Sulphur speciation and biogeochemical cycling in long-term arable cropping of subtropical soils: evidence from wet-chemical reduction and S K-edge XANES spectroscopy

D. Solomon[a], J. Lehmann[a], I. Lobe[b], C. E. Martinez[c], S. Tveitnes[d], C. C. Du Preez[e] & W. Amelung[b]

[a]*Department of Crop and Soil Sciences, Cornell University, Bradfield and Emerson Hall, Ithaca, NY 14853, USA,* [b]*Berlin University of Technology, Institute of Ecology, Department of Soil Science, Salzufer 12, 10587 Berlin, Germany,* [c]*Department of Crop and Soil Sciences, 418 ASI Building, The Pennsylvania State University, University Park, PA 16802, USA,* [d]*Department of Soil and Water Sciences, Agricultural University of Norway, PO Box 5028, 1432 Ås, Norway, and* [e]*Department of Soil, Crop and Climate Sciences, University of the Free State, PO Box 339, Bloemfontein 9300, Republic of South Africa*

Summary

Agriculture has claimed a large share of terrestrial environments in the tropics and subtropics through cultivation of native grasslands or forests. The impact of this anthropogenic change on speciation, dynamics, and ecological significance of sulphur (S) compounds is still poorly understood. We combined degradative wet-chemical reduction and S K-edge X-ray absorption near edge structure (XANES) spectroscopy techniques to evaluate the impact of long-term agricultural management of native grassland soils in South African Highveld on the amount, form and dynamics of S species. Sulphur XANES in the humic substances extracted by 0.1 M NaOH/0.4 M NaF solution showed the presence of strongly reduced (polysulphides, disulphides, thiols, monosulphides and thiophenes), intermediate (sulphoxides and sulphonates) and strongly oxidized (ester sulphates) organic-S. It showed that strongly oxidized-S is the predominant form (39–54%) of the total organic-S in the humic substances, and organic-S in the intermediate oxidation state represented 30–37% (78–93% of which was attributed to sulphonates). The strongly reduced organic-S comprised only 17–24% of the total organic-S. We did not find a close correlation between the results of a degradative wet-chemical procedure and XANES spectroscopy conducted in both the bulk soils (ester SO_4-S from XANES versus HI-fractionation, $r = 0.27$; $P < 0.05$) and the humic substance extracts (ester SO_4-S from XANES versus HI-fractionation, $r = 0.39$; $P < 0.05$). The ratio of reduced-S to strongly oxidized-S (R-S/O-S) in the humic substances decreased from 0.61 to 0.21, while the ratio of intermediate-S to strongly oxidized-S (I-S/O-S) declined from 0.93 to 0.61 after 90 years of arable cropping of the native grassland soils. Hence, there was a shift in oxidation state towards strongly oxidized-S (+6) and, thereby, a change in the relative proportion of the organic-S moieties associated with each oxidation state following cultivation of the native grassland soils. Therefore, we conclude that changes in land-use practice brought about not only quantitative change but also altered the composition of organic-S functional groups in these native subtropical grassland soils.

Introduction

Sulphur (S) is a component of amino acids, enzymes, vitamins and other biomolecules and is involved in a large number of biochemical processes. Thus, it is essential for the growth and

development of all forms of life and its cycling provides a key to understanding changes in the biosphere.

Agriculture has claimed a large share of productive terrestrial environments in the tropics and subtropics through clearing and cultivation of native grasslands or forests. However, there is a growing perception that current agricultural practices are neither sustainable nor environmentally acceptable.

Anthropogenic impact on the biogeochemical cycling of C and N in tropical agro-ecosystems has been the focus of substantial research (Schnitzer & Khan, 1972; Solomon *et al.*, 2002). In contrast, studies on the speciation, turnover dynamics, and ecological significance of specific S-compounds in tropical and subtropical agro-ecosystems have been limited (Neptune *et al.*, 1975; Solomon *et al.*, 2001; Möller *et al.*, 2002). It is, however, increasingly recognized that the biogeochemical cycling of S is complex, intertwined with other organically bound elements such as C, N, P, O and H and that its impact on the environment extends beyond the scale of a given eco-system. Therefore, systematic studies on the influence of land-use changes on the amount, form and composition of soil-S in various agro-ecosystems are required to understand its import-ance in the structure and functioning of ecosystems and to adopt environmentally sound and sustainable land-use practices.

One of the major limiting factors in studies that involve soil-S, however, is the unsatisfactory status of the current analyti-cal techniques to speciate S. Several approaches have been used in the past to study the speciation of S following long-term agricultural management (Neptune *et al.*, 1975; Stanko-Golden & Fitzgerald, 1991; Solomon *et al.*, 2001) and although quantitative and qualitative changes in soil-S due to cultiva-tion have been found, no consistent trends in organic-S trans-formation are apparent. Most studies are also based only on indirect wet-chemical degradation that involves reduction of S-compounds to H_2S by hydroiodic acid (HI) (Kowalenko, 1993a,b). In this method, the total-S is speciated into opera-tionally defined fractions, some of which are directly deter-mined while others are calculated as differences between analytically determined fractions. Thus, error propagation, especially for the calculated S-fractions, can result in consider-able inaccuracy (Prietzel *et al.*, 2003). This chemical reduction technique also cannot identify intermediate oxidation states of S and consequently no information is available about oxida-tion state changes and accompanying turnover rates of differ-ent organic-S species following land-use changes (Solomon *et al.*, 2003). Therefore, new approaches to soil-S studies are needed to help understand better the effects of agricultural management on soil-S cycles (Prietzel *et al.*, 2003; Solomon *et al.*, 2003).

Recently, synchrotron-based S K-edge X-ray absorption near edge structure (XANES) spectroscopy has been successfully applied to speciate and quantify S in petroleum (Waldo *et al.*, 1991), marine sediments (Vairavamurthy *et al.*, 1993), humic substances extracted from bulk soils (Morra *et al.*, 1997; Xia *et al.*, 1998; Hundal *et al.*, 2000; Hutchison *et al.*, 2002) and size separates of mineral soils (Prietzel *et al.*, 2003; Solomon *et al.*, 2003). XANES spectroscopy has also proven to be a valuable tool in the identification and quantification of S oxidation states in soils collected from organic horizons (Martinez *et al.*, 2002; Prietzel *et al.*, 2003). Sulphur K-edge XANES spectroscopy is a solid-state technique that can overcome the limitations of degradative wet-chemical reduction and provides a means to

determine directly the various S species based on the energy required for core electron transitions to bound states or ejection into a continuum (Morra *et al.*, 1997; Xia *et al.*, 1998; Solomon *et al.*, 2003). In a previous study, we demonstrated that char-acterization of oxidation states and various S moieties following agricultural management of native forest soils could indicate a relationship between the proportions of S in each oxidation state (ranging from S^{-2} to S^{6+}) and identify labile and stable S-pools present (Solomon *et al.*, 2003). The impact of long-term agricultural management (up to 90 years) on oxidation states and S moieties in soils collected from native subtropical grass-land biomes has not been determined but our hypothesis is that it will describe important reactions of S to such changes in these agro-ecosystems.

The objectives of this study were therefore (i) to assess the potential of S K-edge XANES spectroscopy to characterize the various oxidation states and S moieties in subtropical grassland soils and (ii) to evaluate how long-term agricultural management of native grassland soils affects the amount, form, distribution and dynamics of S species in South African Highveld soils using both degradative wet-chemical reduction and S XANES spectroscopy.

Materials and methods

Site description

The present study was conducted in three agro-ecosystems near Harrismith (29°7′60″E, 28°16′60″S), Kroonstad (27°13′60″E, 27°38′60″S) and Tweespruit (26°10′0″E, 29°46′0″S) in the Free State Province of South Africa. The altitude of the sites ranges from 1350 to 1800 m above sea level. The three agro-ecosystems are in the summer rainfall region with annual rainfall ranging from 516 to 625 mm and annual temperature ranging from 14 to 17°C. The soils of the area have coarse to medium texture and are classified as Dystric to Eutric Plinthosols according to FAO–UNESCO (1997).

The three agro-ecosystems belong to the Grassland Biome (Harrismith: moist cold Highveld grassland; Kroonstad: dry sandy Highveld grassland; Tweespruit: moist cool Highveld grassland), the botanical composition of which is dominated by *Cymbopogon plurinodis* (Stapf ex Burtt-Davy), *Themeda triandra* (Forssk.), *Setaria sphacelata* (Stapf & Hubbard ex Chipp), *Elionurus muticus* (Kuntze) and *Eragrostis curvula* (Nees) in Harrismith; *Eragrostis lehmanniana* (Nees), *Eragros-tis obtusa* (Munro ex Ficalho & Hiern), *Panicum coloratum* (Cav.), *Stipagrostis uniplumis* (Licht. de Winter) and *Pentzia globosa* (Less.) in Kroonstad, and *Themeda triandra* (Forssk.) in Tweespruit. In each agro-ecosystem, we selected nine indi-vidual sampling sites, comprising arable land that has been under cultivation for 2–3.5, 7.5–8.5, 10–12, 20–22, 30–32, 40–45, 57–68 and 90–98 years and adjacent native grassland. The arable land was ploughed to a depth of 20–30 cm (the

Table 1 Climatic data and selected physical and chemical characteristics of the surface soils (0–20 cm) of the native grassland and cultivated soils of the three agro-ecosystems in South Africa

Site	MDC[a] /years	MAT[b] /°C	MAP[c] /mm	Clay /%	SOC[d] /g kg^{-1}	N /g kg^{-1}	C/N	pH(H$_2$O)	pH(KCl)	CEC$_{pot.}$[e] /mmol$_c$ kg^{-1}
Harrismith	90	13.8	625	13–19	20.6–6.3	1.6–0.7	12.9–9.0	4.6–5.7	3.8–4.6	63–130
Kroonstad	98	16.6	563	10–15	8.0–2.7	0.9–0.4	9.4–7.0	5.2–6.8	4.0–5.5	42–84
Tweespruit	90	16.0	516	10–16	11.7–4.3	1.1–0.5	10.5–8.6	5.4–6.3	4.2–5.2	49–120

[a]MDC, maximum duration of cultivation. [b]MAT, mean annual temperature. [c]MAP, mean annual precipitation. [d]SOC, soil organic carbon. [e]CEC$_{pot.}$, potential cation exchange capacity.
We reported the maximum and minimum values of clay, SOC, N, C/N, pH and CEC for each agro-ecosystem. Details can be found in Lobe *et al.* (2001).

30- and 68-year-old plots in Harrismith had been ploughed to 40 cm), and cultivated with a rotation of wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), with occasional sunflower (*Helianthus* sp.). Selected site characteristics and soil physical and chemical properties are given in Table 1, with further detail in Lobe *et al.* (2001). Inorganic fertilizer was applied regularly (maize: 50–70 kg N ha^{-1}, 10–25 kg P ha^{-1}, 0–10 kg K ha^{-1}; wheat: 10–40 kg N ha^{-1}, 10–25 kg P ha^{-1}, 0–15 kg K ha^{-1}; sunflower: 20–50 kg N ha^{-1}, 10–20 kg P ha^{-1}, 2–6 kg K ha^{-1}), with 50% of N, 100% of P and 100% of K applied at planting time, while the remaining N was applied 6 weeks after planting. With the exception of the 2-year-old plot at Tweespruit where water has been applied by irrigation, the plots were neither irrigated nor organically fertilized.

Sampling

Surface soil samples (0–20 cm) were taken from five subsites on each of the native grassland and cultivated sites with a horizontal distance of > 3 m in a radial sampling scheme, and composite samples for each grassland and cultivation period were prepared from the subsamples. All samples were then air-dried and sieved to < 2 mm size before grinding them for chemical analysis. However, due to limitations in availability of instrument and allocation of beam-time, we mixed soil samples (not ground) from the three native grassland sites and from each age group of the cultivated sites of the three agro-ecosystems together and prepared one representative sample for S K-edge XANES measurement. The average cultivation period in the pooled samples was also rounded to the nearest decade.

Chemical analysis

Total soil organic carbon (SOC) and N concentrations of the soils were determined by C/H/N/S analyser (Elementar Analysensysteme GmbH, Hanau, Germany). The pH(H$_2$O) and pH(KCl) were determined in 1:2.5 soil:water (w/v) suspension. Potential cation exchange capacity (CEC$_{pot.}$) was determined
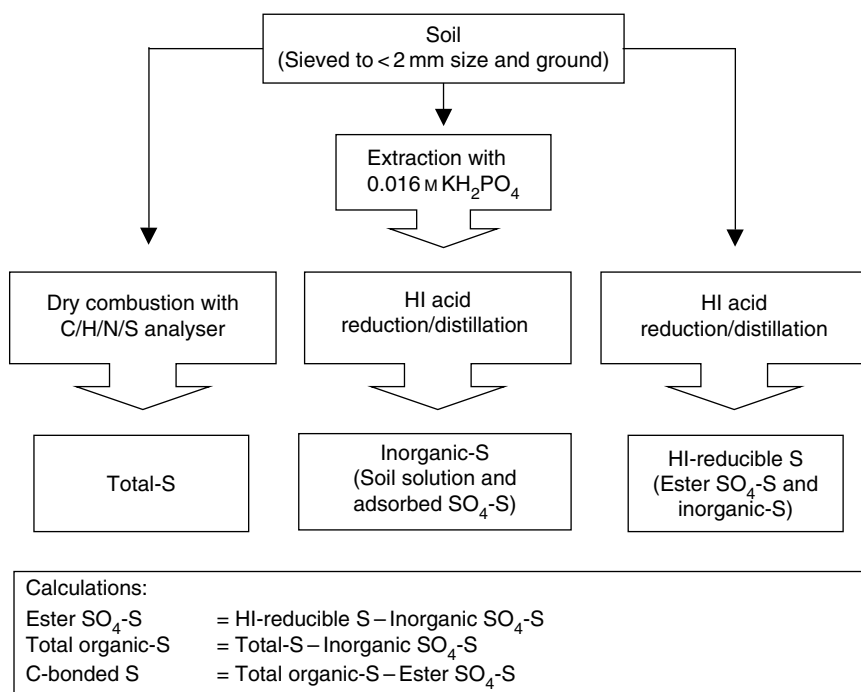
with 1 M NH$_4$OAc (pH 7.0, soil:solution ratio 1:10) according to Hendershot & Lalande (1993).

Sulphur fractionation from the bulk soils

Total-S content of the bulk soils was determined from the ground samples by C/H/N/S analyser (Elementar Analysensysteme GmbH). Inorganic SO$_4$-S (soil solution and adsorbed inorganic SO$_4$-S) from the sieved and ground soil samples was extracted with 16 mM KH$_2$PO$_4$, and the inorganic sulphate in the extracts was determined colorimetrically using the modified hydroiodic acid (HI) reduction/distillation procedure of Kowalenko (1993a). Hydroiodic acid-reducible S, consisting of ester SO$_4$-S and inorganic SO$_4$-S, in the soils was determined by direct reduction/distillation with HI-reducing mixture according to Kowalenko (1993b) and measuring the reduced-S as bismuth sulphide by spectrophotometer. Total organic-S from the soils was calculated as shown in Figure 1 as the difference between the total-S, and inorganic SO$_4$-S extracted by KH$_2$PO$_4$. The difference between HI-reducible S and inorganic SO$_4$-S was considered as ester SO$_4$-S. Because HI acid will not reduce the C-S (amino acid) or R-SO$_3$-H (sulphonate) linkages, C-bonded S was considered to be that fraction of the total organic-S that was not reduced by HI acid.

Sulphur fractionation from the humic substances

Total organic-S in the humic substances was determined directly by dry combustion using a CHNS Elemental Analyser (Carlo Erba Instruments, Milan, Italy). Determination of inorganic SO$_4$-S from the humic substances using KH$_2$PO$_4$ extraction and the HI reduction/distillation procedure (Kowalenko, 1993a) provided inconsistent and often negative results, which could be attributed to the loss of water- and alkali-soluble inorganic SO$_4$-S during the filtration and dialysis steps of the humic substance extraction. Thus, the inorganic SO$_4$-S results were not considered in the present study. HI-reducible S from the humic substances was analysed by direct reduction with HI-reducing mixture as described for the soil samples and ester

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                    ┌─────────────────────────────┐
                    │            Soil             │
                    │ (Sieved to < 2 mm size and  │
                    │          ground)            │
                    └─────────────────────────────┘
                                  │
                    ┌─────────────────────────────┐
                    │      Extraction with        │
                    │    0.016 M KH2PO4           │
                    └─────────────────────────────┘
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Figure 1 flowchart (boxes):

- Dry combustion with C/H/N/S analyser → Total-S
- HI acid reduction/distillation → Inorganic-S (Soil solution and adsorbed SO_4-S)
- HI acid reduction/distillation → HI-reducible S (Ester SO_4-S and inorganic-S)

Calculations:
Ester SO_4-S   = HI-reducible S − Inorganic SO_4-S
Total organic-S   = Total-S − Inorganic SO_4-S
C-bonded S   = Total organic-S − Ester SO_4-S

Figure 1 Flowchart showing the main steps of S fractionation.

SO_4-S was estimated as outlined in Figure 1. Carbon-bonded S in the humic substances was calculated by subtracting ester SO_4-S from the total organic-S.

X-ray absorption near-edge structure spectroscopy (XANES)

Solid-state characterization of S oxidation states in the humic substances extracted from the soils was carried out using S K-edge XANES spectroscopy at beam-line X-19 A of the National Synchrotron Light Source (NSLS), at Brookhaven National Laboratory. The sieved soil samples were extracted three times with a 0.1 M NaOH/0.4 M NaF solution (pH 12.9, soil:solution ratio of 1:5) under N_2 and the combined extracts were filtered twice through a 0.2-μm pore-size membrane filter (Gelman Supor; Pall Gelman Laboratory, MI, USA) under pressure using a vacuum pump to remove fine clay, which may interfere with the S K-edge XANES measurements. The extracts were transferred into dialysis tubes (Spectra/Por Membrane, MWCO, 12,000–14 000 Da; Spectrum Laboratories, CA, USA) and dialysed against distilled–deionized water in plastic buckets in order to eliminate soluble salts (Solomon *et al.*, 2002, 2003) and finally were freeze-dried. This NaOH/NaF procedure extracted 52–69% of the soil organic matter (SOM) from the bulk soils.

The XANES measurements of the humic substance extracts were conducted under standard operating conditions. 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 S-absorption edge of S were collected with a step size of 0.2 eV. Each XANES spectrum was averaged from three scans.

We used a monochromator consisting of double-crystal Si (111) with an entrance slit of 0.5 mm and a minimum energy resolution of 2.5 GeV at the S K-edge. The spectra were recorded in fluorescence mode using a passivated implanted planar silicon (PIPS) detector (Canberra Industries, CT, USA). The beam path from incident ion chamber to the sample chamber was purged with He gas. The samples were pressed into a 0.5 mm-thick acrylic holder and covered with 2.5 μm-thick Mylar film (Complex Industries, NY, USA).

The S K-edge data collection, justifications for the assumptions taken in the present experiment and the details of the fitting procedures were as described by Xia *et al.* (1998), Martinez *et al.* (2002) and Solomon *et al.* (2003). Background correction, normalization and deconvolution of XANES spectra for each sample into pseudocomponents were done using the non-linear least squares fitting routine solver supplied by MS-Excel. Normalization of the spectra was done to avoid spectral dependence on the total organic-S content; therefore, spectral properties are 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 white-lines (1s→3p photoelectron transition peaks). The first (AT1) and the second (AT2) arctangent functions represent the transition of ejected photoelectrons to the continuum for the unoxidized S (white-lines located between 0 and 5 eV above the elemental-S K-edge) and for the oxidized-S forms (white-lines located between 5 and 12 eV above the elemental-S K-edge), respectively. We used the sum of these two arctangent functions (AT1 and AT2) to approximate the X-ray absorption step heights (background) of the spectra. The energy positions (eV) of the Gaussian curves were used to identify the oxidation

states of S present in the sample and the peak areas were used to calculate the percentage of S present at each oxidation state. The linear component of the spectral base-line was removed before fitting and the peak areas were corrected 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; Martinez *et al.*, 2002). Due to the higher electro negativity of O, the differences are not significant for higher-valence ($\geq +4$) S species and S atoms bound to multiple oxygen atoms. Therefore, we have reported the electronic oxidation states rather than formal oxidation states as they reflect the actual electron density in the valence shell of S. Moreover, because of the above reasons, integer values were used to report the electronic oxidation states of the high-valence S species ($\geq +4$), while non-integer values were used for the low-valence ($\leq +4$) S-compounds.

Decay models

By assuming that organic matter reaches an equilibrium concentration, exponential models have been used to describe the response of C and N in soil to the duration of cultivation (Lobe *et al.*, 2001). This approach was also used successfully by Du Toit & Du Preez (1995) to describe the effect of cultivation period on total-S concentration. We have therefore employed similar approaches and used a mono-exponential model to describe the dynamics of S in the soils under investigation following long-term cultivation:

$$X_t = X_e + (X_0 - X_e)\exp(-kt), \qquad (1)$$

where X_t is the concentration of S in the soil at cultivation time t, X_e is the concentration of S in the soil at equilibrium, X_0 is the initial concentration of S in the soil ($t = 0$, S concentration in grassland), and k is a rate constant (year^{-1}).

In models for predicting concentrations of SOC in long-term experiments, the stability of the organic matter is taken into consideration by different pools (Lobe *et al.*, 2001). Assuming the presence of labile and stable pools, we also used the following bi-exponential model to describe the dynamics of the different S-pools in our soils:

$$X_t = X_1 \exp(-k_1 t) + X_2 \exp(-k_2 t), \qquad (2)$$

where X_t is as above the concentration of S in the soil at cultivation time t, X_1 is the concentration of S of the labile pool, X_2 is the concentration of S of the stable pool ($X_2 = X_0 - X_1$), X_0 is the initial concentration of S in the soil ($t = 0$, S concentration in grassland), k_1 is the rate constant of the labile pool (year^{-1}), and k_2 is the rate constant of the stable pool

(year^{-1}). The data set for the organic-S ($n = 9$), ester SO$_4$-S ($n = 9$) and C-bonded S ($n = 9$) fractions in our experiment was fairly small and would have resulted in over-parameterization if used to calculate organic-S concentrations at equilibrium (X_e) using an extended bi-exponential model, $X_t = X_1\exp(-k_1 t) + X_2\exp(-k_2 t) + X_e$. Therefore, we did not determine the equilibrium concentrations of the different organic-S forms following cultivation using the bi-exponential model.

Statistical analyses

The depletion of S was described by Equations (1) and (2) using Regression Wizard in SigmaPlot 4.0 for Windows (automatic determination of initial parameters, 100 iterations, step size 0.1, and a tolerance of 0.000001) (SPSS Inc., Munich, Germany). For the curve fittings of the organic-S fractions (organic-S, ester SO$_4$-S and C-bonded S) data set, we used the Marquardt–Levenberg algorithm. Kinetic parameters of the models were calculated from the mean S concentrations in the soils of the three agro-ecosystems. To reduce the variability between SOM concentrations in the agro-ecosystems, the S losses were expressed relative to the S concentrations of the native grassland, which we assumed to be close to those of the original grassland site before cultivation. Correlation coefficients showing the relationship between the different S-compounds measured by the HI acid reduction technique and S K-edge XANES spectroscopy were run by Pearson product moment correlation using the software package STATISTICA 5.0 for Windows (StatSoft Inc., OK, USA).

Results and discussion

S status and forms extracted by wet-chemical techniques from the bulk soils

The total-S contents in surface soils of the grassland agro-ecosystems were 144–241 mg S kg^{-1} soil at Harrismith, 75–121 mg S kg^{-1} soil at Kroonstad and 94–238 mg S kg^{-1} soil at Tweespruit (Table 2). Du Toit & Du Preez (1995) measured total-S contents of 19–300 mg kg^{-1} in a greater diversity of South African soils. Our results fall within the range reported by Acquaye & Kang (1987) for Ghanaian soils (44–281 mg S kg^{-1}) and by Neptune *et al.* (1975) for Brazilian soils (59–398 mg S kg^{-1}). However, they are smaller than the range of values found for surface soils from Puerto Rico (353–1231 mg S kg^{-1}; Stanko-Golden & Fitzgerald, 1991), Ethiopia (520–1041 mg S kg^{-1}; Solomon *et al.*, 2001) and Thailand (149 and 802 mg S kg^{-1}; Möller *et al.*, 2002). The difference between these sites may be attributed to differences in SOM contents resulting from differences in vegetation, climate, depth of sampling and soil type. The total S in the South African soils was highly significantly correlated ($P < 0.001$) with organic C ($r = 0.92$) and total N ($r = 0.91$). The C/S and N/S ratios of our surface soils

Table 2 Concentrations of various S forms and C/S and N/S ratios of the surface soils (0–20 cm) of the native grassland and cultivated sites in South Africa

Land use	Total-S	Inorganic-S	Organic-S /mg kg^{-1} soil	C-bonded S	Ester-S	C/S	N/S
Harrismith							
Native grassland	241.0	4.3	236.7	186.7	50.0	85.3	6.6
3.5 years cultivated	186.0	3.5	182.5	134.4	48.1	59.2	5.6
8 years cultivated	194.0	10.9	183.1	131.2	51.9	69.8	5.7
10 years cultivated	202.0	15.5	186.5	131.6	54.9	52.2	4.7
20 years cultivated	173.0	1.9	171.1	133.3	37.8	45.3	4.2
30 years cultivated	167.0	2.9	164.1	121.2	42.9	55.6	5.3
45 years cultivated	180.0	0.7	179.3	141.8	37.5	47.9	4.4
68 years cultivated	144.0	2.9	141.1	119.2	21.9	46.9	4.6
90 years cultivated	147.0	2.2	144.8	121.6	23.2	43.0	4.5
Kroonstad							
Native grassland	121.0	1.8	119.2	85.5	33.8	65.8	7.0
2.5 years cultivated	103.5	2.8	100.7	78.3	22.4	51.0	5.9
7.5 years cultivated	95.0	2.4	92.6	70.0	22.6	45.7	5.6
12 years cultivated	94.0	1.2	92.8	65.7	27.1	41.4	5.2
20 years cultivated	94.0	1.9	92.1	77.9	14.2	42.1	5.2
30 years cultivated	74.5	1.8	72.7	59.3	13.5	38.3	6.1
40 years cultivated	85.0	1.9	83.1	73.7	9.4	39.8	5.3
57 years cultivated	83.0	1.8	81.2	68.7	12.6	35.2	5.2
98 years cultivated	76.5	1.5	75.0	66.4	8.7	35.0	5.3
Tweespruit							
Native grassland	238.4	1.4	237.0	187.6	49.5	49.1	4.7
2 years cultivated	169.0	4.3	164.7	120.4	44.3	67.7	6.7
8.5 years cultivated	126.0	2.8	123.2	95.8	27.4	56.4	5.8
12 years cultivated	114.0	1.5	112.5	87.0	25.5	56.5	5.9
22 years cultivated	110.0	2.1	107.9	80.2	27.7	53.0	5.3
32 years cultivated	115.0	2.4	112.6	78.7	34.0	46.1	5.3
40 years cultivated	94.0	2.2	91.8	71.8	20.0	45.7	5.3
60 years cultivated	112.0	8.8	103.2	76.1	27.1	47.5	5.4
90 years cultivated	105.0	2.8	102.2	78.6	23.6	41.1	5.2

ranged from 35 to 85 and from 4 to 7, respectively (Table 2) and are in good agreement with the ranges reported for a variety of tropical soils (Neptune *et al.*, 1975; Acquaye & Kang, 1987; Solomon *et al.*, 2001).

Inorganic-S occurs in both oxidized (e.g. sulphate) and reduced (e.g. elemental-S, thiosulphate, sulphide) forms in soils. The reduced inorganic-S forms are transitory in aerobic soils and their concentrations are usually negligible. In soils under oxic conditions, however, inorganic-S occurs predominantly in sulphate (SO_4^{2-}) form (Kowalenko, 1993a). Thus in the present experiment, the total concentration of soil inorganic SO_4-S was estimated by soil solution and adsorbed SO_4-S. Inorganic SO_4-S accounted for about 3% of the total S content (Table 2). The small concentrations of inorganic SO_4-S suggest that most of the mineralized SO_4^{2-} is likely directly re-metabolized by the soil microflora, taken up by plants or leached to a greater soil depth during the few intensive rain events in these subtropical sites.

The concentration of organic-S in our soils was 141–237 mg S kg^{-1} soil at Harrismith, 75–119 mg S kg^{-1} soil at Kroonstad and 92–237 mg S kg^{-1} soil at Tweespruit, representing 97%, 98% and 97% of the total S in the three grassland agro-ecosystems, respectively (Table 2). The large proportion of organic-S in the soils is similar to that reported for tropical soils (Neptune *et al.*, 1975; Stanko-Golden & Fitzgerald, 1991; Solomon *et al.*, 2001). Unlike inorganic-S, organic-S was highly significantly correlated ($P < 0.001$) with organic C ($r = 0.91$) and total N ($r = 0.91$) in the soils under investigation, reflecting the close coupling between these elements. Expressed as the percentage of the total soil organic-S, ester SO_4-S ranged from 11 to 30% at these sites (Table 2). Although its exact form in these soils is unclear, ester SO_4-S is believed to consist largely, if not entirely, of organic sulphate containing ester linkages (R-O-SO$_3$-H), i.e. aryl sulphates, glucose sulphate, choline sulphate, chrondrotin sulphate and keratin sulphate. On the other hand, C-bonded S, which is believed to consist largely of sulphonate-S (R-SO$_3$-H) and amino acid-S (R-S)

represented 70–89% of the total soil organic-S fraction, showing that it is the dominant form of organic-S in both the native grassland and arable soils of the South African Highveld.

Dynamics of organic-S forms with long-term cropping in the bulk soils

The mean concentrations of organic-S in the cultivated soils decreased exponentially to 55% of the original amount following clearing and long-term cropping (Figure 2). This decline, however, is less pronounced than those reported for organic C (35%) and total N (45%) concentrations in these soils (Lobe *et al.*, 2001). This result corroborated earlier findings of McLaren & Swift (1977) and Solomon *et al.* (2001) for a variety of cultivated temperate and tropical soils and reflects a lower susceptibility of organic-S than of organic C and N to losses from soils under long-term arable cropping.

According to the mono-exponential model, Equation (1), used in the present study, the concentration of organic-S in the arable soils approached an equilibrium after about 19 years of cultivation relative to that of the ecological reference sites (Table 3). Lobe *et al.* (2001) indicated that the point of kinetic change, where loss of organic matter from the relatively stable pool starts controlling the overall loss of organic matter, can be defined by using the bi-exponential model, Equation (2). At this stage, the first derivative of $X_1 \exp(-k_1 t)$ will be equal to that of $X_2 \exp(-k_2 t)$. The results of the present study indicate that the stable organic-S pool dominated the rate of organic-S decline in these soils after 10 years ($t_{kc} = 10.2$) of cultivation (Table 3). Hence, we suggest that the capacity of these native grassland soils to supply S from the easily mineralizable organic-S pools declined considerably after 10 years of continuous cropping. The time of kinetic change (t_{kc}) value for the

labile organic-S fraction was of similar magnitude to that reported for microbial residues at the sites under study ($t_{kc} = 12.6$ years; Amelung *et al.*, 2002) but considerably shorter than that reported for organic C and N ($t_{kc} = 17$ and 22 years, respectively; Lobe *et al.*, 2001).

The average concentration of C-bonded S declined significantly with long-term cultivation (Table 3), rapidly so in the initial stage of cultivation, where more than one third of the C-bonded S pool was lost (Figure 2). In contrast to C-bonded S, the exponential fit for ester SO_4-S revealed that its average concentration declined slowly during the first 20 years of cropping, indicating that at the early stage of cultivation, organic-S mineralization occurred predominantly from C-bonded S (Figure 2). These results agree with those of Freney *et al.* (1969), McLaren & Swift (1977) and Solomon *et al.* (2001), where greater loss of organic-S following cultivation was found from the C-bonded than the ester SO_4-S pool. With prolonged cultivation, however, the relative depletion of ester SO_4-S surpassed the depletion from C-bonded S, with only 40% of the original ester SO_4-S remaining in the soil after 90 years of cropping (Figure 2). This was also reflected in the slow initial rate of loss of labile ester SO_4-S pool ($k_1 = 0.13$) compared with the labile C-bonded S pool ($k_1 = 0.45$; Table 3). However, with prolonged cultivation, the rates of loss of ester SO_4-S ($k_2 = 0.0068$) and C-bound S pool ($k_2 = 0.0009$) were reversed. We ascribe the relatively rapid depletion of C-bonded S at the early stage to increased biological mineralization as a result of improved soil aeration, and exposure of physically protected organic materials in aggregates due to physical disruption following clearing and cultivation of the native grassland soils. In contrast to C-bonded S, the ester SO_4-S is mobilized by biochemical processes involving extracellular enzymatic hydrolysis and is controlled by the need and
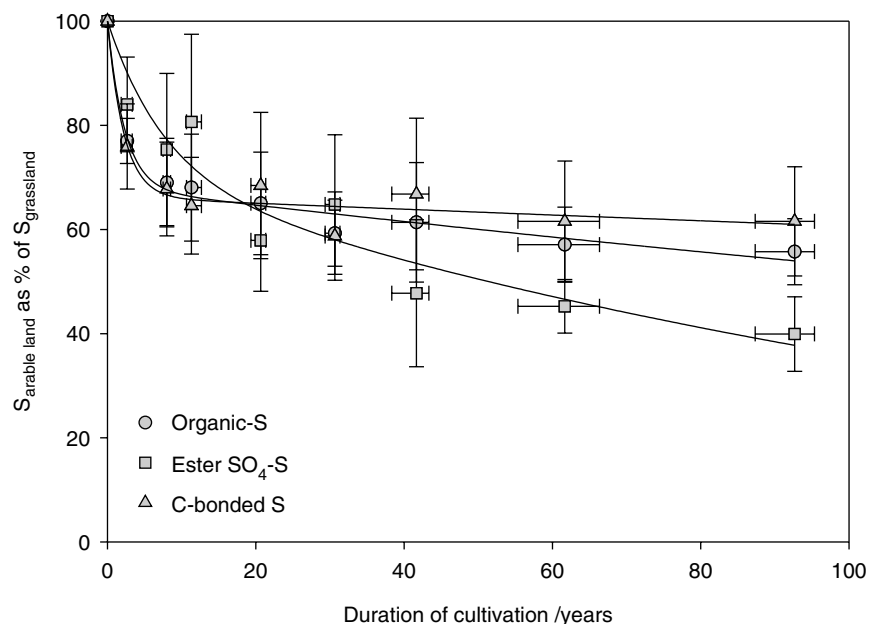
Figure 2 Effect of long-term cultivation on organic-S fractions shown as means of the three agro-ecosystems at Harrismith, Kroonstad and Tweespruit. Horizontal bars represent the span of cultivation period between the three agro-ecosystems, vertical bars indicate the standard error ($n = 3$). The lines represent the fit of the bi-exponential model, Equation (2).

Table 3 Kinetic parameters for the average decline rates of organic-S concentrations as affected by cropping period in semi-arid grassland soils of three agro-ecosystems

	Exponential model				Bi-exponential model				
S forms	k /year^{-1}	X_e /% of grassland	R^2	t_e /years	k_1 /year^{-1}	k_2 /year^{-1}	X_1 /% of grassland	R^2	t_{kc} /years
Organic-S	0.22 (0.05; 0.07[a])	60.3 (1.7; 7.6)	0.93	18.9 (–; 5.0)	0.43 (0.09; 0.21)	0.0025 (0.0005; 0.0004)	32.0 (1.5; 9.9)	0.98	10.2 (–; 2.7)
Ester SO$_4$-S	0.05 (0.01; 0.08[b])	41.9 (5.2; 11.8[b])	0.92	101.1 (–; 31.4[b])	0.13 (0.09; 0.38[b])	0.0068 (0.0030; 0.0068[b])	29.2 (11.8; 8.3[b])	0.93	16.7 (–; 8.9[b])
C-bonded S	0.38 (0.09; 0.10)	63.8 (1.3; 11.0)	0.94	10.6 (–; 1.8)	0.45 (0.13; 0.23)	0.0009 (0.0007; 0.0005)	33.8 (2.2; 10.4)	0.95	12.3 (–; 3.3)

[a]Standard errors were calculated by parameter fitting of the means and the standard errors between the estimates for the single agro-ecosystems.
[b]Only two agro-ecosystems were included in the calculation of the standard error between the estimates for the single agro-ecosystems, as the agro-ecosystem Harrismith showed a linear decline.
k, rate constant; X_e, equilibrium element concentration with $X = $ S form; t_e, time of equilibrium; R^2, coefficient of determination; k_1, rate constant of the labile pool; k_2, rate constant of the stable pool; X_1, element concentration of the labile pool according to Equation (2); t_{kc}, time of kinetic change.

the supply of the end-product (e.g. SO$_4^{2-}$) and not by the requirement of microorganisms for energy sources (McGill & Cole, 1981). Large amounts of C-bonded S have been shown to repress the formation of sulphohydrolases that mobilize ester SO$_4$-S (Fitzgerald, 1978), and thus might reduce the loss of ester SO$_4$-S at the early stage of cultivation of these soils. However, as the duration of cultivation and cropping increases, the amount of labile C-bonded S and soluble inorganic SO$_4$-S in the arable soils decreased significantly. This promotes biochemical mineralization that could lead to the decline in the concentration of ester SO$_4$-S at a later stage of cropping (McGill & Cole, 1981; Maynard *et al.*, 1984).

### S K-edge XANES characterization of organic-S forms in the humic substances

Synchrotron-based XANES spectroscopy at the S K-edge is a powerful tool that helps to determine the various oxidation states of S based on the energy position of the white-line resulting from the s →p photoelectron transition. The intensity and energy dependence of white-line features allow us to probe and determine quantitatively S functional groups in complex matrixes such as soils. We have previously demonstrated that the main features of XANES spectra taken directly from mineral soil particles seem to resemble the spectra taken from the humic substances extracted from the same size separates (Solomon *et al.*, 2003). Therefore, we have employed XANES to determine the oxidation states of S, and characterize and estimate quantitatively the proportions of the major functional groups containing S in the humic substance extracts from the soils under investigation. The use of humic substance extracts for subsequent analysis is also supported by the fact that they often represent up to of 75% of the total SOM (Schnitzer & Khan, 1972; Hutchison *et al.*, 2002). Also, in

most cases, the chemical composition of organic C and P in mild NaOH extracts seems representative of the total soil organic C and P (Solomon *et al.*, 2002, 2003). Schoenau & Bettany (1987) suggested that artificial changes such as autooxidation and alkaline hydrolysis of SOM occur following NaOH extraction of humic substances. However, recent studies of humic substance extracts from soils using S K-edge XANES (Hutchison *et al.*, 2002) failed to detect change in the oxidation states of S in humic substances aerated at various pH levels for up to 44 hours.

The spectra recorded from the humic substance extracts in the present experiment generally show four major absorption bands in the energy ranges of 2473–2476 eV, 2477–2478 eV, 2480–2483 eV and 2483–2486 eV (data not shown). These features were consistent throughout the S XANES spectra, thus allowing us to deconvolute and fit the experimental spectra of the humic substance extracts using a series of Gaussian curves (G1 to G5) and the sum of the two arctangent step functions (AT1 and AT2) as shown in Figure 3. In order to provide a detailed fingerprint of organic-S species, we also present the base-line corrected and normalized S K-edge XANES spectra in Figure 4. The relative energy position, predicted electronic oxidation states, and structure of representative S-compounds are shown in Table 4.

The spectra in Figure 4 show the presence of several whitelines in the range of 0–10 eV above the S K-edge energy. These results indicate the presence of numerous oxidation states of S in the humic substances extracted from these soils under investigation. Based on the electronic oxidation states recorded in the present experiment (Table 4) and literature data (Morra *et al.*, 1997; Xia *et al.*, 1998; Solomon *et al.*, 2003), we grouped the different oxidation states and the organic-S functional moieties associated with them into three major oxidation states: (i) organic-S in its
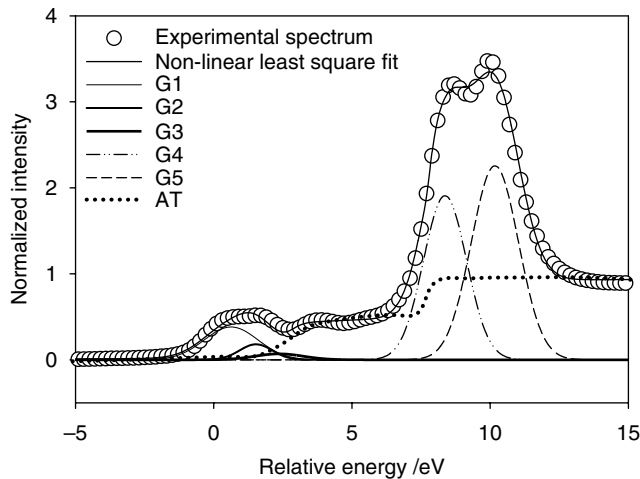
Figure 3 Typical non-linear least square fit of experimental S K-edge XANES spectrum showing the various oxidation states of S in the humic substances extracted from the South African soils (G1 to G5 = Gaussian peak one to five; AT = arctangents 1 and 2).

strongly reduced state (sum of G1 and G2, which include poly-sulphides, disulphides, thiols, monosulphides and thiophenes), (ii) organic-S in its intermediate oxidation state (sum of G3 and G4, which include sulphoxides and sulphonates) and (iii) organic-S in its strongly oxidized state (G5, representing sulphates) (Table 4). Since the absorption cross-section of the different S species increases linearly with increasing oxidation state (Waldo *et al.*, 1991; Xia *et al.*, 1998), the relative proportion of each organic-S functional group (Table 5) at the specific electronic oxidation state for the spectra shown in Figure 4 was computed from the areas under each Gaussian curve shown in Figure 3.

Morra *et al.* (1997) have shown that humic acids from poorly drained soil, peat and aquatic sediments produce white-lines indicative of high-valence S predominantly in the +5 oxidation state with smaller proportions in the +6 state. These results have been corroborated by XANES analysis of the high-valence S species in peat by Martinez *et al.* (2002) and in humic substances extracted from clay-size separates of forest-derived tropical soils by Solomon *et al.* (2003). Contrary to these results, the spectral features of our stacked S K-edge XANES (Figure 4) indicate the presence of a large and distinctive peak in the positive high-valence (+6) region representing strongly oxidized-S in our humic substance extracts. According to Table 5, this strongly oxidized-S is the predominant form representing 39–55% of the total organic-S in the humic substances extracted from these soils. Because inorganic SO_4-S species are not present in significant proportions in these extracts (Solomon *et al.*, 2003), we considered the large proportion of S found in the strongly oxidized state to be ester SO_4-S. The white-line patterns of the S XANES spectra and the relative abundance of S species at each oxidation state resemble, both qualitatively and quantitatively, the spectra
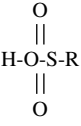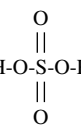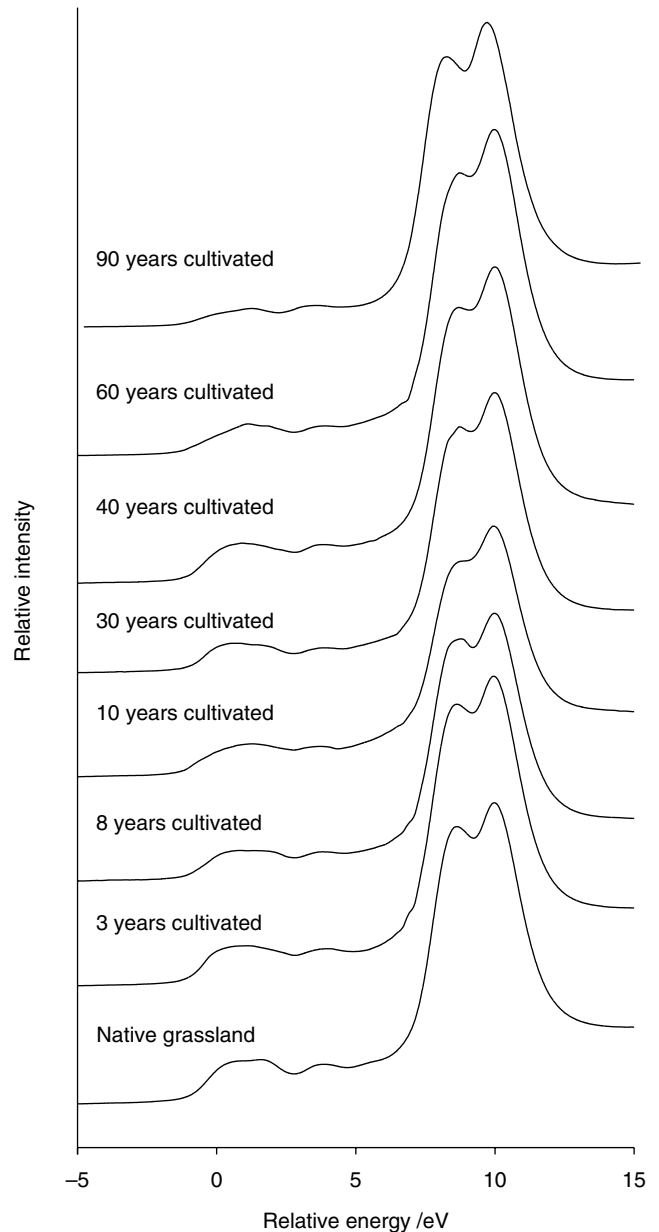

Figure 4 Stacked S K-edge (2472 eV) XANES spectra of the humic substances extracted from the native grassland and cultivated soils from South Africa.

recorded from humic substances extracted from silt-size separates of subhumid tropical soils (Solomon *et al.*, 2003). These results are also consistent with the results from humic substance extracted from a silty loam soil from Wisconsin by Xia *et al.* (1998), where organic-S in the +6 oxidation state was reported to be more dominant than organic-S in the +5 oxidation state.

Our investigation provided evidence that organic-S in the intermediate oxidation state was the second most abundant form of S (30–37% of the total organic-S in the humic substances). Similar to our previous findings from tropical

Table 4 Peak maxima (relative energy position), predicted oxidation states and structures of representative organic-S compounds in the humic substance extracts of native grassland and cultivated soils from South Africa

Organic-S compounds[a]	Gaussian curve	Peak maxima /eV	Electronic oxidation state	Structure
Most reduced-S				
Polysulphide	G1	0.43–0.63	0.14–0.30	R-S-S-S-R'
Disulphide				R-S-S-R'
Thiol				R-S-H
Monosulphide				R-S-R'
Thiophene	G2	1.5–1.8	0.80–0.96	(thiophene ring structure)
Intermediate-S				
Sulphoxide	G3	2.3–3.2	1.3–1.8	R-S-R' with =O
Sulphonate	G4	7.7–8.4	5.0	H-O-S-R with two =O
Highly oxidized-S				
Ester sulphate	G5	9.5–10.2	6.0	H-O-S-O-R with two =O

[a]Waldo *et al.* (1991); Vairavamurthy *et al.* (1993); Morra *et al.* (1997); Xia *et al.* (1998); Prietzel *et al.* (2003); Solomon *et al.* (2003).

Table 5 Changes in the relative proportion of organic-S forms using XANES spectroscopy and degradative wet-chemical reduction techniques in native grassland and continuously cultivated soils of three agro-ecosystems in South Africa

	XANES spectroscopy					Wet-chemical	
	Gaussian curve						
Land use	G1 0.14–0.30[a]	G2 0.80–0.96	G3 1.3–1.8	G4 5.0	G5 6.0	Ester SO$_4$-S /%	C-bonded S
Native grassland	19.1	4.8	2.4	34.3	39.3	34.8	65.2
3 years cultivated	18.0	3.5	5.2	28.0	45.3	36.4	63.6
8 years cultivated	17.5	1.6	4.7	28.2	48.0	30.6	69.4
10 years cultivated	18.5	4.0	6.6	24.0	46.8	37.7	62.3
20 years cultivated	13.7	4.0	3.9	28.9	49.6	40.8	59.2
30 years cultivated	16.0	2.4	3.3	28.5	49.8	28.0	72.0
40 years cultivated	17.6	3.1	5.1	25.7	48.5	35.0	65.0
60 years cultivated	13.1	2.9	2.9	26.8	54.3	40.1	59.9
90 years cultivated	10.4	1.3	3.4	30.0	55.0	42.5	57.5

[a]Electronic oxidation state in eV.

climates (Solomon *et al.*, 2003), the organic-S in the intermediate oxidation state was enriched with sulphonates (78–93%). Xia *et al.* (1998) reported a similar range (26–38%) for the percentage of S in the intermediate oxidation states in humic substances extracted from aquatic and terrestrial environments. Our results agree with those of Strehl & Prietzel (1999) who found that, within the C-bonded S pool, a large proportion of more oxidized C-bonded S fraction comprised sulphoxides, sulphones and sulphonates at the expense of more reduced C-bonded S species in studies conducted in Germany. It should be noted, however, that the results obtained from the humic substances by S XANES spectroscopy were contrary to results of earlier studies using degradative wet-chemical techniques (e.g. Maynard *et al.*, 1984), where C-bonded S in soils

has been mainly attributed to amino acids such as cysteine, cystine and methionine.

The strongly reduced-S compounds in the form of monosulphide, disulphide, polysulphides, thiols and thiophenes comprised only a small fraction of the total organic-S (17–24%) in the humic substances extracted from these South African soils. These values are similar to those reported from well-drained arable soils from Wisconsin by Xia *et al.* (1998) and from size separates of arable soils in the tropics by Solomon *et al.* (2003). However, our samples contained significantly smaller proportions of S in reduced forms than reported for humic extracts from aquatic environments (Xia *et al.*, 1998), peat (presumably under strongly reduced conditions) (Martinez *et al.*, 2002) and organic horizons of seasonally wet forest soils (Prietzel *et al.*, 2003). It appears therefore that S K-edge XANES is a powerful tool for detecting links between the relative proportions of organic-S moieties at each oxidation state, their environmental origin and current agricultural management practice.

S speciation by wet-chemical reduction and XANES spectroscopy techniques

Even though both HI-reduction (Freney *et al.*, 1969; Neptune *et al.*, 1975) and S XANES spectroscopy (Morra *et al.*, 1997; Xia *et al.*, 1998) were used in the past to speciate S in soils, there are only few studies that attempt to compare the conventional wet-chemical technique with XANES spectroscopy in soils and humic substance extracts. Recently, Hundal *et al.* (2000) found that the relative proportions of reduced- and oxidized-S species determined by selective dissolution and by XANES spectroscopy were of similar magnitude in biosolid-derived fulvic acid, and concluded that the two S-speciation methods are in close agreement. Prietzel *et al.* (2003) reported that the average proportion of reduced organic-S analysed by near edge X-ray absorption fine structure spectroscopy (NEX-AFS) agreed with that obtained by wet-chemical analysis for organic horizons at Schluchsee, Germany, while this similarity was not found for organic horizons sampled from Rotherd-bach, Germany. In the present investigation, we did not find a close relationship between the relative proportions of organic-S species identified by XANES spectroscopy in the humic substances and by degradative wet-chemical fractionation techniques of the bulk soils (ester SO_4-S as revealed by XANES versus HI-fractionation, $r = 0.27$; $P < 0.05$). Our study shows that the proportion of ester SO_4-S measured by wet-chemical analysis from the bulk soils (11–30%) was generally less than the proportion of ester SO_4-S determined by XANES spectroscopy (39–55%) (Tables 2 and 5). In order to compare the two S-speciation techniques on a similar matrix, we also determined the proportions of ester SO_4-S and C-bonded S in the humic substances extracted from the bulk soils using the degradative wet-chemical technique. We found that C-bonded S was the major form of organic-S associated with the humic substance extracts, representing 58–72% of the total organic-S (Table 5). This is in the range reported for S-speciation studies using mild-alkali extractable humic substances from soils by McLaren & Swift (1977) and Möller *et al.* (2002). The proportion of ester SO_4-S accounted for 28–42% of the total organic-S from the humic substances extracted from our soils, and compares well with the results of Bettany *et al.* (1979), where a slightly larger proportion of HI-reducible S fraction was found in the humic substances compared with the corresponding bulk soils. However, as for the bulk soils, the proportion of ester SO_4-S measured by wet-chemical reduction from the humic substances did not correlate significantly with the proportion of ester SO_4-S identified by the solid-state S XANES spectroscopy (ester SO_4-S as revealed by XANES versus HI-fractionation, $r = 0.39$; $P < 0.05$). These results agree, in turn, with our previous findings using both differential S-reduction and XANES spectroscopy (Solomon *et al.*, 2001, 2003), where the solid-state spectroscopic technique determined a larger proportion of strongly oxidized-S species compared with wet-chemical fractionation. The discrepancy in these sets of values could be caused by limitations in the comparability of the two S-speciation techniques for different subgroups of organic-S fractions. Prietzel *et al.* (2003) and Solomon *et al.* (2003) suggested that the wet-chemical technique relies on the differential reduction of organic-S compounds to H_2S and thus it might not recover all the organic-S fractions compared with XANES, which is a more direct and non-destructive technique. The results of the present experiment show that with the help of XANES spectroscopy, it is possible to speciate directly and quantify successfully the proportion of the various organic-S functional groups and their respective oxidation states in humic substances extracted from mineral soils under different land-use practices. Unlike earlier studies (Fitzgerald, 1978), where a significant proportion of ester SO_4-S was assumed not to be an integral part of the humic substances, our study also provided evidence for the presence of a substantial amount of ester SO_4-S in humic substances. The shortcoming of S K-edge XANES spectroscopy, however, is its inability to discriminate precisely between soil organic-S forms with an oxidation state of 0 to +1 (polysulphides, disulphides, thiols, monosulphides and thiophenes). Sulphur L-edge XANES spectroscopy could provide complementary information to identify these S moieties (Kasrai *et al.*, 1996), but this technique only works well in samples that have large amounts of S such as in coal (M. Kasrai, personal communication), and our attempt to resolve the organic-S species in humic substance extracts using S L-edge XANES spectroscopy did not provide satisfactory results (Solomon, unpublished data).

Land-use effects on organic-S compounds in the humic substances

We have previously suggested that in undisturbed natural ecosystems where the supply of S from the atmosphere is small, SOM plays a major role in S nutrition of plants by affecting the release of S present as part of complex organic

polymers (Solomon *et al.*, 2001, 2003). In such ecosystems, S cycling is essentially closed with minimal short-term losses or gains, as a balance exists between the input and output processes. The distribution of organic-S across various oxidation states and functional forms thus reflects the history, present structure and functioning of the natural ecosystem. However, the steady state attained under undisturbed conditions and, thereby, the forms and dynamics of organic-S can be influenced dramatically by anthropogenic interventions such as clearing and cultivation that often involve changes in vegetation cover, biomass production and nutrient cycling in the ecosystem (McLaren & Swift, 1977; McGill & Cole, 1981; Solomon *et al.*, 2001, 2003). Our study of humic substances reflected such changes in the soils of these natural and managed subtropical grassland agro-ecosystems. Examination of the organic-S fractions isolated by the wet-chemical method revealed an apparent increase in the proportion of ester SO_4-S and a decline in the proportion of C-bonded S in the humic substance extracts following cultivation of the native grassland sites (Table 5). The S K-edge XANES spectra (Figure 4) and the relative proportions of the various S moieties in each oxidation state (Table 5) also indicate that land-use changes resulted in a substantial change in both oxidation state and composition of the organic-S in the humic substances. The major shift occurred in the strongly reduced-S species (G1 and G2), the relative proportions of which decreased from about 24% in the native grassland soils to about 12% in soils cultivated for 90 years (Table 5). Our results also indicate that the proportion of organic-S compounds in the intermediate oxidation states (G3 and G4) showed a relatively small but consistent decrease (from 37% in the native grassland to 30% in the 60 years cultivated soils). In contrast, the proportion of strongly oxidized-S (G5) as a fraction of the total organic-S increased considerably from 39 to 55% following clearing and long-term cultivation of the grassland (Table 5 and Figure 4).

The shift in oxidation state of organic-S towards the strongly oxidized-S (+6) region as a result of anthropogenic changes was further illustrated by the ratios of the strongly reduced-S to strongly oxidized-S (R-S/O-S) and intermediate-S to strongly oxidized-S (I-S/O-S) (Figure 5). These ratios decreased exponentially following clearing and long-term cultivation of the native grassland soils: that of R-S/O-S from 0.61 to 0.21 and that of I-S/O-S from 0.93 to 0.61 after 90 years of cultivation. The shift in the oxidation state towards the more oxidized organic-S species is accompanied by an increase in the ratio of phenolic acids to aldehydes. This indicates side-chain oxidation of lignin (Lobe *et al.*, 2002) following long-term cultivation, suggesting that agricultural management influenced the oxidation state not only of organic-S but also of the C moieties in these subtropical agro-ecosystems. The results from the present experiment agree well with our previous study of subhumid tropical agro-ecosystems (Solomon *et al.*, 2003), where we observed a sharp decline in R-S/O-S and I-S/O-S ratios of humic substances extracted from clay- and
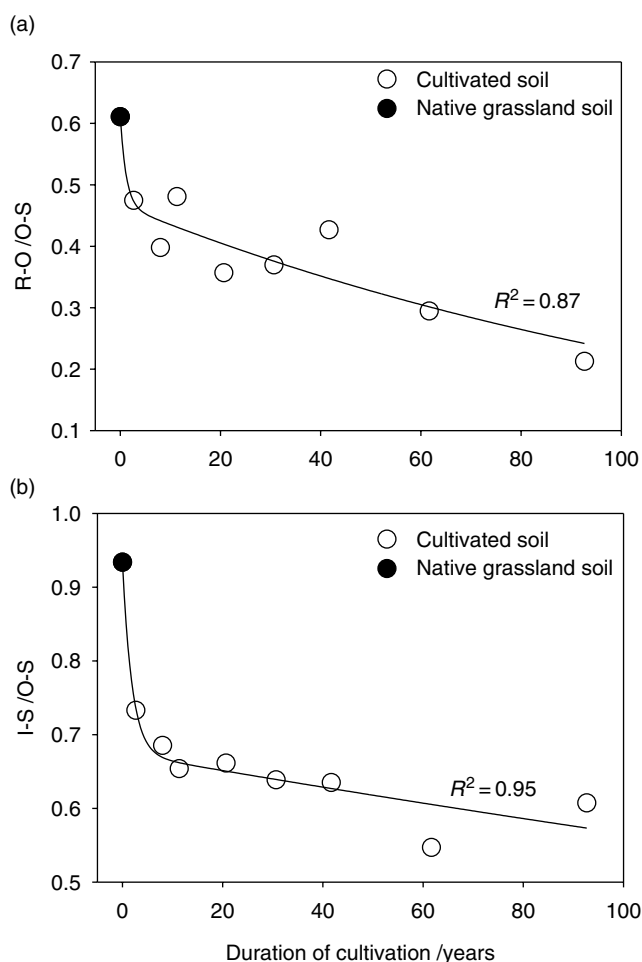
Figure 5 Effect of cultivation period on (a) strongly reduced-S to strongly oxidized-S (R-S/O-S) ratio and (b) intermediate-S to strongly oxidized-S (I-S/O-S) ratio in the humic substances extracted from the native grasslands and cultivated soils of South Africa.

silt-size separates following deforestation of natural forests and conversion to low-input cultivation. The relative shift in oxidation state towards organic-S found at the high-valence (+6) state could be explained by the more transitory nature of strongly oxidized organic-S fractions. McLaren & Swift (1977) and McGill & Cole (1981) suggested that ester SO_4-S is a transitory structure. These authors argue that the enhanced microbial activity associated with cultivation of native soils could lead to accelerated transformation of the more reduced organic-S fractions (C-bonded S) to more oxidized organic-S forms (ester SO_4-S), before S is released as inorganic SO_4-S. This mechanism would tend to shift the balance towards strongly oxidized organic-S species, whereas the relative proportion of the C-bonded S fraction (especially S in the strongly reduced oxidation state) falls continuously in the humic substances (McGill & Cole, 1981; Solomon *et al.*, 2001, 2003). Our data do not provide final proof for or against this hypothesis, but suggest the need for further research that involves land-use

and management changes in contrasting agro-ecosystems to understand the reasons behind the shift in oxidation state of organic-S species in humic substances. The shift in oxidation state and thereby a change in the relative proportion of the various organic-S moieties associated with each oxidation state, however, indicated that conversion of native subtropical grasslands to long-term cultivation has an impact on the composition and oxidation states of the organic-S. Therefore, changes in land-use practice not only brought quantitative changes in the organic-S content but also affected the composition of the various functional groups of the organic-S in the humic substances extracted from these soils, which we were able to detect with the help of XANES spectroscopy.

Conclusion

We were able to determine the various species of S with the help of differential reduction and S K-edge XANES spectroscopy, and to follow the dynamics of S both quantitatively and qualitatively in subtropical grassland agro-ecosystems following land-use changes.

Most loss of soil organic-S during the first 20 years of arable cropping occurred from labile C-bonded S pools, suggesting that S dynamics in the short term was controlled mainly by biological mineralization. The long-term (> 20 years) loss, however, occurred from the ester SO_4-S pool probably due to biochemical mineralization.

Organic-S in the strongly oxidized state was the dominant form of S followed by S in the intermediate and strongly reduced oxidation states in the humic substances extracted from these subtropical grassland soils. Long-term arable cropping of these native grassland soils led to a shift in oxidation states from the strongly reduced and intermediate oxidation states towards the strongly oxidized-S (+6) region and, thereby, a change in the relative proportion of the organic-S moieties associated with each oxidation state. Thus, we conclude that land-use changes not only brought about quantitative changes but also altered the composition of organic-S functional groups in the humic substances extracted from these soils.

The present study indicated that S K-edge XANES is a powerful tool for detecting links between the relative proportions of organic-S moieties of each oxidation state and their environmental origin, and for investigating the long-term impact of land-use changes on soil organic-S in subtropical agro-ecosystems.

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