Sulphur speciation and turnover in soils: evidence from sulphur K-edge XANES spectroscopy and isotope dilution studies

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Abstract

Sulphur K-edge X-ray absorption near edge structure (XANES) spectroscopy was used to quantify S species in humic substance extracts from ten soils from the UK, China and New Zealand, which differ in land use and agricultural management. XANES spectroscopy showed the presence of most reduced (sulphides, disulphides, thiols and thiophenes), intermediate (sulphoxides and sulphonates) and highly oxidised S (ester sulphates) forms, with the three groups representing 14–32%, 33–50% and 22–53% of the organic S in the humic substance extracts, respectively. Land use had a profound influence on the relative proportions of S species. Well-drained arable soils generally had a higher proportion of organic S present in the most oxidised form than the grassland soils collected nearby, whereas paddy soils showed a more reduced profile due to episodic flooding. In the Broadbalk Classical Experiment at Rothamsted, reversion of an arable system to grassland or woodland in the 1880s resulted in an increase of the most reduced and intermediate S species at the expense of the most oxidised S species. Long-term applications of farmyard manure to an arable plot also shifted S species from the most oxidised to the intermediate and the most reduced species. Sulphur immobilisation and gross mineralisation were determined in seven soils using the ^{35}S isotope dilution method. Gross mineralisation during a 53-day incubation correlated more closely with the amounts of the most reduced and intermediate S species than with the most oxidised S species, suggesting that the former (C-bonded S) were the main source of organic S for mineralisation in the short-term.
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1. Introduction

Sulphur is an essential plant nutrient, but can also be an environmental pollutant if it is present in acidic forms in excess. Excessive emissions of SO_2 from man-made sources have caused widespread acid rain and acidification of freshwaters and soils in Europe and North America in the past (Reuss et al., 1987; Rodhe, 1999), and in Southern China at present (Smith et al., 2001; Feng et al., 2002). In contrast, S deficiency in crops has increased in Europe (McGrath et al., 2002) and other parts of the world (Blair, 2002), as a result of decreased atmospheric inputs, changes in fertiliser uses and increased crop yields. To predict the supply of S from the soil to crops or to assess the impact of excessive S on ecosystems requires an understanding of the forms of S and its transformations in soil.

Most soils contain > 90% of the total S in organic forms (Zhao et al., 1996). Traditionally, soil organic S has been fractionated into HI-reducible and non-reducible S, which are thought to represent ester sulphates and C-bonded S, respectively (Freney, 1986). Additionally, C-bonded S may be further divided into Raney Ni-reducible or non-reducible S. However, the S fractions thus determined are operationally defined, and the exact species of S cannot be ascertained. Recently, synchrotron-based S K-edge X-ray absorption near-edge structure (XANES) spectroscopy has been successfully used to determine different oxidation states of S in humic substances extracted from soils (Morra et al., 1997; Xia et al., 1998; Hundal et al., 2000; Hutchison et al., 2001; Solomon et al., 2003; Solomon et al., 2005) and in size separates of mineral soils (Prietzel et al., 2003; Solomon et al., 2003). Sulphur XANES spectroscopy is a solid-state technique that provides a means to directly determine the various S species based on the energy required for core electron transitions to bound states or ejection into continuum. The technique can overcome the limitations of wet chemical reduction methods and provides unambiguous information on the species of S that are present. Previous studies showed that long-term agricultural management had a significant impact on S speciation in humic substance extracts from tropical

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and sub-tropical regions in Africa (Solomon et al., 2003; Solomon et al., 2005). So far, only a very limited number of soils around the world have been analysed for S species with XANES spectroscopy. There are no studies linking the S speciation measurements by XANES with the processes of S mineralisation and immobilisation.

The objectives of the present study were to investigate the influence of different land uses and long-term applications of organic manure on S speciation in soil organic S, using humic substance extracts by the application of the S XANES spectroscopy, and to evaluate the relationship between S speciation measurements and S mineralisation and immobilisation determined using ^{35}S isotope dilution. The ^{35}S isotope dilution technique allows us to measure mineralisation and immobilisation simultaneously (McLaren et al., 1985; Nziguheba et al., 2005)

2. Materials and methods

2.1. Soils

We collected ten soils from contrasting agroecosystems from various parts of the world, including four from the Broadbalk Classical Experiment at Rothamsted, England, and two each from Scotland, New Zealand and China. The Broadbalk experiment at Rothamsted started in 1843, and has run continuously for more than 160 years (see Goulding et al. (2000) for further details of the experiment). The original objective of the experiment was to test the effects of different fertilisers on wheat yield. In 1882, 0.2 ha of arable land on the western side of Broadbalk, which was unmanured for many years, was fenced off and left uncultivated. This area is named the 'Wilderness' and is divided into three parts: (a) an undisturbed area which has developed into a mixed

deciduous woodland; (b) an area where invading woody plants have been regularly removed and the resulting grassland is not grazed; and (c) a third area created in 1957, when half of the ungrazed grassland changed to a regime of annual grazing. In the present study, we collected four samples from this experiment from two arable plots (plot 3: unmanured control; plot 22: farmyard manure (FYM; 35 t ha^{-1} fresh weight) annually; both from the continuous wheat section) and two Wilderness areas (woodland and ungrazed grassland).

The Scottish soils were sampled from Pitcaple (grassland) and Inschfield (arable), near Aberdeen. The two locations were 1.5 km apart and both soils belong to the Insch series. The New Zealand soils were collected from an arable and a pasture field on the Crop & Food Research farm at Lincoln, Canterbury. The two fields were within 100 m of each other, and have been either arable or pasture for over 15 years. The two Chinese soils were collected from paddy fields in Nan Ling County, Anhui Province. Except the four Broadbalk soils which were sampled to a depth of 23 cm, all other soils were sampled to 20 cm. Soils were air-dried and gently ground to pass a 2 mm sieve. Soil classifications and textures are shown in Table 1.

2.2. Chemical analysis

Soil pH was determined in a suspension of 1:2.5 soil:water (w:v). Soil total C and N were determined by dry combustion with a LECO analyser (St Joseph, MI, USA). Organic C was calculated as the difference between total and inorganic C (measured as $CaCO_3$). The soil total S concentration was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES) (Fisons-ARL Accuris, Ecublens, Switzerland), after a digestion with a mixture of HNO_3 and $HClO_4$ (85/15, v/v) (Zhao et al., 1994). Adsorbed and

Table 1
Selected properties of the soils studied

Soil	Soil classification	Soil texture	pH	Organic C (g kg^{-1})	Total N (g kg^{-1})	Total S (mg kg^{-1})	SO_4^{2-}–S (mg kg^{-1})	Organic S (mg kg^{-1})	Ratio		
									C/N	C/S[a]	N/S[a]
Broadbalk woodland	Aquic paleudalf	Silty clay loam	7.2	43.3	3.9	467	17.8	449	11.1	96	8.6
Broadbalk grassland	Aquic paleudalf	Silty clay loam	7.4	39.7	3.4	531	10.6	520	11.7	76	6.6
Broadbalk arable control	Aquic paleudalf	Silty clay loam	8.0	8.2	0.9	154	3.3	151	9.4	54	6.4
Broadbalk arable +FYM	Aquic paleudalf	Silty clay loam	7.6	32.2	3.0	545	6.0	539	10.7	60	5.8
Scottish pasture	Typic dystrochrept	Sandy loam	4.8	49.5	3.9	652	3.3	648	12.8	76	6.0
Scottish arable	Typic dystrochrept	Sandy loam	6.7	44.6	3.9	582	6.3	576	11.5	77	6.7
New Zealand pasture	Udic ustochrept	Silty loam	5.7	34.5	2.7	338	4.6	333	12.8	103	8.1
New Zealand arable	Udic ustochrept	Silty loam	5.8	26.5	2.2	282	16.8	265	12.3	100	8.1
Chinese paddy A	Typic haplaquept	Clay	7.1	27.6	2.5	832	394.1	438	10.9	63	5.8
Chinese paddy B	Typic haplaquept	Clay	4.8	26.5	2.3	493	186.0	307	11.4	86	7.6

[a] C/S and N/S ratios were calculated using organic S data.

water-soluble inorganic sulphate was extracted with 0.016 M KH_2PO_4 in a soil to solution ratio of 1:5. Sulphate concentrations in the extracts were determined with ion chromatography (Dionex DX500, Sunnyvale, CA, USA), using an IonPac AS9A separation column fitted with an IonPac AG9A guard column. The eluent consisted of 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ at a flow rate of 2 ml min^{-1}. Solutions were filtered through a 0.2 μm membrane filter before analysis. Selected soil properties are shown in Table 1.

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

Humic substances were extracted from the soils with a mixture of 0.1 M NaOH and 0.4 M NaF (pH 12.4) at a soil to extraction solution ratio of 1:5 (w/v) under N_2 environment 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 dialyzed against distilled-deionized water in order to remove soluble salts (Solomon et al., 2005) and finally were freeze-dried. This NaOH/NaF procedure extracted 51–72% of the soil organic matter (SOM) from the bulk soils. Solid-state characterisation of the oxidation states of S in the humic substances was carried out using S K-edge X-ray Absorption Near-Edge Structure (XANES) spectroscopy at beamline X-19A of the National Synchrotron Light Source (NSLS), at Brookhaven National Laboratory. Scans ranging from 150 eV below to 300 eV above the absorption edge of S at 2472 eV were collected with a step size of 0.2 eV. Each XANES spectrum was composed of an average of 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 2.8 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 the incident ion chamber to the sample chamber was purged with He gas. The samples were pressed into 0.5 mm thick acrylic holder and covered with 2.5 μm thick Mylar film (Complex Industries, NY, USA). Deconvolution of the spectra into pseudo-components was accomplished using the nonlinear least-square fitting as described by Xia et al. (1998). The linear component of the spectral baseline was removed before fitting and 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) according to Xia et al. (1998). The Gaussian curves correspond to different oxidation states and organic S forms (Xia et al., 1998; Solomon et al., 2003). The first and the second arctangent functions represent the transition of ejected photoelectrons to the continuum for the unoxidised S (white-lines located between 0 and 5 eV above the elemental S K-edge) and for the oxidised-S forms (white-lines located between 5 and 12 eV above the elemental-S K-edge), respectively. We combined these two arctangent functions (AT) to approximate the X-ray absorption

step heights (background) of the spectra. Previous studies showed that the spectral characteristics of organic S obtained from extracts and those obtained from whole soil are almost identical (Solomon et al., 2003). Therefore, S XANES analyses of the soil extracts adequately represent organic S forms in total soil.

2.4. Sulphur mineralisation and immobilisation

Seven soils were used in this experiment, including Broadbalk arable control, arable + FYM and grassland (not subject to grazing), two Scottish and two New Zealand soils. The two Chinese paddy soils were not used in this experiment because they contained very large concentrations of extractable sulphate (Table 1). The Broadbalk woodland soil was also not used because of insufficient sample. Soil samples (< 2 mm) equivalent to 0.5 kg dry weight were placed in 1 L Kilner jars. Soils were amended with 10 mg S kg^{-1} soil, added as K_2SO_4 which was labelled with $^{35}SO_4^{2-}$ to an activity of 100 kBq mg^{-1} S. K_2SO_4 was added in solution and mixed thoroughly with the soils. The soil moisture content was raised to 70% of the water holding capacity (WHC) with deionised water, and maintained at this level throughout the incubation period at 20 °C in the dark, with the jars being closed loosely. Soils were mixed periodically. Immediately after the addition of K_2SO_4 and on day 53, three portions of soil samples equivalent to 20 g dry weight (DW) were taken from each jar and extracted with 100 ml 0.016 M KH_2PO_4 for 1 h. The suspensions were filtered through Whatman No. 42 filter paper. A 2-ml aliquot of the extract was mixed with 10 ml of cocktail (Ultima Liquid Gold, Packard Ltd, UK) in a polypropylene scintillation vial. The ^{35}S-radioactivity was determined using a liquid scintillation counter (Tri-Carb 2500TR Packard, UK). Counts were automatically corrected for quenching using internal standardisation. Radioactivities were corrected to the time of addition to allow for radioactive decay. Sulphate concentrations in the KH_2PO_4 extracts were quantified with ion chromatography. Sulphur immobilisation and gross mineralisation were calculated according to the following equations (McLaren et al., 1985; Knights et al., 2001):

Immobilisation

$$= (\% \text{ of } ^{35}S \text{ not extracted on day 53})$$
$$\times (\text{Initial concentration of soil } SO_4^{2-} - S$$
$$+ 10 \text{ mg S kg}^{-1} \text{ added to the soil}) \quad (1)$$

Gross mineralisation

$$= (\text{Concentration of soil } SO_4^{2-}–S \text{ measured on day 53}$$
$$+ S \text{ immobilised})$$
$$- (\text{Initial concentration of soil } SO_4^{2-}–S$$
$$+ 10 \text{ mg S kg}^{-1} \text{ added to the soil}) \quad (2)$$

In the Eq. (1), % of ^{35}S not extracted on day 53 was corrected for the recovery rates of ^{35}S measured on day 0. Nziguheba et al. (2005) recently proposed a different mathematical equation for calculating S immobilisation, which requires measurements of extractable $^{35}SO_4^{2-}$ and $^{32}SO_4^{2-}$ over a time course to allow curve fittings. Their equation could not be used here because we did not follow the dynamics of extractable $^{35}SO_4^{2-}$ and $^{32}SO_4^{2-}$ over a time course. However, the difference in the estimated amount of S immobilisation between the two ways of calculations is likely to be small. It is possible that some of the immobilised ^{35}S may have been re-mineralised over the 53-d period; thus the values obtained from Eqs. (1) and (2) should be regarded as minimum estimates for immobilisation and the gross mineralisation (McLaren et al., 1985).

3. Results

3.1. Soil properties

The soil pH ranged from 4.8 to 8.0 (Table 1). Soil organic C varied by 5.2-fold, and total N and S by 3.9- and 5.4-fold, respectively. The two Chinese paddy soils contained large concentrations of extractable sulphate, accounting for 38–47% of the total S, probably as a result of the use of sulphate-containing fertilisers such as single superphosphate and ammonium sulphate. In the other eight soils, organic S was the predominant form of S, accounting for $>94\%$ of the soil total S. While the C/N ratio remained relatively stable among the soils used (9.4–12.8), the C/S$_{organic}$ ratio varied widely from 54 to 103. The two soils from New Zealand had the highest C/S$_{organic}$ ratio. In the Broadbalk experiment, reversion from arable to woodland or grassland resulted in an increase of C/S$_{organic}$ ratio. The N/S$_{organic}$ ratio ranged from 5.8 to 8.6 and showed a significant correlation ($r=0.89$, $n=10$, $P<0.01$) with the C/S$_{organic}$ ratio.

3.2. Sulphur speciation by XANES spectroscopy

The XANES 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). Fig. 1 shows the base-line corrected and normalised S K-edge XANES spectra for the 10 soils used in this study. The spectra in Fig. 1 show the presence of several white-lines in the range of 0–10 eV above the S K-edge energy, indicating the presence of several oxidation states of S in the extracted humic substances. The relative proportions of the five Gaussian curves, representing different electronic oxidation states, for each soil extract are presented in Table 2. According to literature data (Morra et al., 1997; Xia et al., 1998; Solomon et al., 2003; Solomon et al., 2005), we grouped the different oxidation states and the organic-S functional moieties associated with them into three major groups: (i) organic-S in its strongly reduced state (R-S = sum of $G1$ and $G2$, which include polysulphides, disulphides, thiols, monosulphides and thiophenes), (ii) organic-S in its
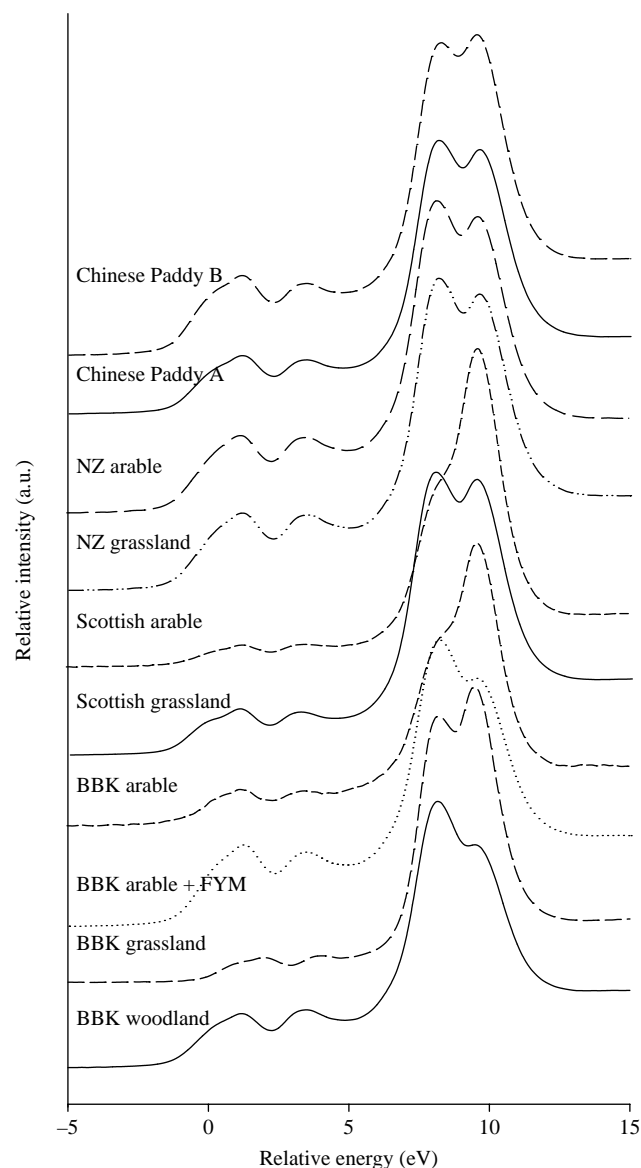

Fig. 1. Normalised S K-edge XANES spectra of the humic substances extracted from ten soils used in the study. BBK, Broadbalk Experiment; NZ, New Zealand; FYM, farmyard manure.

intermediate oxidation state (I-S = sum of $G3$ and $G4$, which include sulphoxides and sulphonates) and (iii) organic-S in its strongly oxidized state (O-S = $G5$, representing ester sulphates).

The 10 soils studied varied considerably in the relative proportions of the three major organic-S groups, with ranges of 14.4–32.4%, 32.8–49.7% and 21.5–52.5%, respectively, for the reduced, intermediate and oxidised S (Table 2). Land use and, in the case of the Broadbalk experiment, manure application were two notable factors influencing the relative distribution of different S species. Applications of FYM have greatly increased the proportions of the reduced and intermediate S species at the expense of the oxidised S species (Table 2). Thus, the soil from the Broadbalk arable + FYM plot had the highest ratios of R-S/O-S and I-S/O-S among the 10 soils studied

Table 2
Percentage distribution of different oxidation states of organic S determined using XANES spectroscopy

Soil	Gaussian curve					Reduced S $(G1+G2)$	Intermediate S $(G3+G4)$	Oxidised S $(G5)$
	$G1$ (0.17–0.26)[a]	$G2$ (0.96–1.24)	$G3$ (2.42–2.47)	$G4$ (5.0)	$G5$ (6.0)			
Broadbalk woodland	15.3	9.5	12.3	33.7	29.1	24.8	46.0	29.1
Broadbalk grassland	14.5	3.8	9.1	28.3	44.3	18.4	37.4	44.3
Broadbalk arable control	13.5	1.2	6.4	26.4	52.5	14.7	32.8	52.5
Broadbalk arable +FYM	21.5	7.2	16.5	33.2	21.5	28.7	49.7	21.5
Scottish pasture	19.2	5.1	11.1	24.6	40.0	24.3	35.7	40.0
Scottish arable	11.6	2.8	5.7	28.9	51.0	14.4	34.6	51.0
New Zealand pasture	13.8	5.3	7.1	27.3	46.6	19.0	34.4	46.6
New Zealand arable	14.2	5.6	7.4	26.9	45.9	19.7	34.3	45.9
Chinese paddy A	20.4	7.3	14.5	23.7	34.1	27.7	38.2	34.1
Chinese paddy B	20.7	11.8	17.0	23.2	27.4	32.4	40.2	27.4

[a] Values in the parentheses are electronic oxidation state in eV.

(Fig. 2). In the Broadbalk experiment, reversion of the land use from arable to grassland or woodland resulted in an increase of the reduced and intermediate S species at the expense of the oxidised S species (Table 2 and Fig. 2). Also, S species from the woodland soil were more reduced than the grassland soil from the same site. Similar difference between arable and grassland soils was observed in the Scottish samples. However, the adjacent grassland and arable soils from New Zealand showed an almost identical distribution pattern of S species. As might be expected, S speciation in the two paddy soils from China was dominated by the reduced and intermediate species (>65%; Table 2), and consequently the R-S/O-S and I-S/O-S ratios were relatively high compared to other well-drained aerobic soils (except Broadbalk arable + FYM and woodland; Fig. 2).
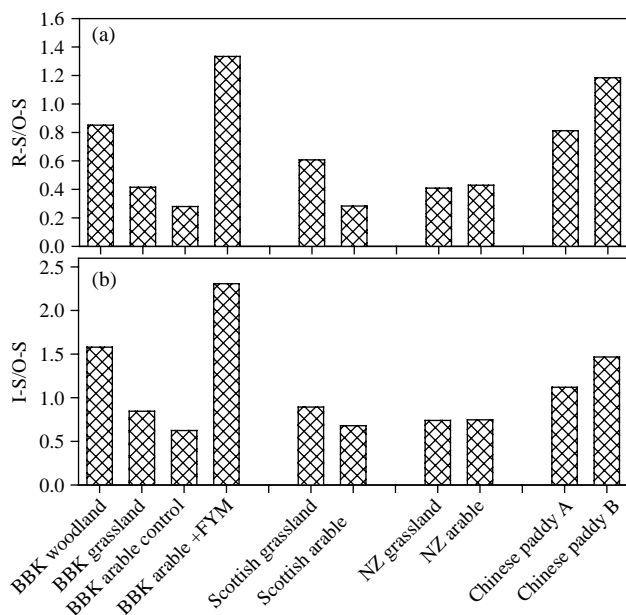

Fig. 2. Ratios of most reduced-S to most oxidised-S (R-S/O-S) (a) and intermediate-S to most oxidised-S (I-S/O-S) (b) in the humic substances extracted from the ten soils used in this study. BBK, Broadbalk Experiment; NZ, New Zealand; FYM, farmyard manure.

3.3. Sulphur mineralisation and immobilisation and relationships with XANES fractions

The amounts of S immobilisation and gross mineralisation during the 53-d incubation varied from 2.2 to 7.2 mg kg^{-1} and from 0.9 to 8.2 mg kg^{-1}, respectively (Table 3). Where gross mineralisation was larger than immobilisation, net mineralisation occurred. This was observed only in three soils (Broadbalk grassland, Broadbalk arable +FYM and Scottish pasture).

Gross S mineralisation correlated significantly with the amounts of S present in all five fractions identified by XANES, although correlation was closer with $G1$–$G4$ individually ($r=$ 0.91, 0.83, 0.83 and 0.92, respectively, $n=7$, $P<0.01$) than with $G5$ ($r=0.67$, $n=7$, $P<0.05$). Fig. 3 shows the relationships between gross S mineralisation and the amounts of S in the reduced fractions ($G1+G2$), intermediate fractions ($G3+G4$), the sum of reduced and intermediate fractions ($G1+G2+G3+G4$), and the oxidised fraction ($G5$). It is clear that the reduced and intermediate fractions of S could explain the variation among soils in gross S mineralisation much better than the oxidised S.

In contrast to gross S mineralisation, S immobilisation did not correlate significantly ($r=0.24$–0.53, $n=7$, $P>0.05$) with any of the XANES fractions. Nor did it correlate significantly

Table 3
Amounts of S immobilisation and gross mineralisation during a 53-day incubation, determined by ^{35}S dilution. Values in parentheses are standard errors ($n=3$)

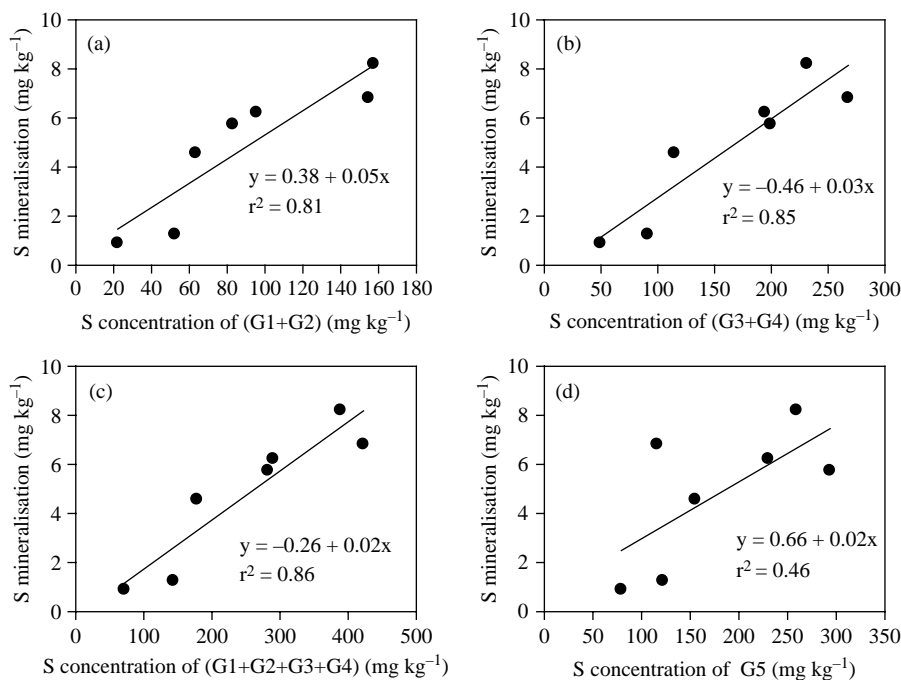| Soil	S immobilisation (mg kg^{-1})	Gross S mineralisation (mg kg^{-1})
Broadbalk grassland	3.9 (0.20)	6.2 (1.08)
Broadbalk arable control	3.5 (0.32)	0.9 (0.05)
Broadbalk arable + FYM	4.5 (0.96)	6.8 (1.26)
Scottish pasture	6.5 (0.05)	8.2 (1.31)
Scottish arable	6.0 (0.07)	5.7 (0.32)
New Zealand pasture	7.2 (0.15)	4.6 (0.89)
New Zealand arable	2.2 (0.54)	1.3 (0.98)

Fig. 3. Relationships between gross S mineralisation during 53-day incubation and the concentrations of various S fractions determined by XANES spectroscopy.

with soil organic C content or the $C/S_{organic}$ ratio. However, there was a significant negative correlation between S immobilisation and the initial concentration of soil extractable sulphate ($r = -0.69$, $n = 7$, $P < 0.05$).

4. Discussion

Sulphur K-edge XANES spectroscopy is a non-invasive technique that allows a quantitative determination of different oxidation states of S in organic compounds. Sulphur species present in SOM can be deduced from the information of its oxidation states. The methodology is a leap forward from the traditional wet chemical fractionation methods, which are operationally defined and do not provide accurate information on S speciation. The main limitation of the XANES technique lies in its relatively low sensitivity when applied to mineral soils with low S content. For example, previous studies conducted by Solomon et al. (2003) indicated that S XANES spectra measured directly on soil particle size separates tend to have large background, deflated signal, and an upward curving baseline compared with the spectra obtained from the humic substances extracted from the same size separates. These features make uniform data analysis (background correction and normalisation of the data) from the direct measurements difficult and hinder reliable data analysis and quantitative comparison of the results. For this reason, XANES is normally applied to humic substances extracted from soils (Morra et al., 1997; Xia et al., 1998; Solomon et al., 2003; Solomon et al., 2005), except in soils with high organic matter and S contents (Prietzel et al., 2003). It is possible that the XANES spectra of the humic substance extracts may not reflect the in situ S speciation in soil, because humic substances may have

undergone possible changes during the extraction. However, previous studies (Solomon et al., 2003) showed that the spectral characteristics of organic S obtained from extracts and those obtained from the corresponding clay or silt fractions are almost identical. This gives confidence to the approach used in the present and previous studies (Solomon et al., 2005). To minimise the possibility of auto-oxidation of the extracted organic S, humic substances were extracted at pH 12.4 under a N_2 environment according to the recommendation by Hutchison et al. (2001). These authors investigated the influence of aeration and pH on the stability of organic sulphur in humic substances using XANES spectroscopy. They found no detectable change in the different oxidation states of S in samples exposed to oxygen at various pH levels from 3.5 to 12.4 for 44 h.

The ten soils studied were collected from diverse temperate regions around the world with contrasting land uses, including both paddy soils and upland aerobic soils. The XANES results indicate that the organic S in the extracted humic substances is dominated by either O-S or I-S, while the R-S group in the forms of sulphides, disulphides, thiols and thiophenes comprised only 14–32% (Table 2). Within the I-S group, sulphonate (oxidation state $= 5$) was the most abundant, accounting for 58–84% of the total I-S. These results are in general agreement with those reported for two soils from the United States (Xia et al., 1998), six soils from Ethiopia (Solomon et al., 2003) and nine soils from South Africa (Solomon et al., 2005). In contrast, significantly larger proportions of organic S have been reported to be present in the most reduced form in the humic extracts from aquatic environments (Xia et al., 1998), peat (Martinez et al., 2002) and organic horizons of seasonally wet forest soils (Prietzel

et al., 2003). These differences probably reflect the environmental conditions, particularly the redox potential, under which the soils occur. The two paddy soils from China, which undergo episodic flooding during the rice growing season, also showed larger proportions of reduced S and smaller proportions of ester sulphates than other well-drained soils (Table 2).

Apart from the effect of redox conditions, land use and long-term applications of organic manure also have a profound impact on the S speciation in the humic substance extracts. Reversion of arable land use to grassland or woodland clearly led to an increase in the proportion of the reduced-S and intermediate-S, with a concomitant decrease in the proportion of the oxidised-S, in the Broadbalk experiment. These changes mirror those that were observed when native grassland was converted to arable cropping, which resulted in a shift in soil organic S toward the more oxidised species (Solomon et al., 2005). A similar pattern occurred when the pair of pasture and arable soils from Scotland was compared. Increased aeration brought about by arable cultivation may have led to enhanced mineralisation of C-bonded S, or transformation of C-bonded S to ester sulphates (McLaren and Swift, 1977; McGill and Cole, 1981); either process would have increased the relative proportion of the oxidised-S at the expense of the reduced-S or intermediate-S. The pair of pasture and arable soils from New Zealand appears to be an exception, with little difference in the S speciation between them. The two soils from New Zealand had the highest $C/S_{organic}$ ratio (Table 1), suggesting that their organic matter is undersaturated with S compared to other soils.

Among the ten soils studied, the arable + FYM plot from the Broadbalk experiment showed the highest ratios of R-S/O-S and I-S/O-S (Fig. 2), reflecting the dominance of the I-S and R-S species over the ester sulphate species. On average, this plot receives annual additions 38 kg S ha^{-1}, of which 12.5% is as sulphate and the remainder organic S (Knights et al., 2000). The organic S in FYM is derived from both plant residues and animal excreta. Although organic S species in FYM were not determined in the present study, they are likely to be dominated by C-bonded S. In plant residues, 60–90% of the S usually exists in the C-bonded form (Zhao et al., 1996). In a study of the forms of S in sheep excreta, Williams and Haynes (1993) showed that sulphate-S accounted for 74% of the urine S, whereas 80% of the S in dung was C-bonded organic S. When applied to an undisturbed pasture, sulphate from urine and dung was leached rapidly, whereas the C-bonded S in dung remained in the soil for a long period of time (Williams and Haynes, 1993).

It is often thought that organic ester sulphates represent a more labile form of S than C-bonded S. However, there is contradictory evidence regarding the relative availability of ester sulphates versus C-bonded S for mineralisation or plant uptake (Zhao et al., 1996). For example, some researchers (Tsuji and Goh, 1979; Maynard et al., 1985; Blair et al., 1994) showed that ester sulphates, as determined by the HI reduction method, were more readily available to plant uptake than C-bonded S. In contrast, Ghani et al. (1991; 1992) found that S mineralised during short-term incubations appeared to be derived almost exclusively from C-bonded S. Other studies showed that both ester sulphates and C-bonded S contributed to plant uptake or mineralisation (Freney et al., 1975; Goh and Pamidi, 2003). In the present study, we found that gross S mineralisation was correlated more closely with the amounts of S present in the reduced and intermediate S species (oxidation state of ≤5) than with ester sulphates (oxidation state of =6), suggesting that C-bonded S was the main source for mineralisation during the 53-d incubation. Similarly, Ghani et al. (1991) reported that the best predictor of mineralised S was the amount of C-bonded S in the soil. It is worth noting that the same conclusion was obtained in the two studies despite different methods being used for S speciation (HI reduction versus XANES) and for measurement of S mineralisation (open leaching versus ^{35}S isotope dilution). Therefore, the results from these short-term incubation studies are consistent with those from studies looking into the long-term changes in organic S fractions following cultivation of native grasslands and forests (McLaren and Swift, 1977; Solomon et al., 2001; Solomon et al., 2005), indicating that C-bonded S, or organic S in the reduced and intermediate valences, is the main source for S mineralisation.

There was no significant correlation between S immobilisation and the amounts of different S species quantified by XANES. The only significant, but negative, correlation was with the initial concentrations of sulphate in the soils. This may be because the initial level of sulphates reflects the equilibrium of immobilisation and mineralisation; the higher the initial sulphate level, the lower the capacity of soil to immobilise sulphate.

In conclusion, our study has provided further evidence of a profound influence of land uses and organic manure applications on S speciation in soil humic substances, and that S species in the reduced and intermediate oxidation states appeared to be the main source of organic S for mineralisation in short-term.

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