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Sulphur immobilization and availability in soils assessed using isotope dilution

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Abstract

Increasing recognition of S deficiency in soils has raised the need for understanding processes governing S cycling and availability in soils. However, the quantification of the two main processes of S cycling, i.e. mineralization and immobilization, remains difficult as these processes occur simultaneously. A modified isotope $^{35}SO_4$ dilution technique was developed and used to measure the effect of sulphate (SO_4) fertilization on S mineralization and immobilization in planted (pot experiment with ryegrass (*Lolium multiflorum* L.)) and unplanted soils (incubation). The immobilization and mineralization of S was calculated from the dynamics of stable and labelled S in soil KH_2PO_4 extracts containing an anion exchange membrane that concentrates SO_4 and mainly excludes other S species. The mathematical analysis of the isotope dilution data differs from methods proposed earlier. The radiolabile S in unplanted soil (*E* value) and in ryegrass (*L* value) were used as a measure of total available S in soils. Sulphate immobilization rate significantly declined during incubation. Sulphate application reduced gross mineralization but surprisingly reduced SO_4 immobilization. The *E* value significantly increased during the incubation in all soils as a result of gross mineralization, e.g. from 3.8 mg S kg^{-1} at day 0 to 11.5 mg S kg^{-1} at day 43 in the sandy soil with no sulphate addition. A full recovery in the *E* value of S added in (+S) treatments was achieved. Similarly, radiolabile S in the above-ground ryegrass biomass (*L* value) increased with S addition, with a full recovery of added S. The *E* and *L* values nearly fit a 1:1 line suggesting identical S dynamics in a planted and unplanted soil. The method proposed has operational advantages compared to methods used earlier.
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Keywords: *E* value; Immobilization; *L* value; Mineralization; Radioactive S; Ryegrass; Sulphate fertilization

1. Introduction

There is growing evidence of sulphur deficiency in agricultural systems both in temperate and tropical soils as a result of reduced S inputs from atmospheric deposition and from S containing fertilizers (Eriksen, 1997a; Weil and Mughogho, 2000; McGrath et al., 2002a). Sulphur occurs in surface soils predominantly in organic form (Ghani et al., 1993; Zhao et al., 1996). Mineralization of this organic S pool together with immobilization of inorganic sulphate regulates the cycling of S in soils as well as its availability to crops. Understanding the dynamics of soil organic S is, therefore, important to predict the availability of sulphur. However, mineralization and immobilization take place simultaneously in soils, rendering their quantification difficult (McLaren et al., 1985; Ghani et al., 1993). Often, the net mineralization defined as the difference between gross mineralization and immobilization is determined. To-date, there is no generally accepted method to quantify these processes separately. Previously, S mineralization has been assessed either by the sulphate periodically leached in open incubations (Ghani et al., 1991; Zhou et al., 1999; Knights et al., 2001; Pamidi et al., 2001) or by the sum of S uptake by plant and soil sulphate increase during the period of plant growth (Bettany et al., 1974; Eriksen et al., 1995; Pamidi et al., 2001). The open incubation with periodic leaching limits sulphate immobilization and, therefore, can be used to quantify gross mineralization but the alteration of soil conditions by successive leaching can lead to overestimation of S mineralization (Pamidi et al., 2001; McGrath et al., 2002b). Isotope technique can be applied to quantify fluxes separately. Based on this technique, immobilization has

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often been assessed by the decrease in $^{35}SO_4$ activity in extracts of soil labelled with $^{35}SO_4$ (McLaren et al., 1985; Knights et al., 2001). In this approach, immobilization is estimated by multiplying the decrease in applied $^{35}SO_4$ at a given incubation time with the extractable SO_4 before labeling the soil as shown by Eq. (1).

$$I(t) = \frac{^{35}SO_4(t0) - ^{35}SO_4(t)}{^{35}SO_4(t0) \times t} \times C_i^{32}SO_4(t0) \quad (1)$$

where $I(t)$ (mg kg^{-1} soil day^{-1}) is the SO_4 immobilized rate between $t=0$ and t, $^{35}SO_4$ ($t0$) is the radioactivity added or recovered at day 0 in Bq kg^{-1} soil, $^{35}SO_4$(t) is the radioactivity recovered at day t in Bq kg^{-1} soil and $C_i^{32}SO_4(t0)$ is the initial sulphate concentration before soil labeling in mg kg^{-1} soil.

This methodology has some shortcomings. First of all, the immobilization rate calculated according to Eq. (1) is an underestimate of the real immobilization rate because the $^{35}SO_4/^{32}SO_4$ ratio (specific activity) decreases during incubation due to mineralization (McLaren et al., 1985; McGrath et al., 2002b). Eq. (1) effectively assumes that the specific activity (SA) remains at the (highest) initial value found at the start of the incubation, thereby underestimating immobilization at prolonged incubation. Secondly, other extractable ^{35}S-labelled species may be produced during incubations and which can be incorrectly attributed to $^{35}SO_4$. In this paper, we present a methodology to quantify immobilization rate based on the dynamics of the specific activity in the extractable SO_4 pool, thereby overcoming the 2 issues described above.

Sulphur availability has been studied by monitoring phosphate extractable sulphate (Blair et al., 1991; Zhao and McGrath, 1994) and a positive correlation between extractable sulphate and plant S uptake has been reported (Eriksen, 1997a). Isotope techniques can also be used to quantify the total available S in soils by the so-called 'L value' and/or 'E value'. This technique is widely used to estimate the available pool of nutrients such as phosphorus (Frossard et al., 1994). The *L* value (based on a plant essay) may differ from an *E* value (based on a batch extraction) of the same soil because S-mineralization in planted soils might be different from that in unplanted soils. Recently, a modification of the assay for the determination of the radiolabile phosphorus in soil has been proposed to avoid artifacts related to the presence of non-labile forms of P in solution and to overcome detection limits (Hamon and McLaughlin, 2002; Maertens et al., 2003). This method may also be applicable to the S isotope dilution assay because variable amounts of the S-label may exchange with soluble organic S. A speciation of both stable and labelled S in solution is required to calculate SO_4 immobilization in soil based on isotope dilution. A decrease in specific activity of SO_4 on the resin membrane during incubation allows the quantification of the gross mineralization of unlabelled (native) S.

The objectives of this study were to measure the immobilization/mineralization and available S in soil using isotope techniques. Effects of soil type and effects of sulphate application are given as an illustration and validation of the methodology.

2. Materials and methods

2.1. Soils

Two agricultural soils from Flanders, Belgium, were used in this study: a sandy soil from Bocholt and a sandy loam soil from Zwanenberg. Soils were collected from the top 0 to 20 cm layer, air dried and sieved to 4 mm. The characteristics of the soils are given in Table 1.

2.2. Description of experiments

2.2.1. Incubation

Soils were pre-incubated for 7 days at 20 °C, and at 88% of field capacity (FC) for the sandy soil and at 50% FC for the sandy loam soil prior to soil amendments. Two treatments were set up in three replicates for each soil: soil without sulphate addition (−S) and soil with sulphate addition (+S). Pre-incubated soil, equivalent to 500 g oven dry weight was mixed with carrier-free ^{35}S

Table 1
Selected characteristics of soils used in incubation and pot experiments

Site	Soil texture	pH[a] 0.01 M $CaCl_2$	CEC[b] (cmolc kg^{-1})	Organic *C*[c] (g kg^{-1})	Total S[d] (mg kg^{-1})	SO_4[e] (mg kg^{-1})	Total *N*[f] (g kg^{-1})	H_2O content at field capacity[g] (%)	Bulk density[g] (g cm^{-3})
Bocholt	Sandy	5.4	6.0	28	297	4.7	1.7	28	1.21
Zwanenberg	Sandy loam	7.0	8.9	9.8	149	4.1	1.2	29	1.19

[a] At soil pH.
[b] Ag Tu method (Chhabra et al., 1975).
[c] Dry combustion CN analyzer.
[d] Aqua regia total digestion.
[e] KH_2PO_4 extraction.
[f] Kjeldhal digestion.
[g] Sandbox method using 100 cm^3 soil cores.

(Amersham Biosciences, UK) at 88.8 kBq kg^{-1} soil. In (+S) treatment, S was added as $(NH_4)_2SO_4$ at 10 mg S kg^{-1} soil through a stock solution. To compensate for probable change in pH due to the ammonium salt, an equivalent amount of N was added in the (−S) treatment as NH_4Cl. At the end, the moisture content was adjusted to FC for the sandy soil and 67% FC for the sandy loam soil. The difference in water content between soils was based on a preliminary test, which showed that at field capacity the sandy loam soil became too wet to handle. Soils were transferred in covered 1 l plastic pots and placed randomly in an incubation room at 20 °C. Pots were regularly opened during incubation and the soil was gently mixed.

2.2.2. Pot experiment

The treatments in the pot experiment were similar to those of the incubation experiment. Pre-incubated soil, equivalent to 1 kg oven dry weight, was mixed with carrier-free ^{35}S and $(NH_4)_2SO_4$ or NH_4Cl at the same rates and same moisture content as in the incubation. Three replicates were used. Soils were then transferred to 1 l plastic pots (upper diameter = 11.7 cm, lower diameter = 9.5 cm). Italian ryegrass (*Lolium multiflorum* L.) (0.978 g) was sown in the shallow layer in each pot. A thin layer of polythene beads was added on top to reduce water loss by evaporation and to prevent soil disturbance during watering. Two set of pots were prepared to allow 2 harvests. Pots were placed randomly in a plant growth chamber (Weiss klimatechnik, Reiskirchen, Germany) at 70% humidity and 20 °C during the 12 h day periods and 70% humidity and 15 °C for the 12 h night periods. An opaque ring was placed around each pot to avoid light penetration in the pots through the sides and subsequent algae growth. Water loss was restored daily using distilled water.

The above-ground biomass of one set of pots was harvested at day 20 after emergence (referred to as day 20 harvest in the text). Plants in this set were allowed to re-grow for another 20 days. N and P removed by plants until this harvesting time (20 days) were compensated by addition of 100 mg N and 13 mg P per pot as stock solution from KNO_3 and KH_2PO_4 in all pots during three watering. At day 40 after emergence, the above-ground biomass of the second set of pots was harvested as well as the re-growth from the previously harvested set and is referred to as 40 day harvest and 20 day re-growth in the text. For each harvest, grasses were oven dried at 70 °C to a constant weight prior to digestion for total S determination.

2.3. Soil and plant analyses

For the incubation trial, soil samples were taken from each pot immediately after addition of treatments (day 0) and at day 1, 3, 7, 15, 23 and 43 during the incubation. At each sampling time, three soil samples equivalent to 10 g oven dry weight each were weighed in 40 ml Oak Ridge centrifuge tubes. One set of samples was extracted with 10 mM KH_2PO_4, the second set with 10 mM KH_2PO_4 plus 2 anion exchange resin membranes (5 cm^2, BDH Laboratory Supplies, Poole, England; anion exchange capacity: 0.037 meq cm^{-2}) in bicarbonate form and the third set with deionized water plus 2 anion exchange resin membranes. In all extractions, the ratio soil: solution was 1:2. All soil extractions were shaken for 16 h on an end-over-end shaker, after which the KH_2PO_4 extracts without resin were centrifuged for 25 min at $3260 \times g$ and filtered through 0.45 μm Millipore membrane filters. Sulphate in the filtrate was determined by Ion Chromatography (IC, Dionex Automatic Analyzer with AS4A-SC column) and total extractable S by Inductively Coupled Plasma Optical Emission Spectromerty (ICP-OES, Perkin Elmer, Norwalk, USA) at 181.975 nm wavelength. In the other soil extractions, resin membranes were removed, rinsed with deionized water and transferred to new centrifuge tubes containing 20 ml of 0.5 M HCl. Sulphate adsorbed on resin was desorbed by 0.5 M HCl during a 16 h shaking period. Desorbed S was determined by ICP-OES. The three extraction protocols aimed at S speciation. KH_2PO_4 extracts inorganic SO_4 as well as soluble organic S. The incorporation of ^{35}S in the soluble organic S can be a source of error in the isotope dilution assay. The quantification of the immobilization is based on the specific activity (SA) of the SO_4 in solution (see below). This SA is not identical to the ratio of dissolved ^{35}S activity to dissolved stable SO_4 if the ^{35}S has reacted with soluble organic S-species. To avoid such error, anion resin membranes were included in the second set of KH_2PO_4 extraction to allow an extraction of sulphate only. In a preliminary test, the resin extraction was conducted on these soils and the comparison between extracted sulphate, determined either with ICP-OES or IC gave identical results, implying that the anion resin adsorbs inorganic sulphate only. For this reason and for convenience, S extracted with anion resin was measured with ICP-OES in this study. The deionized water + resin is used for the determination of E value as proposed for phosphorus (Maertens et al., 2003). The extractability of S in water might be smaller than KH_2PO_4 extractable S if SO_4 adsorption is significant in the soil. The determination of the E value only requires the specific activity in solution, i.e. a water extract is sufficient for the purpose (Maertens et al., 2003). Note, however, that a complete SO_4 extraction is required for the determination of the net mineralization.

Plant samples were digested with concentrated HNO_3 and total S determined on ICP-OES. A plant reference sample, (*Brassica oleracea* L.) supplied by the Wageningen Evaluating Programme for analytical laboratories in the Netherlands, was digested each time along with the samples for quality control.

For each soil or plant extract, a 1-ml aliquot was mixed with 9 ml cocktail (Ultima Gold) in 20 ml scintillation vial and ^{35}S radioactivity determined on a Parkard Tri-Carb 1600 CA liquid scintillation counter. In a preliminary test,

the different extractants used in this study for soil or plant analyses were checked for quenching by mixing each extractant with the cocktail in ratios (extractant: cocktail) ranging from 1:9 to 4:6. It was found that at the 1:9 ratio, quenching was negligible.

2.4. Data analysis

2.4.1. Assessment of S immobilization

The fluxes of S in soil can be described as follows:

$$\text{Organic S} \xrightarrow{M} \text{SO}_4 \xrightarrow{I} \text{S immobilized} \quad (2)$$

where M is mineralization and I immobilization, both in mg kg^{-1} soil day^{-1}.

Some of the S immobilized during incubation can re-mineralize but this cannot be measured.

The mass balance of SO_4 (mg kg^{-1} soil) reads

$$\frac{d\text{SO}_4}{dt} = M - I \quad (3)$$

The mass balance of $^{35}SO_4$ (Bq kg^{-1} soil) after amendment with $^{35}SO_4$ reads

$$\frac{d^{35}\text{SO}_4}{dt} = -I \times \frac{^{35}\text{SO}_4}{\text{SO}_4} \quad (4)$$

Mineralization of S changes the SO_4 pool, i.e. SO_4 is time dependent. Our results show a linear increase of SO_4 with incubation time (t), which is described as

$$\text{SO}_4 = a + bt \quad (5)$$

Likewise, immobilization changes the $^{35}SO_4$ concentration and the percentage of $^{35}SO_4$ remaining extractable during incubation could be fitted to an exponential decay described as:

$$^{35}\text{SO}_4 = r + (\text{S0} - r) \times \exp(-kt) \quad (6)$$

where S0 is the percentage of added $^{35}SO_4$ recovered in the day 0 extraction, and r and k are parameters to be fitted and were estimated by SAS (1999). (S0 − r) indicates the maximum $^{35}SO_4$ that may be immobilized, and k the shape of the curve. S0 varied between 80 and 95% of the added dose and was preferred over the added dose in contrast with other studies (Knights et al., 2001). The time elapsed between spiking and soil extraction at $t=0$ is about 5 h.

Combining (4)–(6) yields:

$$\frac{d(r + (S0 - r) \times \text{Exp}(-kt))}{dt} = -I \times \frac{r + (S0 - r) \times \text{Exp}(-kt)}{a + bt} \quad (7)$$

After integration Eq. (7) becomes

$$\ln\left(\frac{r + (\text{S0} - r) \times \text{Exp}(-kt)}{\text{S0}}\right) = -\frac{I}{b} \times \ln\left(\frac{a + bt}{a}\right) \quad (8)$$

Eq. (8) predicts the immobilization rate of SO_4 from the dynamics of both extractable $^{32}SO_4$ and $^{35}SO_4$. The parameter fitting was made for each of the three replicates, thereby yielding three replicates M or I from which mean and SE's could be calculated.

2.4.2. Assessment of S available pool (E value)

The plant available S (L value, mg kg^{-1} soil) is calculated as:

$$L = \frac{^{32}\text{S}}{^{35}\text{S}} \times R \quad (9)$$

where ^{32}S (mg kg^{-1}) and ^{35}S (Bq kg^{-1}) are concentrations of S in harvested rygrass, R (Bq kg^{-1} soil) is the radioactivity added to the soil.

Based on a similar concept, the isotopically pool in soil (E value, mg kg^{-1} soil) can be quantified from the specific activity (SA) of SO_4 in soil, i.e.

$$E(t) = \frac{^{32}\text{SO}_4}{^{35}\text{SO}_4} \times R \quad (10)$$

Immobilization alone does not decrease the specific activity since $^{32}SO_4$ and $^{35}SO_4$ are immobilized at similar rates. Mineralization of unlabelled S, however, decreases the SA leading to an increase in E value.

The E value is assessed from the specific activity of the SO_4 pool after a set equilibration time. The SA is obviously

Table 2
Extractable S and SO_4–S (mg S kg^{-1} soil dry weight) during incubation of a sandy soil and a sandy loam soil with or without S application. Means of three replicates ± standard errors

Soil	Type of extraction	(−S)			(+S)		
		Day 0	Day 3	Day 43	Day 0	Day 3	Day 43
Sandy	KH_2PO_4 extractable S (mg kg^{-1})	5.9 ± 0.04	6.0 ± 0.04	8.1 ± 0.06	15.7 ± 0.15	15.8 ± 0.15	17.5 ± 0.21
	KH_2PO_4 extractable SO_4 (mg kg^{-1})	3.3 ± 0.21	3.5 ± 0.21	6.6 ± 0.01	12.2 ± 0.15	12.4 ± 0.14	15.3 ± 0.10
	KH_2PO_4 + resin extractable SO_4 (mg kg^{-1})	3.2 ± 0.04	3.4 ± 0.04	6.6 ± 0.02	12.7 ± 0.10	13.0 ± 0.10	16.4 ± 0.07
	Water + resin extractable SO_4 (mg kg^{-1})	3.2 ± 0.05	3.4 ± 0.05	6.8 ± 0.03	13.0 ± 0.10	13.3 ± 0.09	16.6 ± 0.08
Sandy loam	KH_2PO_4 extractable S (mg kg^{-1})	5.3 ± 0.05	5.4 ± 0.05	5.7 ± 0.04	14.3 ± 0.35	14.3 ± 0.34	14.8 ± 0.11
	KH_2PO_4 extractable SO_4 (mg kg^{-1})	4.3 ± 0.18	4.4 ± 0.17	5.1 ± 0.02	13.3 ± 0.28	13.4 ± 0.28	14.1 ± 0.13
	KH_2PO_4 + resin extractable SO_4 (mg kg^{-1})	4.2 ± 0.02	4.2 ± 0.02	5.1 ± 0.02	12.8 ± 0.23	12.9 ± 0.22	13.9 ± 0.11
	Water + resin extractable SO_4 (mg kg^{-1})	4.3 ± 0.04	4.3 ± 0.04	5.3 ± 0.01	13.4 ± 0.31	13.5 ± 0.30	14.5 ± 0.08

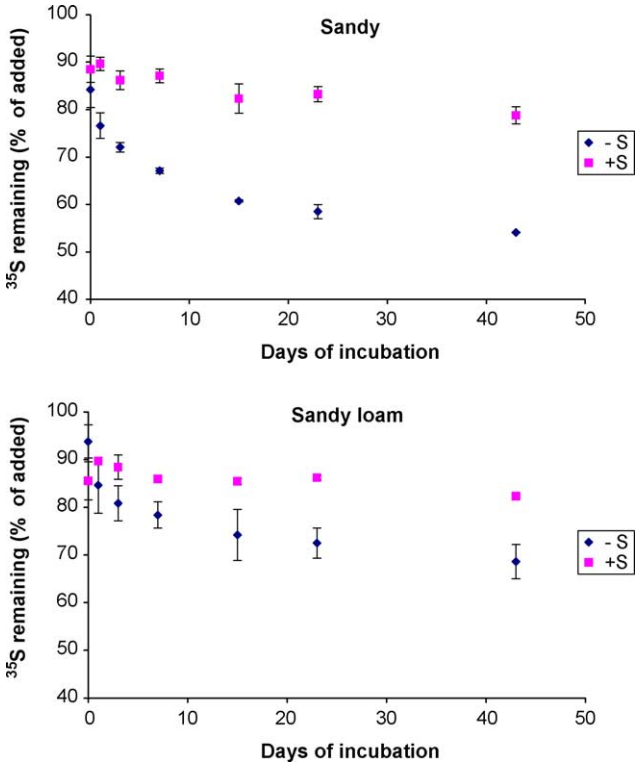

Fig. 1. ^{35}S recovered in KH_2PO_4 extract during incubation of a sandy soil and sandy loam with or without S addition. Bars denote the standard deviations from the means of three replicates.

lower than the initial value due to mineralization of S. In this study, *E* value was assessed from the SA on the resin in a water extract.

A *t*-test was applied to compare the different extraction protocols and to compare the effect of sulphate application on S mineralization and immobilization.

3. Results

3.1. Extractable sulphate and sulphur

The total S (ICP-OES) extracted with KH_2PO_4 was slightly higher than SO_4–S (IC) in the same extract (Table 2). These differences were more pronounced in the sandy soil than in the sandy loam soil and can be attributed to soluble organic S. In general, there was no significant difference in SO_4–S between different extraction protocols (KH_2PO_4, KH_2PO_4 + resin, and deionized water + resin), suggesting that SO_4 sorption is small in these soils. Extractable sulphate increased in the (−S) treatments during the incubation in the sandy soil with an average increase of 3.3 mg kg^{-1} at day 43 compared to day 0 values. Smaller increases were observed in the sandy loam soil (0.8 mg kg^{-1} at day 43 in the (−S) treatment). Sulphate application increased extractable sulphate of (−S) treatments, an increase representing 87–98% of added S in the sandy soil and 87–90% in sandy loam soil during incubation. The increase in extractable SO_4 (i.e. net mineralization) was almost identical between the (−S) and (+S) treatments for both soils.

3.2. Immobilization of $^{35}SO_4$ and $^{32}SO_4$, specific activity and E values

The fraction of ^{35}S which remained extractable by KH_2PO_4 during incubation was assessed as an indicator of $^{35}SO_4$ immobilization. In the day 0 extraction, 10–15% of added $^{35}SO_4$ were lost for which we could not find a clear explanation. The large proportion of $^{35}SO_4$ immobilized took place during the first week of incubation (Fig. 1). Thereafter, the incorporation of ^{35}S into non-extractable S

Table 3
Cumulative immobilization and mineralization of S estimated by isotopic dilution during incubation of two soils from Belgium, calculated from SO_4 and $^{35}SO_4$ dynamics in KH_2PO_4 + resin extracts. Means of three replicates

Soil	Days of incubation	Net mineralization[a] (mg kg^{-1})		Immobilization[b] (mg kg^{-1})		Gross mineralization[c] (mg kg^{-1})	
		(−S)	(+S)[d]	(−S)	(+S)[d]	(−S)	(+S)[d]
Sandy	1	0.08	0.09*	0.18	0.05**	0.26	0.14**
	7	0.54	0.60*	0.82	0.32**	1.36	0.92**
	23	1.79	1.96*	1.23	0.70*	3.02	2.66*
	43	3.34	3.66*	1.46	0.90*	4.80	4.56*
Sandy loam	1	0.02	0.02 ns	0.09	−0.05*	0.11	−0.03 ns
	7	0.15	0.17 ns	0.27	−0.21*	0.42	−0.04*
	23	0.49	0.55 ns	0.43	−0.31*	0.92	0.24*
	43	0.92	1.04 ns	0.52	−0.37*	1.44	0.67 ns

*Significantly different from value in (−S) treatment at $P=0.05$. **Significantly different from value in (−S) treatment at $P=0.01$. ns, not significantly different from the value in (−S) treatment.

[a] Net mineralization = extractable sulphate at day d − extractable sulphate at day 0.

[b] Immobilization values are calculated based on Eq. (8):

$$\ln\left(\frac{r+(S0-r)\times\mathrm{Exp}(-kt)}{S0}\right)=-\frac{I}{b}\times\ln\left(\frac{a+bt}{a}\right)$$

[c] Gross mineralization = net mineralization + immobilization.

[d] Values are compared to those in (−S) treatments.

Table 4
Parameters of equations used to estimate sulphate immobilization with isotope dilution technique

Soil	$^{35}SO_4$ recovered (%): $^{35}SO_4 = r + (S0 - r) \times \exp(-kt)$			Extractable SO_4 (mg kg^{-1}): $SO_4 = a + bt$		
	$r \pm$ SE	$k \pm$ SE	R^2	$a \pm$ SE	$b \pm$ SE	R^2
Sandy						
(−S)	58.48 ± 0.22	0.21 ± 0.01	0.99	3.20 ± 0.04	0.08 ± 0.00	0.99
(+S)	81.29 ± 0.30	0.05 ± 0.01	0.99	12.74 ± 0.10	0.08 ± 0.00	0.98
Sandy loam						
(−S)	65.01 ± 0.07	0.08 ± 0.07	0.99	4.20 ± 0.02	0.02 ± 0.00	0.83
(+S)	79.18 ± 0.10	0.05 ± 0.00	0.99	12.82 ± 0.23	0.02 ± 0.01	0.75

S0 represents $^{35}SO_4$ recovered at day 0 in KH_2PO_4 + resin extraction.

fraction proceeded slowly. Sulphate application reduced $^{35}SO_4$ immobilized and by day 43, 21% of $^{35}SO_4$ added were lost in the (+S) treatments compared to 46% in the (−S) treatments in the sandy soil. The immobilization of $^{35}SO_4$ was less pronounced in the sandy loam soil where by day 43, 32% of added ^{35}S was lost in the (−S) treatments compared to 18% in the (+S) treatments.

The specific activity expressed as ^{35}S activity to the stable SO_4 during incubation in KH_2PO_4 represented 101 ± 6% of that measured in the KH_2PO_4 + resin extract and 97 ± 9% of that in the deionized water + resin extract (data not shown). The similarity in the SA from the three extractions implies that there was no loss of $^{35}SO_4$ in organic form despite the higher KH_2PO_4 extractable S compared to SO_4 shown in Table 2.

The cumulative immobilization values of $^{32}SO_4$, predicted by Eq. (8), are given in Table 3 (values are calculated with reference to $^{35}SO_4$ recovered at day 0 and from the KH_2PO_4 + resin extraction). Parameters for Eq. (8) are given in Table 4. In (−S) treatments, immobilization proceeded rapidly in the first week of the incubation, then decreased slowly. For example, immobilization rate in the sandy soil decreased from 0.18 mg kg^{-1} day^{-1} between day 0 and day 1 to 0.03 mg kg^{-1} day^{-1} between day 23 and day 43. Addition of S significantly reduced immobilization rate in all soils. The gross mineralization, calculated as the sum of immobilization and net mineralization, showed a similar trend as immobilization (Table 3). The small but negative immobilization in the sandy loam ((+S) treatments) is related to increased ^{35}S in the extracts during incubation compared to ^{35}S measured at day 0 (Fig. 1). The observation at day 0 is made about 5 h after spiking by which some ^{35}S may already be immobilized that could be re-mineralized in the subsequent 43 days. However, these observations may be resulting from experimental error as the increased extractable ^{35}S is only 5% of the observation at day 0. Moreover, if microbial immobilization of S in the (+S) treatment were identical as in the (−S) treatments (in term of mg S kg^{-1}), then only a 6% decrease of ^{35}S would be predicted. It is clear that such small value may be not detectable.

The specific activity on resin in the deionized water + resin extraction decreased rapidly in the (−S) treatment of the sandy soil during the first week of incubation with about 50% of the reduction in specific activity occurring in that week (Fig. 2). The decrease in specific activity was less pronounced in the sandy loam soil though it was also rapid in the first 3 days of incubation in the (−S) treatments. In both soils, S application decreased dramatically the specific activity at day 0 compared to the values in the (−S) treatments but remained relatively unchanged during the incubation.

The decrease in SA (Fig. 2) are reflected in the increase of the E values (Fig. 3). The difference in E values between the (-S) and (+S) treatments was about 10 mg S kg^{-1}, representing the amount of S applied in (+S) treatments.

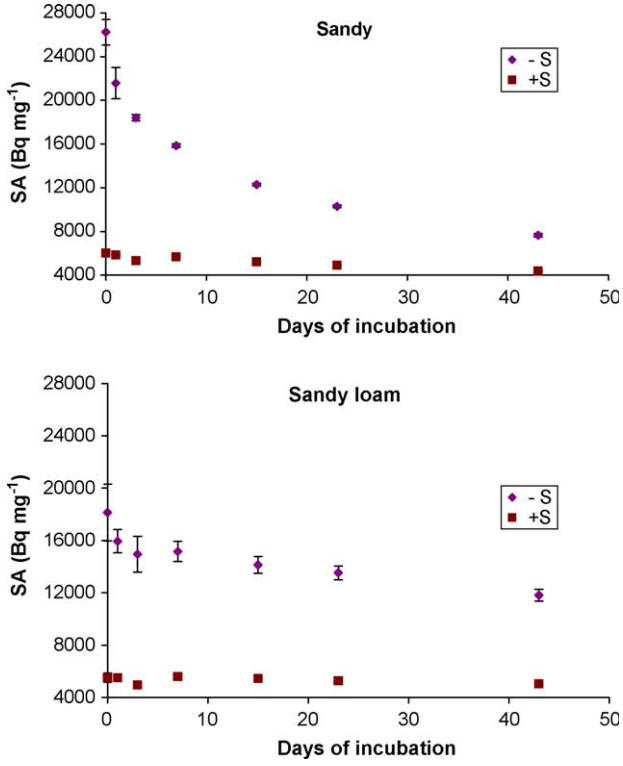

Fig. 2. Specific activity $^{35}SO_4/^{32}SO_4$ in water + resin extract during incubation of a sandy soil and a sandy loam soil with or without S addition. Bars denote the standard deviations from the means of three replicates.

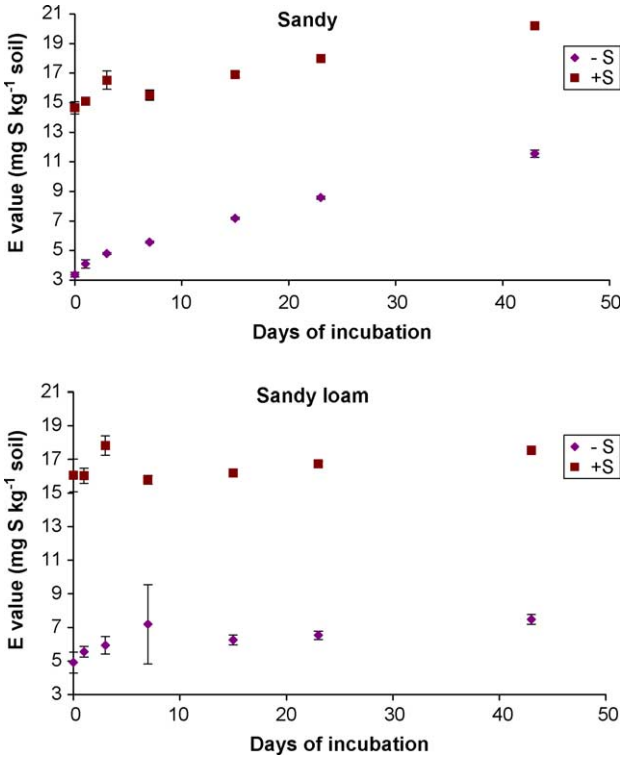

Fig. 3. E values during incubation of a sandy soil and a sandy loam soil with or without S addition. Bars denote the standard deviations from the means of three replicates.

3.3. Shoot dry matter, S uptake and L value

Sulphate application did not affect the dry weight of ryegrass at the first harvest but a slight increase was observed at the second harvest (Table 5). Plants of the (−S) treatments were visibly S deficient and shoot S concentration was increased by S addition in all soils (Table 5). S content in (−S) treatments in the 20 day re-growth was not different from that of the 40 day harvest while higher concentrations were observed in the (+S) treatments.

The percentages of added ^{35}S that was present in the shoot were 25–41% in the 20 day harvest, 32–48% in the 40 day harvest, and 5–22% in the 20 day re-growth (data not shown).

Plant available S (*L* values) in (−S) treatment at day 20 harvest were 9.6 in the sandy soil and 7.7 mg kg^{-1} for the sandy loam soil (Table 5). These values were at least doubled by S application. The difference in *L* values between (−S) and (+S) treatments was about the amount of applied S (10 mg S kg^{-1}). *L* values were very close to *E* values measured at the same period during the incubation (Fig. 4). The deviation of (*E*, *L*) points from 1:1 line was smaller at the 40 day harvest compared to 20 day harvest.

4. Discussion

The higher total extractable S than extractable sulphate in the KH_2PO_4 indicated that a fraction of extracted S was not in the inorganic form, implying the presence of dissolved organic S (Watkinson and Kear, 1996; Zhao and McGrath, 1994). This means that S speciation in solution is required to quantify the immobilization and mineralization with isotope dilution where labelled SO_4 is introduced. The presence of organic S in solution can be a source of error in the interpretation of isotope dilution data. It is for this reason that the specific activity from the three extraction protocols was compared. The SA's were not different among the three extraction protocols showing that the isotope exchange with organic S in solution is not detectable within 43 days. However, this finding may not be applicable to other soils. For non-S sorbing soil with low organic matter content, the use of any of the three extraction protocols to determine either the specific activity or SO_4–S does not affect the results. However, the resin membrane method facilitates speciation because the time consuming IC step can be avoided and the SA is restricted to the SO_4 form, as required conceptually. An additional advantage of the anion exchange resin membrane for sulphate extraction is that no filtration is required and it can be used for multiple extractions after regeneration. The use of water for extracting sulphate has been limited by the high risk of soil dispersion, generating colloids interferences, compared to salt extractions (Tabatabai, 1982). This study showed that such interference could be avoided if an anion resin is included in the extraction. For SO_4 sorbing soils, however,

Table 5
Yield, plant *S* and *L* values in a pot trial with ryegrass grown on a sandy soil and a sandy loam soil with or without S application. Means of three replicates ± standard errors

	Harvest time	Sandy		Sandy loam	
		(−S)	(+S)	(−S)	(+S)
Dry weight (g)	20 days	2.2±0.05	2.3±0.06	1.7±0.12	1.7±0.06
	40 days	4.1±0.14	5.5±0.06	6.7±0.11	7.4±0.02
	20 days re-growth	1.6±0.03	1.4±0.07	2.4±0.02	3.0±0.02
S content	20 days	1.4±0.03	2.5±0.05	1.9±0.10	2.8±0.09
(g kg^{-1} plant)	40 days	0.9±0.03	1.3±0.08	0.7±0.01	1.2±0.02
	20 days re-growth	0.9±0.06	1.7±0.12	0.7±0.04	1.4±0.11
L value	20 days	9.6±0.16	19.4±0.23	7.7±0.15	18.8±0.11
(mg kg^{-1} soil)	40 days	11.4±0.23	21.5±0.42	8.8±0.56	19.2±0.38

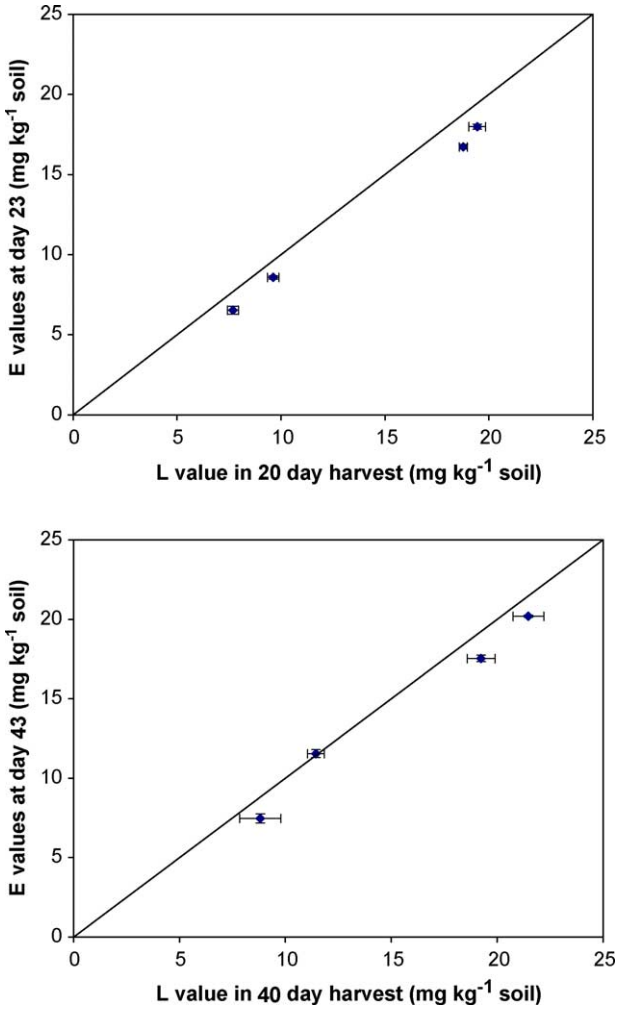

Fig. 4. Relationship between *L* values in ryegrass and *E* values measured at the same period in incubation and pot trials with a sandy soil and sandy loam soil with or without S addition. Error bars denote the standard deviations from the means of three replicates. The 1:1 line is indicated.

the deionized water+resin method may underestimate the amount of extractable SO_4 whereas it remains useful for the specific activity and *E* values determination.

The rapid decrease in the ^{35}S during incubation is in agreement with other studies. Ghani et al. (1993) reported 18% loss of ^{35}S in 1 day of incubation. In a field experiment, Eriksen (1997b) reported ^{35}S immobilization ranging from 10 to 40% of added ^{35}S 2 days after soil labeling, immobilization which depended on the size of unprotected organic matter in soil. In the present study, 10–23% was lost at day 1. Larger ^{35}S immobilization in sandy soil than in sandy loam can be attributed to the differences in organic C content between the 2 soils (Table 1).

Previous studies have reported either increase (Nguyen and Goh, 1992) or no effect of S fertilization on mineralization–immobilization processes (Bettany et al., 1974; Wu et al., 1995; Knights et al., 2001). Our study showed reduced immobilization and gross mineralization with fertilization. The inhibition of gross mineralization by SO_4 application may reflect a feed back effect. The inhibition of immobilization by SO_4 is somewhat surprising and cannot be explained.

The immobilization rate calculated from Eq. (1) as used by several authors is lower than that calculated by Eq. (8). After 43 days of incubation, our data for the sandy soil predict cumulative immobilization of 0.86 mg kg^{-1} in the (−S) treatment with Eq. (1) (data not shown) whereas Eq. (8) predict an immobilization of 1.46 mg kg^{-1} (Table 3). The differences between both methods become larger as incubation proceeds because the error involved in assuming constant specific activity (see Eq. (1)) becomes more important. If, we take into account the error introduced by the use of a constant specific activity in other studies, and differences in soil type and soil conditioning, immobilization values estimated in this study would be within the range of immobilization reported by other authors in Europe. For example, using Eq. (1); Vong et al. (2003) found a cumulative immobilization of 0.678 mg kg^{-1} after 35 days of incubation of a calcareous soil from Lorraine (France), whereas Knights et al. (2001) estimated immobilization in control plots of the long-term Broadbalk experiment to 4.0 mg kg^{-1} after 132 days of incubations.

The change in *E* value (gross mineralization) can be combined with net mineralization data (Table 3) to also predict immobilization rates. The cumulative immobilization calculated based on *E* values was 4.53 mg kg^{-1} after 43 days in (−S) treatments of the sandy soil. However, the *E* value method has a conceptual shortcoming that is similar to the shortcoming of the Eq. (1) based estimates: the *E* value estimates the gross mineralization based on the SA after an incubation. The SA decreases during incubation and an estimate of immobilization from the final SA only overestimates the immobilization. Eq. (4) predicts that immobilization is a function of SA that is also varying with time. The simplification of either using the initial SA (Eq. (1)) or the final specific activity (*E* value method) ignores this change and results in an underestimated or overestimated immobilization rates, respectively.

The E value has been shown to be a good estimate of the available pool of nutrients such as phosphorus in soils in temperate soils (Morel and Plenchette, 1994). In this study, the full recovery of added S throughout the incubation presents *E* value as a good estimate of available S in soils (Fig. 3). In addition, *E* values during incubation were very close to the *L* values in ryegrass at the 2 harvests (Fig. 4), confirming the validity of *E* value as a measure of plant available S. A similarity between *E* value and *L* value indicates that the plant access the same pool as that sampled in the soil extract. This would mean that rhizosphere effects on S mineralization were negligible in our study. Differences in S mineralization between cropped and uncropped soils have been attributed

to enhanced root-derived enzyme activity stimulated by low S status in rhizosphere resulting from plant and microbial S uptake (Li et al., 2001; Vong et al., 2003). However, data on such effects are not uniform. For example, no difference in cumulative S mineralization was observed in a pot experiment with or without perennial ryegrass (Pamidi et al., 2001) whereas Vong et al. (2003) reported increase in arylsulfatase activity in rape and barley rhizosphere soil compared to uncropped soil. They attributed the rhizosphere effects on sulphur mineralization (immobilization) to the presence of an active pool organic matter. This active pool differs between crops and between cropping systems. The type of experiment (laboratory vs. field), its duration and the plant species may determine difference in S mineralization (immobilization) between cropped and uncropped plants. The S content in ryegrass in our study indicated a low S status in these soils, which would stimulate enzyme activity and S mineralization in cropped soils, leading to higher *L* values than *E* values. This was, however, not the case. *E* and *L* values in this study were determined at the growing stage when the specific activity in soil was low (Fig. 2) and somewhat stabilized. It is not known whether these values remain comparable when determined earlier in the plant growth stage. Nevertheless, the full recovery of added S throughout the incubation in *E* values as well as in *L* values confirms the *E* value as a valuable measure of S availability. Note that both the *E* and *L* values represent the total available pool which is a quantity of available elements. Actual S availability is conditioned by additional factors such as its concentration in soil solution (i.e. intensity) and water uptake (Barber, 1984). Therefore, even when *E* and *L* values show consistent means of quantity assessment, there is yet no guarantee that these are predictive of S availability over a wide range of soils.

In conclusion, the isotope dilution technique enables the estimation of sulphur mineralization–immobilization processes. An alternative equation was put forward to avoid conceptual limitations leading to either an overestimation or underestimation of the underlying processes. The isotopically exchangeable S in soil (*E* value) represents the available S pool owing to the full recovery of S added in fertilizers, and the major source of S to ryegrass as indicated by its close similarity with ryegrass available S (*L* value).

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