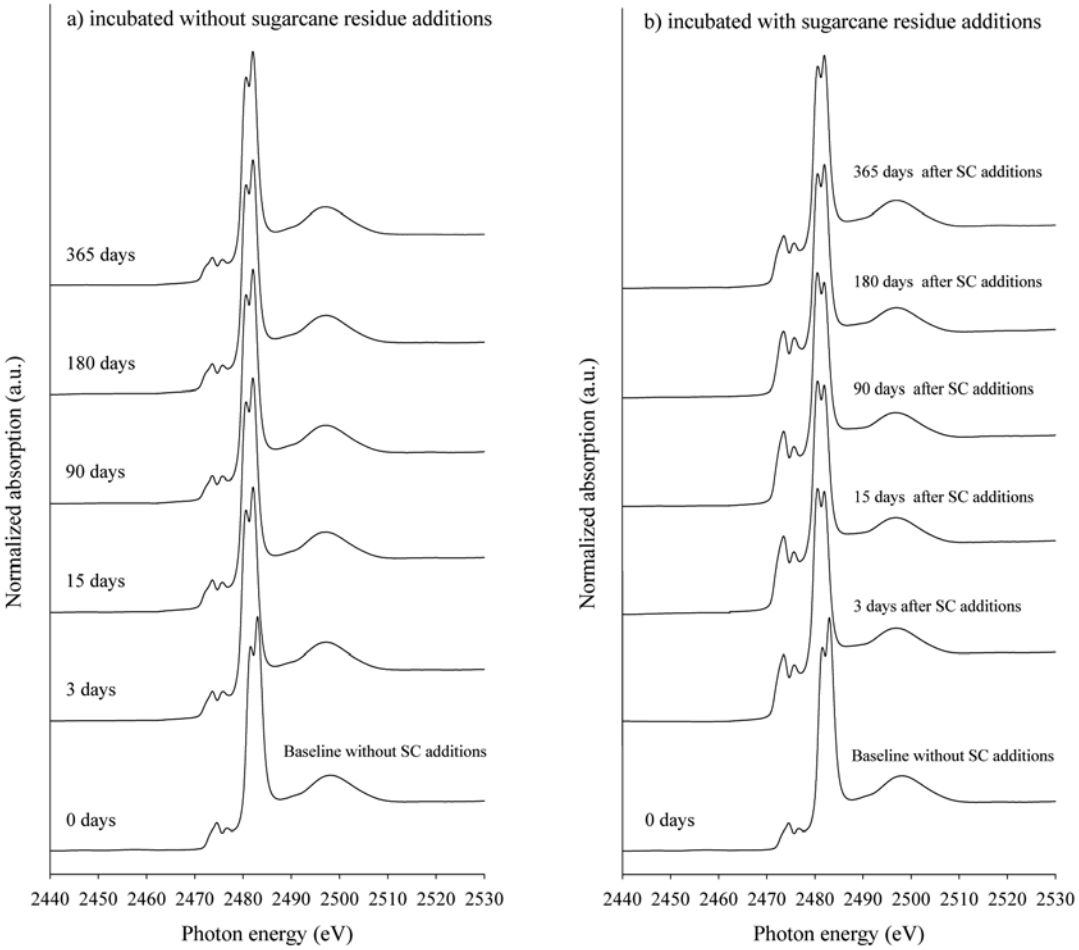

Fig. 1. Stacked S K-edge X-ray absorption near-edge structure spectra of cultivated soils since 1880 incubated (a) without and (b) with the addition of sugarcane (SC) residue for 365 d.

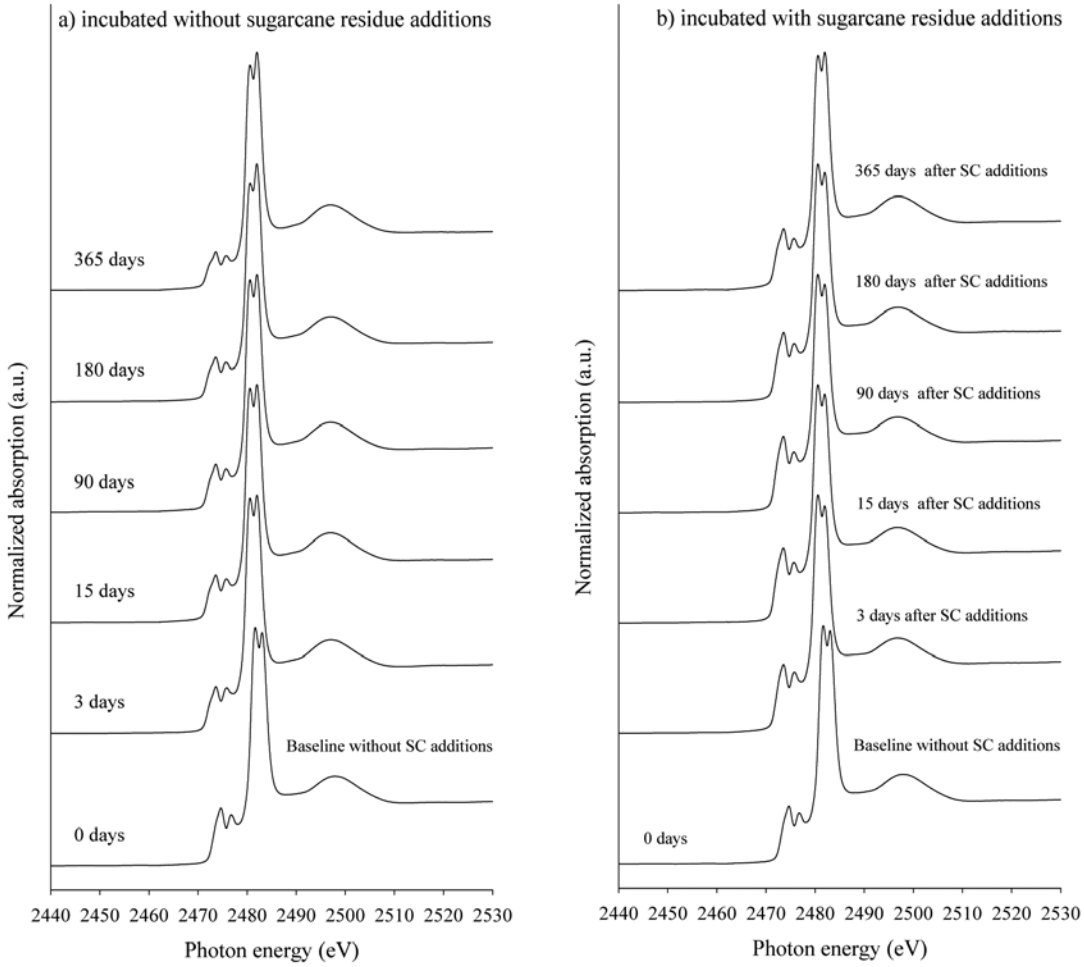

Fig. 2. Stacked S K-edge X-ray absorption near-edge structure spectra of undisturbed soils since 1931 incubated (a) without and (b) with the addition of sugarcane (SC) residue for 365 d.

major S-containing amino acid, which might explain why higher sulfonate levels were found in the surface layers of these undisturbed ecosystems (Solomon et al., 2009).

On the contrary, organic S in its highly oxidized state, where S is directly bonded to O in C-OSO_3–C linkage, represented only 20% of the total organic S detected by XANES spectroscopy in the surface soils of the undisturbed grassland soils since 1880 (Fig. 4). Some amount of ester–SO_4–S is known to occur in plant residues in the form of aryl, alkyl, phenol, and polysaccharide sulfates and might be deposited in soils to a smaller extent along with leaf litterfall input on the surface layers of these grassland soils (Maynard et al., 1984; Houle and Carignan, 1992). However, this organic S fraction in general is believed to be a transitory product synthesized in situ predominantly through biochemical processes by microflora in the presence of adequate inorganic SO_4^{2-}. Usually its contribution to the total soil organic S pool in undisturbed ecosystems is minimal (McGill and Cole, 1981; Houle and Carignan, 1992; Saggar et al., 1998; Solomon et al., 2003, 2009).

Long-term Soil Organic Sulfur Compositional Dynamics after Human Intervention

Long-term human intervention markedly changed the molecular-level composition of soil organic S and led to a shift in the apparent oxidation state of organic S from undisturbed grassland soils primarily composed of S moieties in highly reduced and intermediate oxidation states toward managed agroecosystems dominated by organic S rich in strongly oxidized or high-valence S species. Examination of the molecular-level dynamics of the various organic S moieties clearly indicates that the largest loss of organic S as a result of cultivation for up to 112 yr occurred from organic S in strongly reduced oxidation states (44%) that encompass thiols, monosulfides, disulfides, polysulfides, and thiophenes, followed by organic S in the intermediate oxidation states (10%) that include sulfoxides and sulfonates (Fig. 4). However, the detected decline in the relative proportions of organic S in the strongly reduced (1%) and intermediate oxidation (3%) states was almost negligible in the grassland soils that remained undisturbed for the last 61 yr (Fig. 4). Conversely, our results show that the proportion of organic S in strongly oxidized state increased by up to 108% in the soils collected from fields that have been under various levels of cultivation since 1880, whereas a minimal increase (7%) was observed in the proportion of these strongly oxidized organosulfur compounds in the soils that remained undisturbed since 1931 (Fig. 4). These compositional chemistry and oxidation state changes were further demonstrated by the changes observed in the ratios of strongly reduced organic S species (thiols, monosulfides, disulfides, polysulfides, and thiophenes) to organic S in strongly oxidized (ester SO_4–S) states (R-S/O-S) and organic S in intermediate oxidation states (sulfoxides and sulfonates) to

strongly oxidized S (I-S/O-S). The R-S/O-S ratio decreased after human intervention from 2.01 in the undisturbed grassland soils to 0.54 in the soils cultivated since 1880, and the I-S/O-S ratio declined from 1.89 in the undisturbed soils to 0.82 in the soils collected from the cultivated fields (Fig. 4). The apparent changes in oxidation states and compositional chemistry changes of organic S manifested in the cultivated soils could be attributed to improved soil aeration, exposure of physically protected organic S, increased microbial activity, and stimulation of aerobic decomposition processes. This is often reported as a result of

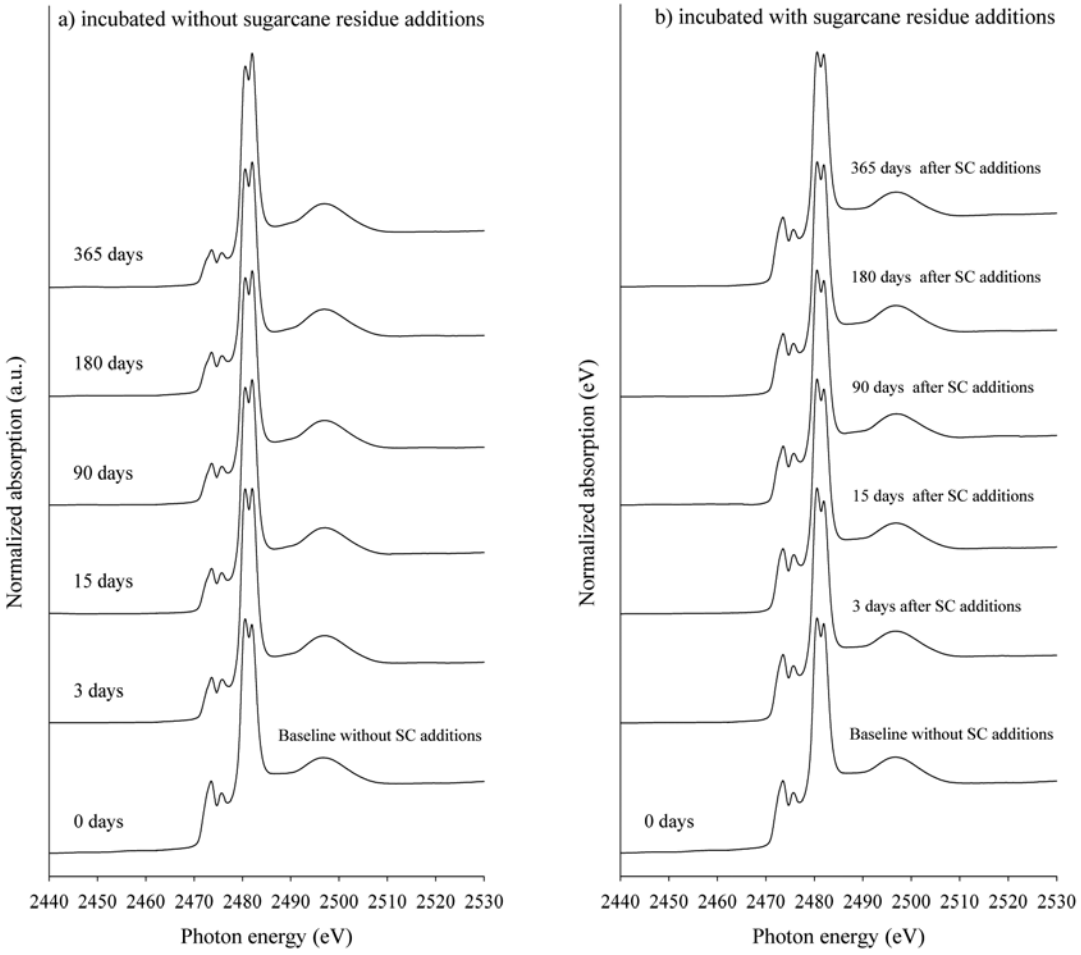

Fig. 3. Stacked S K-edge X-ray absorption near-edge structure spectra of undisturbed soils since 1880 incubated (a) without and (b) with the addition of sugarcane (SC) residue for 365 d.

Table 2. Relative energy positions (peak maxima), predicted electronic oxidation states, and structure of representative soil organic sulfur compounds from long-term agroecosystem experiments at the Columbia Basin Agricultural Research Center near Pendleton, Oregon.

Organic S compounds†	Structure	Gaussian curve	Peak maxima	Electronic oxidation state
			eV	
		Organic S in most reduced oxidation states		
Thiols	R-SH	G1	0.32–0.65	0.13–0.36
Organic monosulfides	R-S-R′			
Organic disulfides	R-S-S-R′			
Organic polysulfides	R-S-S_n–S-R′			
Thiophenes		G2	1.4–1.8	0.70–0.96
		Organic S in intermediate oxidation states		
Sulfoxides	R-SO-R′	G3	2.7–3.5	1.5–1.9
Sulfonates	R-S$(O)_3$–H	G4	7.4–8.5	5
		Organic S in highly oxidized state		
Ester–SO_4–S	R-O-SO_3–H	G5	9.5–9.9	6

† Representative soil organic S compounds were compiled from literature data collected from Vairavamurthy et al. (1993, 1998), Morra et al. (1997), Xia et al. (1998), Prietzel et al. (2003), and Solomon et al. (2003, 2005, 2009).

clearing of the native grassland vegetation and disruption of the soil aggregates through frequent exposure to rain drop impact, rapid wetting and drying, and the shearing action of agricultural implements during the long-term cultivation practices, the net effect of which is accelerated transformation of the C-bonded S functionalities to possibly ester–SO_4–S before being released as inorganic SO_4^{2-} (McGill and Cole, 1981; Saggar et al., 1998; Wang et al., 2006; Solomon et al., 2001, 2003, 2009). This mechanism could increase the level of ester–SO_4–S in the soils that have been under various levels of cultivation since 1880 (Fig. 4) while continually diminishing the level of organic S directly bound to C in sulfide, thiol, sulfoxide, and sulfonate linkages in these soils. This finding is in line with the results of the studies by McLaren and Swift (1977) and Solomon et al. (2003, 2009). Our results also agree with the earlier conclusion by McGill and Cole (1981), Saggar et al. (1998), Zhao et al. (2006), Schroth et al. (2007), and Solomon et al. (2001, 2003, 2009) that S directly bonded to C in highly reduced oxidation states seems to be the most biologically reactive organic S pool to long-term anthropogenic disturbances, followed by organic S in the intermediate oxidation states; together these organic S forms seem to represent the major source of biologically mineralizable soil S in these grassland agroecosystems. Our S K-edge XANES spectroscopy analyses also suggest that the soil S cycles might have recovered after the elimination of long-term soil disturbance through agricultural operations since 1931, returning the organic S distribution back to undisturbed grassland ecosystems, because the values for R-S/O-S (1.86) and I-S/O-S (1.71) in the undisturbed soils since 1931 appear to have reverted in the direction of values observed in the ecological reference sites, with a greater proportion of organic S present in more reduced and intermediate oxidation states.

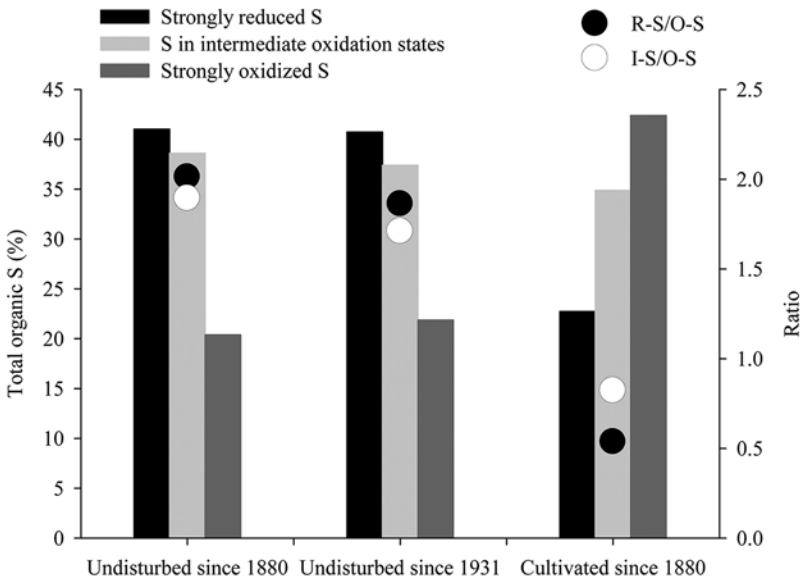

Fig. 4. Organic S speciation, long-term dynamics of organic S after human intervention, and the ratios of highly reduced S (thiols, monosulfides, disulfides, and polysulfides) to highly oxidized S (ester sulfates) (R-S/O-S) and organic S in the intermediate oxidation states (sulfoxides and sulfonates) to highly oxidized S (I-S/O-S) resolved by S X-ray absorption near-edge structure spectroscopy of grassland-derived soils under different management. Total organic S (%) represents the total organic S detected by X-ray absorption near-edge structure spectroscopy; these soils are not incubated.

Short-term Molecular-level Organic Sulfur Dynamics after Aerobic Incubation

Short-term molecular-level transformations of organic S and the various factors controlling its reactivity are critical for understanding its long-term mobility and fate within the soil environment, as well as the transfer of S between soil system and other reservoirs in the global S cycle on decadal or centurial scales. However, a thorough understanding of the various forms of easily mineralizable S moieties, their bioavailability, the interchange between the different organic S functionalities, and the influence of organic matter decomposition on the speciation dynamics and short-term cycling of S in mineral soils remains elusive (Janzen and Ellert, 1998; Schroth et al., 2007; Solomon et al., 2001, 2003, 2009). The S K-edge XANES spectral features (Fig. 1–3) and the changes observed in proportions of organic S functionalities present in the mineral soils subjected to aerobic incubation with and without the addition of fresh sugarcane plant residue for up to 365 d (Fig. 5) seem to suggest that the general trends in the short-term molecular-level transformations of the various organosulfur compounds in these grassland-derived soils are similar. Despite marked differences in the initial concentrations of C-bonded S and the variable level of disturbance that these soils were subjected to, the relative proportions of these organanosulfur compounds decreased in all three agroecosystems by 21% after aerobic incubation without the addition of fresh sugarcane residue for up to 365 d (Fig. 5a). The addition of sugarcane residue rich in organic S directly bonded to C (90%), followed by aerobic incubation, led to an initial increase in the relative proportion of these organosulfur compounds, especially in samples collected from cultivated fields with low original C-bonded S level, where we have observed up to 40% more C-bonded S after only 3 d of incubation compared with the amount detected in the unamended baseline (0 d) cultivated soil (Fig. 5b). Although the final proportion of organic S directly linked to C in the cultivated soils receiving fresh residue was up to 13% larger than the unamended baseline soils after 365 d of aerobic incubation, the overall trend shows a steady decline in these soil organosulfur compounds with increasing duration of incubation in all three agroecosystems. Our results are in line with the results of Ghani et al. (1991, 1992), who found that S mineralized during short-term incubations appeared to be derived almost exclusively from C-bonded S. Zhao et al. (2006) reported that gross S mineralization was correlated more closely with the amounts of S present in the reduced (S^0 to S^{1+}) and intermediate (S^{2+} to S^{5+}) S species than with the highly oxidized (S^{6+}) S species, suggesting that C-bonded S is the main source for mineralization during a short-term (53 d) aerobic incubation experiment. The same conclusion was obtained in these two studies despite different methods being used for S speciation (HI reduction by Ghani et al., 1991, 1992 versus XANES by Zhao et al., 2006) and for measurement of S mineralization (open leaching versus ^{35}S isotope dilution, respectively). The results of our short-term aerobic incubation experiments are also consistent with the long-term trend observed in this investigation and with other studies of long-term changes of organic S functionalities

after anthropogenic interventions on native grasslands and forests (McLaren and Swift, 1977; Solomon et al., 2001; Solomon et al., 2003, 2009), indicating that C-bonded S, or organic S in the reduced and intermediate valences, is the main source for S mineralization. However, our results are contrary to the findings of Goh and Pamidi (2003), who reported that ester–SO_4–S decreases rapidly in short-term aerobic incubation experiments and concluded that these organosulfur compounds seem to be more sensitive than C-bound S. In fact, in organic S mineralization studies involving Canadian soils, Lowe (1964) considered C-bonded S to be of little value as a source of mineralizable S.

Detailed examination of the short-term molecular-level dynamics of the various C-bonded S components after 365 d of aerobic incubation without sugarcane residue revealed that the relative proportions of thiols and sulfides declined by 25, 29, and 29% while the of proportion thiophenes decreased by 79, 45, and 59% of the original amount in the cultivated soils since 1880, undisturbed grassland soils since 1931, and undisturbed grassland soils since 1880, respectively (Fig. 6a and 7a). Analogous to the speciation dynamics observed in soils that did not receive sugarcane residue, the proportion of thiols and sulfides declined by 20% in undisturbed grassland soils since 1931 and by 7% in undisturbed grassland soils since 1880 after 365 d of aerobic incubation with the addition of sugarcane residue, whereas the proportion of thiophenes decreased by 22 and 28%, respectively.

The proportions of thiols and sulfides, asa well as thiophenes, in the cultivated soils since 1880 increased by an additional 88 and 43% after 3 d of incubation after the addition of fresh residue, respectively, compared with the amounts of these organic S compounds detected in the unamended baseline soil. The initial increase in thiols, sulfides, and thiophenic S could be largely attributed to the incorporation of these organosulfur compounds along with the fresh sugarcane residue. These organosulfur compounds are known to be the primary constituent of leaves and photosynthetic tissues (Autry and Fitzgerald, 1990; Solomon et al., 2009). Our results also show that thiol and sulfide S contribute up to 39% to the total soil organic S and that thiophenic S constitutes up to 7% of the total organic S detected by S K-edge XANES spectroscopy from the fresh sugarcane residue (Fig. 6b and 7b). These values are in agreement with the results of Jalilehvand (2005), who reported that 44 to 55% of the total S in plant leaves could be present in reduced S forms as thiols, thioethers, and disulfides using S speciation experiments conducted on a variety of intact plant leaves by XANES spectroscopy. With increased duration of aerobic incubation, however, the proportion of these organic S functionalities continued to drop in the soil collected from the managed agroecosystems (Fig. 6b and 7b). Our results seem to suggest that thiols (C–SH), sulfides (C–S_n–C), and thiophenic S might represent the most labile components of the organic S pool and that these C-bonded S moieties might be the major source of short-term mineralizable S in these grassland-derived soils. Our results concur with the results of Schroth et al. (2007), who reported that preferential reactivity of organic sulfide fractions in oxidizing podzolic environments accounted for much of the change in speciation of soil organic S using short-term laboratory incubation experiments. We attribute the rapid decline of thiols, sulfides, and thiophenic S after aerobic incubation to direct biological oxidation of the C–S linkage to inorganic SO_4^{2-}–S or to transient interconversion of the C–S linkage in thiols, sulfides, and thiophenic S compounds possibly to ester–SO_4–S linkages (McGill and Cole, 1981; Edwards, 1998; Schroth et al., 2007; Solomon et al., 2009). According to McGill and Cole (1981) and Solomon et al. (2009), the release of S from organosulfur compounds directly linked to C is a biological process, occurring when the C to which they are attached is oxidized to CO_2 by soil microorganisms. This process occurs internally and is strictly catabolic, controlled more by the requirement for energy and C skeletons than by the need for S. Fitzgerald (1976) demonstrated that various soil fungi and bacteria can convert cysteine (HS–CH_2–CH(NH_2)–COOH) and cystine (S–CH_2–CH(NH_2)–COOH)$_2$ to inorganic SO_4^{2-}–S aerobically using oxidative enzymatic degradation mechanisms. Sulfur present in methionine (CH_3–S–(CH_2)$_2$–CH(NH_2)–COOH) has been also shown to be converted aerobically to this anion by a mixed population of soil microorganisms (Hesse, 1957). McGill and Cole (1981), Zhao et al. (1996), and Solomon et al. (2009) suggested that biological oxidation seems to be the principal pathway for mineralization of thiols and sulfides and possibly in this

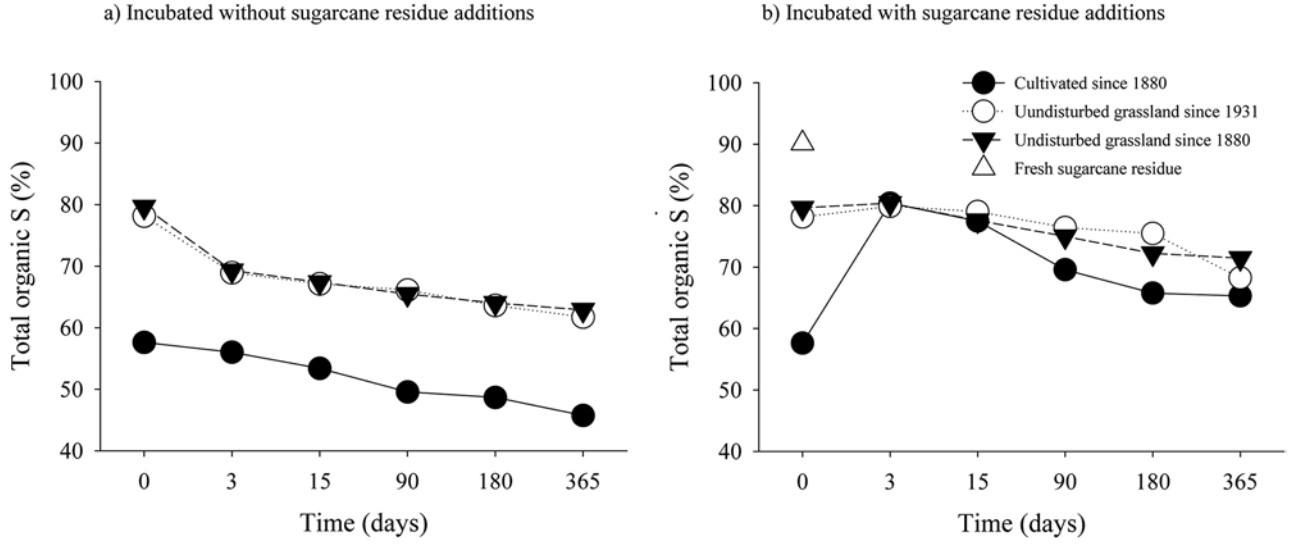

Fig. 5. Short-term speciation dynamics of C-bonded S compounds (organic S in highly reduced and intermediate oxidation states) after aerobic incubation of grassland-derived soils (a) without and (b) with the addition of sugarcane residue.

case of thiophenic S, whereby SO_4^{2-} is comineralized or released as a byproduct of the oxidation process in the soil environment. On the other hand, Kertesz et al. (2007) stated that organic S in the soil environment is not static in nature and could be rapidly interconverted from one organosulfur form to another within the soil system by microbial action. Such interconversion is a highly dynamic process that could lead to accelerated transformation of these C-bonded S functionalities to organosulfur compounds directly bonded to O as an intermediate product before being released as inorganic SO_4^{2-}–S (McLaren and Swift, 1977; McGill and Cole, 1981; Saggar et al., 1998; Solomon et al., 2001) after increased microbial activity due to the incorporation of organic matter that is rich in S directly bonded to C followed by aerobic incubation of the soils under investigation. This mechanism tends to maintain or increase the level of ester–SO_4–S while continually diminishing the level of C-bonded S compounds such as thiols, sulfides, and thiophenic S in the soil environment.

The proportion of sulfoxide (C–O–S–C) S decreased by 71, 31, and 30% in the soils collected from cultivated fields since 1880, from undisturbed grassland fields since 1931, and from undisturbed fields since 1880, respectively, after 365 d of aerobic incubation without the addition of sugarcane residue (Fig. 8a and 9a). In comparison, a small but consistent decline (3, 4, and 4%) was observed in the proportion of sulfonate (C–SO_3–C) S. A slight increase in the relative proportion of sulfoxides and sulfonates was observed in the soils of all three agroecosystems at the earlier stage (3 d) of aerobic incubation after the addition of sugarcane residues (Fig. 8b and Fig. 9b). However, similar to the pattern observed in the soils that received no fresh sugarcane residue, the proportion of these organic S moieties continued to fall with increasing amounts of aerobic incubation in all the three agroecosystems regardless of incorporation fresh sugarcane residues rich in these organosulfur compounds. The consistent decline observed in the relative abundance of sulfoxides seems to suggest that these C-bonded organic S compounds could be an important component of relatively reactive soil organic S pool on a short time scale. Similar results have been reported by Schroth et al. (2007), where a consistent decline in the relative

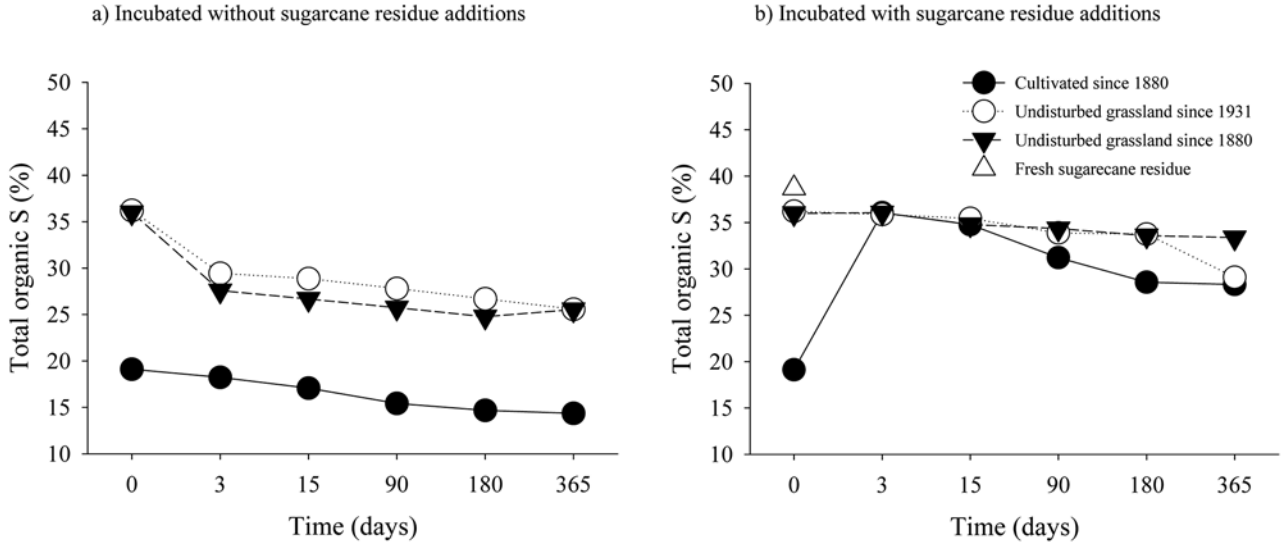

Fig. 6. Short-term speciation dynamics of sulfides and thiols after aerobic incubation of grassland-derived soils.

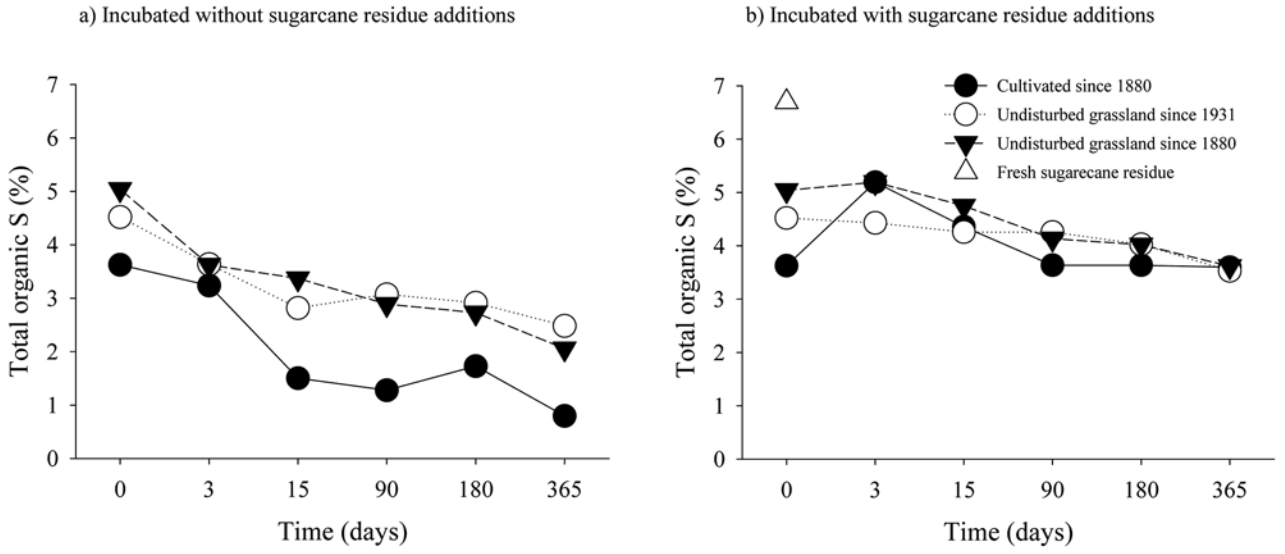

Fig. 7. Short-term speciation dynamics of thiophenic S after aerobic incubation of grassland-derived soils.

abundance of sulfoxides has been observed in forest leaf litter samples subjected to aerobic decomposition. These authors indicated that sulfoxides appear to be another labile fraction of S and a significant source of reactive organic S. Thus, sulfoxy S species should be considered as an integral part of the S cycle in the soil environment. The lower short-term reactivity of sulfonates observed in this experiment is surprising because these C-bonded organic S moieties were thought to be biologically very labile, even though the pathways for the catabolism of these major components of the S cycle are poorly delineated (Autry and Fitzgerald, 1990; Roy et al., 2000). For example, Strickland and Fitzgerald (1983) found that 6-sulfoquinovose was subject to rapid biological mineralization when incubated with forest soil and litter, indicating the dynamic nature of these organic S moieties. Focht and Williams (1970) showed similarly that arylsulfonates, including ρ-toluene sulfonate and benzene sulfonate, are rapidly degraded by bacteria in pure cultures. Roy et al. (2000) reported that several bacterial strains grew on sulfoquinovose as a sole source of C and that some of these strains achieved complete mineralization of these organic S compounds to inorganic SO_4^{2-}–S. Strickland and Fitzgerald (1983), Roy et al. (2000), and Solomon et al. (2009) suggested that the mobilization of sulfonate S and the liberation of SO_4^{2-} ion from these organic S forms in the soil environment seem to be primarily due to biological processes and not due to chemical catalysis.

Contrary to the various organosulfur compounds directly bonded to C, the relative proportion of organic S directly bonded to O in ester–SO_4–S (C–OSO_3–C) linkage increased by 28, 75, and 82% on aerobic incubation without fresh sugarcane residue addition in the cultivated soils since 1880, in the undisturbed grassland soils since 1931, and in the undisturbed grassland soils since 1880, respectively (Fig. 10a). Our results show that incorporation of fresh sugarcane residue low in ester–SO_4–S (10%) but rich in C-bonded S (90%) led to an temporary decline in the proportion of organic S directly bonded to O by 54% in the cultivated soils since 1880, by 8% in the undisturbed grassland soils since 1931, and by 4% in undisturbed grassland soils since 1880 at the initial stage (after 3 d) of aerobic incubation (Fig. 10b).

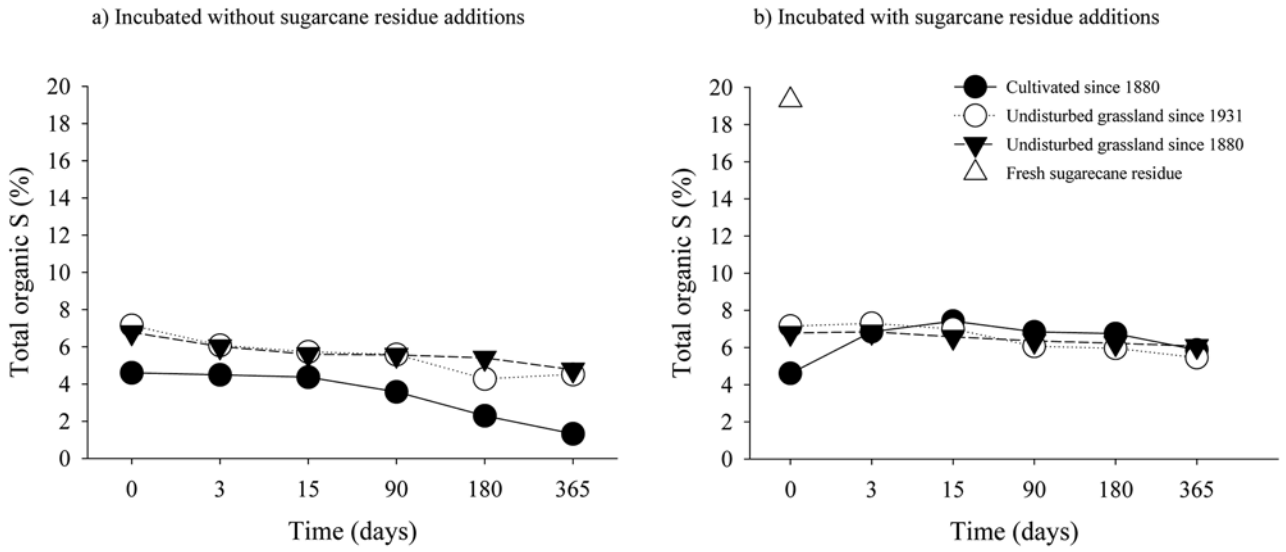

Fig. 8. Short-term speciation dynamics of sulfoxide S after aerobic incubation of grassland-derived soils.

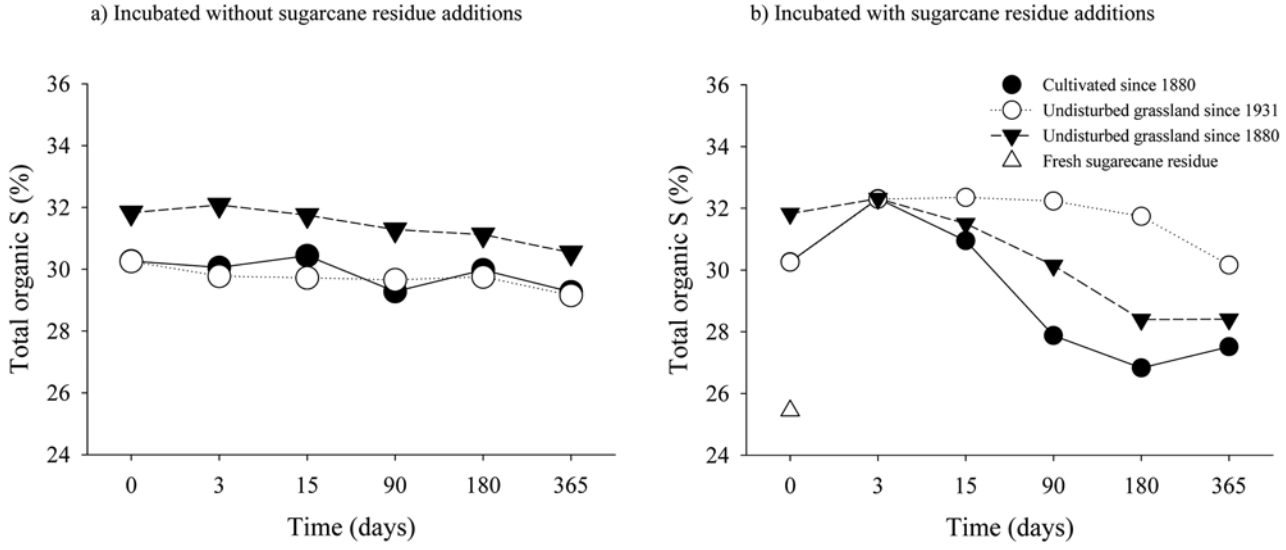

Fig. 9. Short-term speciation dynamics of sulfonate S after aerobic incubation of grassland-derived soils.

Regardless of this initial drop, the proportion of these organic S moieties continued to rise steadily with increasing duration of incubation. This is particularly prevalent in the undisturbed grassland soils since 1931 and 1880, where an increase of up to 45 and 40%, respectively, in organic S directly bonded to O was observed after 365 d of aerobic incubation compared with the proportions detected by S K-edge from the unamended baseline soils (0 d) (Fig. 10b).

The quantitative changes observed in the relative abundance of the different organosulfur compounds after aerobic incubation were equally reflected by the qualitative changes detected from the spectral signatures of the soils under investigation. According to Fig. 1, 2, and 3, the whitelines from the stacked spectra of the soils incubated without and with sugarcane residue additions show a subtle but salient decline in the resonances of the absorption bands present between 2470 and 2476 eV (representing C-bonded S in S^0 to S^{1+} states that include thiols, sulfides, and thiophenes), between 2476 and 2478 eV (representing C-bonded S in S^{2+} states that include sulfoxides), and between 2478 and 2482 eV (representing C-bonded S in S^{5+} oxidation states that include sulfonates) and a corresponding increase in the intensity of a high-valence (S^{6+}) S present in the 2478 to 2482 eV absorption band region, indicating that organic S directly bonded to O in ester–SO_4–S linkage. The shifts in oxidation states from the unamended baseline soils (0 d), where the organic S pool for the most part was dominated by S in the low-valence (S^0 to S^{1+}) and intermediate-valence (S^{2+} to S^{5+}) states, toward a system dominated by high-valence (S^{6+}) S compounds appeared at a much earlier stage (between 3 and 15 d) of aerobic incubation in the soils that did not receive fresh sugarcane residue and continued to intensify with time (Fig. 1a, 2a, and 3a). Our results showed that such qualitative shifts in the spectral signatures occurred at a much later stage (between 180 and 360 d of incubation) in the case of the soils that received fresh sugarcane residue rich in S in highly reduced and intermediate oxidation states (Fig. 1b, 2b, and 3b).

The relative shift in oxidation state toward high-valence (S^{6+}) S and the accompanying apparent change in functional group chemistry of organic S forms from unamended baseline soils dominated by organosulfur compounds in strongly reduced and intermediate states toward a system dominated by organic S compounds directly linked to O after aerobic incubation was further demonstrated by the decline in R-O/O-S and I-S/O-S ratios of the soils under investigation (Fig. 11 and 12). The R-O/O-S ratio declined from 0.54 to 0.28 in the cultivated soils since 1880, from 1.86 to 0.73 in the undisturbed grassland soils since 1931, and from 2.01 to 0.74 in the undisturbed grassland soils since 1880 (Fig. 11a and 12a). In contrast, the I-S/O-S ratio decreased from 0.82 to 0.56, from 1.71 to 0.88, and from 1.89 to 0.95, respectively, after 365 d of incubation without the addition of fresh sugarcane residue. Similarly, despite brief increases in the R-O/O-S and I-S/O-S ratios observed at the early stage of incubation after the addition of plant residue (Fig. 11b and 12b), the values of these ratios declined steadily with the continued duration of aerobic incubation. However, the R-O/O-S and I-S/O-S ratios were much higher in undisturbed soils since the 1880s (1.29 and 1.21), followed by grassland soils that have been under cultivation up until 1930 but remained undisturbed for the last 61 yr (1.0 and 1.12) and soils cultivated since 1880 (0.91 and 0.96). Solomon et al. (2009) suggested that such a shift in oxidation state toward a high-valence state and the accompanying change in the functional group chemistry toward organic S compounds directly linked to O could be the results of several processes occurring simultaneously within the soil environment. The first mechanism could be a result of direct biological oxidation of organic S linked to C, followed by the formation of more transient but strongly oxidized organic S functionalities. The second reason arises from the distinct mobilization mechanism of organosulfur compounds directly linked to O in ester–SO_4–S linkage. McGill and Cole (1981) indicated that these organic S forms are mobilized in soils through a biochemical mineralization process involving extracellular hydrolysis of ester–SO_4–S by sulfohydrolases. This catalytic process occurs independently from the biological need for energy and C. However, Dodgson and Rose (1975) indicated that the formation of sulfohydrolases such as sulfatase in the soil environment can be repressed by the presence of C-bonded S such as sulfites, cysteine, cystine, and methionine. As a result, the supply of substrates containing large amounts of C-bonded S (in this case fresh sugarcane residue)

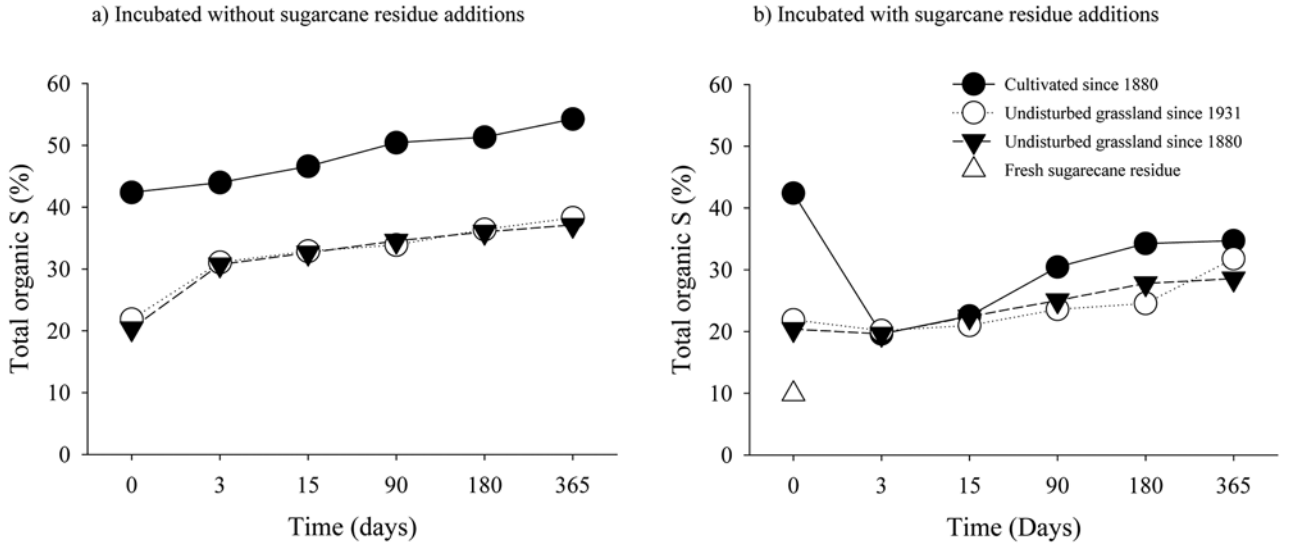

Fig. 10. Short-term speciation dynamics of ester–SO_4–S after aerobic incubation of grassland-derived soils.

could repress the formation of these enzymes and might inhibit the biochemical mineralization of organic S directly linked to O, thereby leading to accumulation of strongly oxidized S species and lowering R-S/O-S and I-S/O-S values (Fitzgerald, 1978; McGill and Cole, 1981). Cooper (1972) and Edwards (1998) suggested that sulfohydrolases are subject to end-product inhibition by SO_4^{2-} ions, which may further control their formation and activity in the soil environment. A similar conclusion was reached by Fitzgerald (1978) regarding the activity of glycerosulfohydrolases in soils. Cooper (1972) reported that increases in the concentration of inorganic SO_4^{2-} could lead to inhibition of sulfatase activity and to an overall reduction of S mineralization from ester–SO_4–S, resulting in the accumulation of organosulfur compounds directly linked to O. The third possible explanation comes from the fact that sulfate ester production might be a safe mechanism used by soil microbes to store S without altering the pH of their surroundings (Fitzgerald, 1976). Ghani et al. (1991) stated that in some cases the occurrence of any short-term biochemical mineralization of ester–SO_4–S could be obscured by the much larger biological mineralization of C-bonded S to inorganic SO_4^{2-}–S and, with subsequent transformation of this anion to ester–SO_4–S, by soil microorganisms. The fourth mechanism that may account for the relative accumulation of S directly linked to O arises from the stabilization pattern of elements exiting in ester forms. Solomon et al. (2009) indicated that organic nutrients existing in ester forms may be stabilized independently of the main moiety of organic matter through reactions of esters with soil components such as clays, sesquioxides, or free cations present in soil solution, reducing their mobilization by extracellular enzymes. The biochemical stability of ester sulfates may also depend on their location in the structure of humic polymers (Nannipieri et al., 1990; Lou and Warman, 1992). These authors indicated that although ester–SO_4 groups located on external surfaces of humic polymers may be accessible to sulfatases, the sulfate groups that are attached to the inner struc-

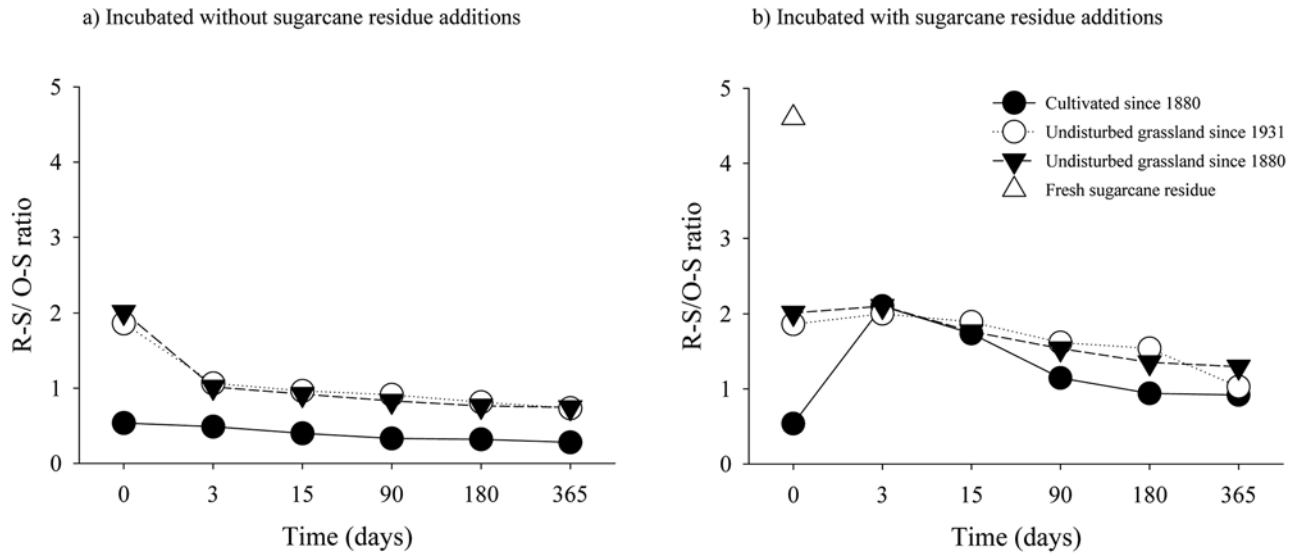

Fig. 11. Ratios of highly reduced S to highly oxidized S (RS/O-S) and organic S in the intermediate oxidation state to highly oxidized S measured using S K-edge X-ray absorption near-edge structure spectroscopy after short-term aerobic incubation of grassland-derived soils.

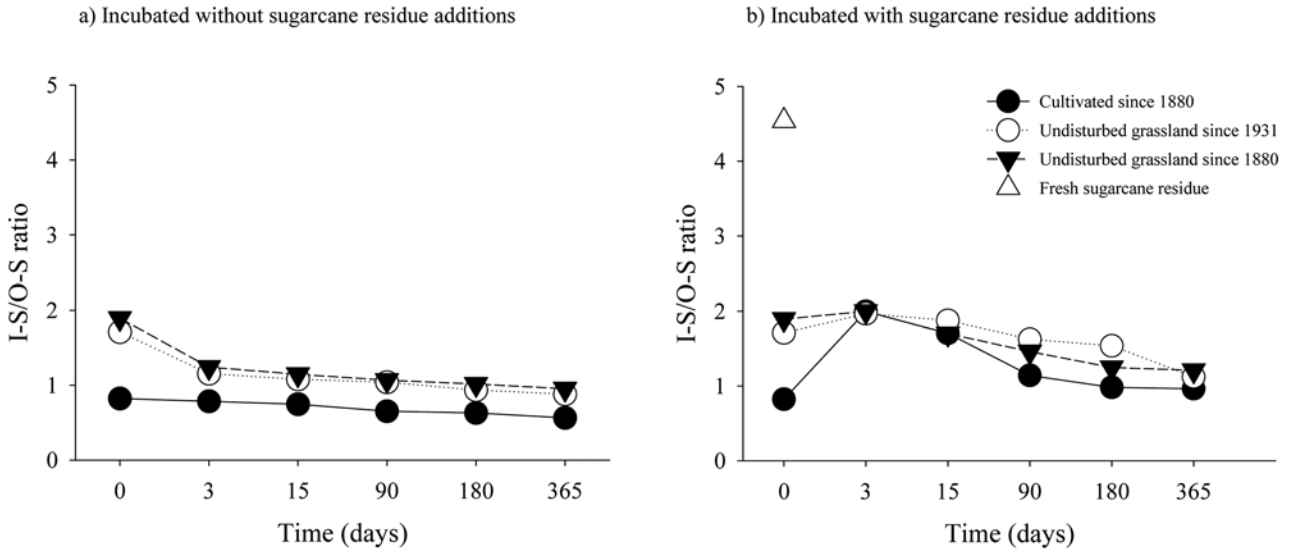

Fig. 12. Ratios of organic S in the intermediate oxidation states to highly oxidized organic S (I-S/O-S) resolved by using S K-edge X-ray absorption near-edge structure spectroscopy after short-term aerobic incubation of grassland-derived soils.

ture are less available to these enzymes, leading to the accumulation of these organosulfur compounds in the soil environment.

Conclusions

The experimental S K-edge XANES spectra recorded from grassland-derived soils correspond to transitions of photoelectrons from the S 1*s* core level to unoccupied S 3*p* σ* antibonding orbitals and represent characteristic fingerprints of the chemical state of the absorber S atom present in these compositionally complex natural matrixes. This study demonstrates that synchrotron-based X-ray spectroscopic technique is an ideal nondestructive tool to identify the various oxidation states of S, characterize the discrete organic S functionalities associated with these oxidation states into distinct groups of organosulfur compounds, and follow their long- and short-term speciation dynamics in the soil environment. Based on our XANES spectroscopy study, we concluded that there are three distinct groups of organosulfur compounds in these grassland-derived soils: (i) organic S in strongly reduced oxidation (S^0 to S^{1+}) states that encompasses thiols, monosulfides, disulfides, polysulfides, and thiophenes; (ii) organic S in intermediate oxidation (S^{2+} to S^{5+}) states that include sulfoxide and sulfonate S forms; and (iii) organic S in strongly oxidized (S^{6+}) states that comprise ester–SO_4–S. Organic S in a highly oxidized state represents a small fraction of the total soil organic S pool, indicating that S directly bonded to C and present in highly reduced and intermediate oxidation states might be the dominant organic S pool in these undisturbed grassland soils of the western United States. Among the organosulfur compounds directly linked to C, S in highly reduced oxidation states seems to be more prevalent in the undisturbed soils than S present in intermediate oxidation states. Long-term human intervention markedly altered the molecular-level composition of organic S and led to a shift in the apparent oxidation state of soil S from undisturbed grassland ecosystems primarily composed of S moieties in highly reduced and intermediate oxidation states toward managed agroecosystems dominated by strongly oxidized S, possibly influencing the bioavailability of this nutrient element. Organic S directly bonded to C in strongly reduced oxidation states, and S in the intermediate oxidation states seems to be the most biologically reactive organosulfur pool to anthropogenic disturbances; together these organosulfur pools represent the major source of biologically mineralizable S in these grassland soils. Reverting the cultivated fields back to grasslands helped the degraded soils to recover and to accumulate organic S directly bonded to C almost to the same level observed in the undisturbed grasslands. Aerobic incubation without residue addition led to a decline in organanosulfur compounds directly linked to C and to an increase in the proportion of high-valence (S^{6+}) organic S directly bonded to O with increasing duration of incubation. Among the C-bonded S compounds, low-valence (S^0 to S^{1+}) thiols, sulfides, and thiophenic S, as well as intermediate-valence (S^{2+}) sulfoxide S functionalities, seem to be highly susceptible microbial attack and may represent the most reactive components of soil organic S pool in these grassland soils. Intermediate-valence (S^{5+}) sulfonate S exhibited a much lower short-term reactivity. The incorporation of sugarcane residue increased organosulfur compounds directly bonded to C at the early stage of incubation. With an increase in the duration of incubation, however, the proportion of these organanosulfur compounds declined, whereas S directly bonded to O showed a steady rise.

Acknowledgments

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