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The humus layer determines SO_4^{2-} isotope values in the mineral soil

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Abstract. Biocycling of sulfur (S) has been proposed to play an important role in the recovery of ecosystems following anthropogenic S deposition. Here, we investigated the importance of the humus layer in the biocycling of S in three forested catchments in the Gårdsjön area of southwestern Sweden with differing S inputs and S isotope signature values. These experimental sites consisted of two reference catchments and the Gårdsjön roof experiment catchment (G1), where anthropogenic deposition was intercepted from 1991 until May 2002 by a roof placed over the entire catchment area. Under the roof, controlled levels of deposition were applied, using a sprinkler system, and the only form of S added was marine SO_4^{2-} with a $\delta\,^{34}S_{SO_4}$ of +19.5‰.We installed ion exchange resin bags at the interface between the humus layer and mineral soil at each of the catchments to collect SO_4^{2-} passing through the humus. The resin bags were installed on four occasions, in 1999 and 2000, covering two summer and two winter periods. The ions collected by each bag during these sampling periods were then eluted and their $\delta\,^{34}S_{SO_4}$ values and SO_4^{2-} concentrations determined. The most striking result is that the average $\delta\,^{34}S_{SO_4}$ value in the resin bags was more than 12‰ lower compared to that of the sprinkler water in the G1 roof catchment. There was no increasing trend in the isotope value in the resin bag SO_4^{2-} despite that the roof treatment has been on-going for almost 10 years; the average value for all resin bags was +7.1‰. The highest $\delta\,^{34}S_{SO_4}$ values found in the G1 roof catchment were between +11‰ and +12‰. However, these values were all obtained from resin bags installed at a single sampling location. Throughfall and resin bag $\delta\,^{34}S_{SO_4}$ values were more similar in the two reference catchments: about +7.5‰ in both cases. There was, however, an increase in resin bag $\delta\,^{34}S_{SO_4}$ values during the first winter period, from about +7‰ to +9‰. The resin bag $\delta\,^{34}S_{SO_4}$ value was linearly and positively related ($r^2 = 0.26, p < 0.001$) to the amount of SO_4^{2-} extracted from the resin bags, if relatively high amounts (> 50 mmol m^{-2}) were excluded. High amounts of resin bag SO_4^{2-} seemed to be related to groundwater inputs, as indicated by the $\delta\,^{34}S_{SO_4}$ value. Our results suggest that rapid immobilization of SO_4^{2-} into a large organic S pool may alter the S isotope value and affect the $\delta\,^{34}S_{SO_4}$ values measured in the mineral soil and runoff.

Introduction

The decline in sulfur (S) deposition in North America and Europe in recent decades (Hedin et al. 1994) has fostered increasing interest in the reversibility of anthropogenic acidification in terrestrial and aquatic ecosystems (Wright et al. 1993; Hultberg and Skeffington 1998; Wilander 2001). A number of catchment scale studies, where the primary source of S is deposition, have indicated that losses of SO_4^{2-} still exceed SO_4^{2-} inputs, although the source of soil SO_4^{2-} is not clear (Torssander and Mörth 1998; Löfgren et al. 2001; Wilander 2001). Considerable attention in previous studies has been focused on geochemical processes such as adsorption–desorption of SO_4^{2-} (Wright et al. 1993; Mörth and Torssander 1995). However, some recent studies have indicated that net mineralization of organically bound S in the humus layer may play an important role, although quantitative data on the contribution of organic S mineralization to soil solution SO_4^{2-} is often lacking Gebauer et al. 1994; Mayer et al. 1995; Novak et al. 1996; Torssander and Mörth 1998; Alewell et al. 2000; Mitchell et al. 2001). In order to better understand processes and sources involved in S dynamics, $\delta\ ^{34}S$ values in different forms of S in precipitation/throughfall, soils, soil solution and stream flow have been measured (Fuller et al. 1986; Krouse and Grinenko 1991; Mayer et al. 1995; Mörth and Torssander, 1995; Alewell et al. 1999; Mörth et al. 1999; Mitchell et al. 2001; Novak et al. 2000, 2001). Despite these efforts, there are still major uncertainties in the interpretation of studies involving $\delta\ ^{34}S$ and S dynamics in forest ecosystems. One problem arises from the generally small differences in $\delta\ ^{34}S$ values between deposition and stream runoff. For instance, Alewell et al. (2000) showed that $\delta\ ^{34}S_{SO_4}$ values in bulk deposition ranged only between approximately $+3.2‰$ and $+5.1‰$ in Hubbard Brook and the difference in average $\delta\ ^{34}S_{SO_4}$ values in bulk deposition and stream water was less than 1‰ (Alewell et al. 1999). With such small differences it is difficult to separate sources and processes involved in S dynamics based solely on $\delta\ ^{34}S$ values. Studies in Europe have found relatively large differences in isotope values, depending on the relative influence of marine, anthropogenic and geogenic sources (Mörth and Torssander 1995; deCaritat et al. 1997; Novak et al. 2001).However, within-site differences seem to be smaller (Novak et al. 2000, 2001), which complicates the interpretation of $\delta\ ^{34}S$ measurements. Besides differences in $\delta\ ^{34}S$ values of the sources, fractionation can affect isotope values. Major fractionation of S isotopes is generally caused by bacterial dissimilatory SO_4^{2-} reduction, which mainly occurs under anaerobic conditions (vanStempvoort et al. 1990; Canfield and Thamrup 1994; Habicht and Canfield 1997; Mörth et al. 1999; Groscheova et al. 2000). Fractionation due to mineralization and immobilization is considered to be of minor importance (Krouse and Grinenko 1991; Durka et al. 1999; Norman et al. 2002). However, relatively large changes in $\delta\ ^{34}S$ with depth in aerated soil profiles have been attributed to this type of fractionation (Mayer et al. 1995; Novak et al. 1996, 2000).

There is a need for large-scale "tracer" experiments using stable S isotopes in order to minimize the interpretation problems associated with the small differences in isotope values and thus exploit the full potential of stable isotope analyses. Such an experiment was performed at Gårdsjön in southwestern Sweden. From 1991 until the experiment was terminated in May 2002, anthropogenica deposition was intercepted by a roof over the G1 catchment (Moldan et al. 1998) and pre-industrial levels of deposition (of naturally occurring marine SO_4^{2-}) were applied by a sprinkling system. The change in SO_4^{2-} input from a mainly anthropogenic source to a marine source increased the isotopic $\delta\ ^{34}S_{SO_4}$ value from about +6.9‰ to about +19.5‰ in May 1991 (Torssander and Mörth 1998). However, this change was not reflected in $\delta\ ^{34}S_{SO_4}$ values observed in the soil and runoff (C.M. Morth et al. submitted), and the $\delta\ ^{34}S_{SO_4}$ values in the runoff were still less than +7‰ in 1998.

We believe that processes in the O-horizon may play a major role in explaining the absence of a change in runoff $\delta\ ^{34}S_{SO_4}$ The isotope value of the humus layer was about +6‰ in the G1 roof catchment at the start of the treatment and turnover of marine SO_4^{2-} in the humus layer is likely to decrease the value of the SO_4^{2-} passing through the humus layer. The magnitude of the change will, however, depend on the turnover rate and size of the "active" portion of the organic S pool. In order to assess the magnitude of the $\delta\ ^{34}S_{SO_4}$ change during the transfer of SO_4^{2-} through the humus layer we installed anion exchange resin bags under the humus layer in the G1 Roof catchment, starting in July 1999, about 8 years after the start of the roof treatment. The anion resin bags were installed and sampled so that the sampling periods covered both the growing seasons and "winter" periods of the two following years. We used a reference catchment (F1 Control) and a nitrogen fertilized experimental plot (G2 Nitrex) as controls.

Material and methods

Study site

Lake Gårdsjön is situated 50 km north of Gothenburg and approximately 10 km from the west coast of Sweden (58°04′ N, 12°03′ E). It has a maritime climate due to the vicinity of the Atlantic Ocean. The length of the growing season (mean daily temperature > 3 °C) ranges from 210 to 240 days (Olsson et al. 1985). The coldest period is January–February, with average temperatures of about −2.5 °C (Andersson et al. 1998). The warmest month is July, with an average temperature of about 16.0 °C (Andersson et al. 1998). The annual precipitation ranges from 700 to 1200 mm and falls mainly as rain (Olsson et al. 1985). The throughfall chemistry of the Gårdsjön area is dominated by the strong marine influence, resulting in high loadings of chloride (110 kg ha^{-1} $year^{-1}$) and sodium (55 kg ha^{-1} $year^{-1}$).

Within the Gårdsjön area three small forested catchments have been studied: the reference catchment. F1 Control (3.70 ha), the roofed catchment G1 roof (0.63 ha), and the nitrogen fertilized catchment, G2 Nitrex (0.52 ha).The throughfall was intercepted at G1 roof from April 1991 to May 2002 when the roof experiment was terminated. The deposition rates for $SO_4^{2-}S$ were

20–24 kg ha^{-1} year^{-1} and that of N was 13 kg ha^{-1} year^{-1} when the roof experiment began (Hultberg and Grennfelt 1992; Moldan et al. 1998). Since then, SO_4^{2-}S deposition rates have decreased by about 40%. In the G1 roof catchment the "throughfall" was reduced from about 20 to about 3 kg SO_4-S ha^{-1} year^{-1} at the onset of the experiment in 1991. Throughfall SO_4^{2-} deposition in the nearby site Hensbacka was about 8 kg ha^{-1} year^{-1} in 1999–2001 (Anonymous 2003). There was, however, a large difference in marine seasalt inputs between the hydrological year 1999/2000 (October 1999 to September 2000) and 2000/2001 (October 2000 to September 2001). The throughfall input of marine SO_4^{2-} was 94% higher in 1999/2000 than in 2000/2001, 3.5 and 1.8 kg marine SO_4 ha^{-1} year^{-1}. The input of chloride in 1999/2000 was 75 kg ha^{-1} year^{-1} and in 2000/2001 39 also reflecting the large difference throughfall composition between the two hydrological years. The differences in precipitation between the two hydrological years were, however, $<10\%$. In the G1 roof experiment only marine S was added during the course of the treatment (Moldan et al. 1998). In the G2 Nitrex experiment ambient N deposition rates were experimentally increased by about 35 kg ha^{-1} year^{-1}, the treatment starting in April 1991 Moldan and Wright 1998; Stuanes and Kjønaas 1998).

The forest cover in all three catchments is dominated by approximately 90–110-year-old Norway spruce [*Picea abies* (L.) Karst] and a large component of Scots pine [*Pinus silvestris* (K.)]. The field layer is dominated by dwarf shrubs, mainly *Vaccinium myrtillus* (L.) and grasses mainly *Deshampsia flexuosa* (L.).

The till soils are generally shallow (<0.5 m) on the steeper sides of the catchments and deeper in the valley bottoms (1–3 m; Andersson et al., 1998). The soil texture ranges from sandy to silty-loam with an average clay content of about 5% (Andersson et al. 1998). The soils are generally spodosols (Typic Haplohumods; Soil Survey Staff, 1998) with well-developed albic and spodic horizons and an overlaying humus layer (O-horizon). The average thickness of the humus layer in the three sites is about 8 cm,the albic E-horizon is about 5 cm thick and the spodic B-horizon about 20 cm thick. The upper part of the humus layer is generally less decomposed (O_i whereas the lower part is slightly or highly decomposed (O_i or O_a) Selected chemical properties are given in Table 1.

Table 1. Selected chemical properties for the humus layer in the three catchments.

Catchment	$pH_{(H_2O)}$	CEC (mmol kg^{-1})	C/N	Total N (mmol kg^{-1})	Total S (mmol m^{-2})
G1 Roof	3.97	352	34	1175	1098[a]
F1 Control	3.69	363	37	1049	na
G2 Nitrex	3.74	390	40	1070	na

Data from Andersson et al. (1998).
[a]Data from C.M. Morth et al. (submitted).

Ion exchange resin

Rectangular bags (6 × 7 cm) made from fine polyethylene fiber web (50 g m^{-2}, BBA Nonwovens Sweden AB, Norrköping, Sweden) were each filled with about 17 cm^{-3} of anion exchange resin (strong basic anion resin, no. 21.740-9, Dowex 1 × 8–50, 20–50 mesh. Sigma-Aldrich, Milwaukee, Wl). This amount of resin provides approximately 28 $mmol_c$ of anion exchange capacity distributed across a 39 cm^2 resin bag surface area,giving an anion exchange capacity of about 7000 $mmol_c$ m^{-2}. The throughfall input of anions (SO_4^{2-}, Cl^- and NO_3^-) is equivalent to approximately 500 $mmol_c$ m^{-2} $year^{-1}$ (Moldan et al. 1998) and the anion exchange capacity is thus well in excess of the throughfall input. The amount of SO_4^{2-} captured by the resin is expressed in mmol m^{-2} resin bag surface area rather than to the amount of resin in the resin bag to allow comparison with estimates of SO_4^{2-} inputs into the soil. The estimates are, however, not quantitative flux estimates since the resin bags may not only recover ions from gravitational water flow. The resin was eluted with 0.5 M NaCl (suprapure grade), washed with distilled water (ELGA Maxima Analytica, > 18.2 MΩ, ELGA, Bucks, UK) three times and then saturated with 0.5 M NaCl (suprapure grade) before use. This procedure assured that the resin contained negligible amounts of SO_4^{2-} prior to the sampling.

Resin bag installation

The resin bags were placed at the humus layer-mineral soil interface (Figure 1) by cutting a slit, approximately 0.1 m long, in the humus layer down to the mineral soil surface and pushing the resin bags horizontally into position with a rod approximately 40 cm from the slit. The resin bags were retrieved with a tagged nylon cord at collection. We assume that the resin bags mainly collect soil water that has percolated through the humus layer (O-horizon). Between 10–20 resin bags were installed randomly within each of the three catchments on the first occasion. On the following occasions, resin bags were installed at the same locations within about 1 m of the first installation. In ail, 173 resin bags were recovered and analyzed. The first set of resin bags was installed on

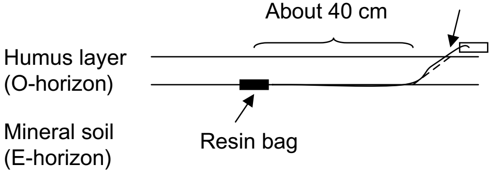

Figure 1. Resin bags were installed in the interface between the humus layer (O-horizon) and the mineral soil about 40 cm from the inserting point.

July 19, 1999 and recovered November 15, 1999. The second set was installed November 15, 1999 and recovered May 15, 2000. The third set was installed May 15, 2000 and recovered October 2000 when the last set also was installed. This was recovered May 22, 2001. The average time in the field for the four sets were 169 days. Two sets covered November–May (i.e. the winter period) and two sets May–November, the main part of the growing season.

After collection from the field, the resin in the bags was transferred carefully to small plastic PE columns and eluted with 200 ml 0.5 M NaCl (suprapure grade). An aliquot of the eluate was used for analyses of SO_4^{2-}. The eluted SO_4^{2-} was precipitated with 0.5 M $BaCl_2$ after boiling for 1 min and adjusting the pH to about 2 with HCl. The precipitate of $BaSO_4$ was then incubated in a water bath for 2 h at about 90 °C. After that the solution was cooled to room temperature and passed through a polycarbonate filter (Isopore, no HTTP 02500 or HTTP 04700, Millipore SA, Malsheim, France, depending on the amount of $BaSO_4$ precipitated). The $BaSO_4$ collected on the filters was washed with ultra-clean water to remove Cl^- from the eluent and dried in an oven at 60 °C overnight. Filters were then weighed and scraped to remove the $BaSO_4$. The recovery is normally over 95%, based on a large number of samples processed at the Department of geology and geochemistry during the last 12 years.

Analyses

The sample and standard mass ratios were measured using Isotope Ratio Gas mass spectrometry (IRMS, Finnigan Delta+, Thermofinnigan Corp., Bremen, Germany). All S isotope measurements were done on SO_2 (g) converted from $BaSO_4$ on-line in a Carlo Erba NC2500 CF-IRMS system. The reference gas was measured before the sample for every determination. Based on standard measurements the precision was better than $\pm 0.2‰$, but this level of precision is only attained if large number of standards are run every day.

$\delta\,^{34}S$ values are reported in ‰ according to

$$\delta\,^{34}S(‰) = \left(\frac{R_{\text{sample}}}{R_{\text{STD}}} - 1\right) \cdot 1000$$

where R refers to the $^{34}S/^{32}S$ mass ratio and R_{STD} is the mass ratio of $^{34}S/^{32}S$ in CDT (Cañon Diablo Troilite).

The SO_4^{2-} concentrations were measured with an ion chromatography system (Dionex, DX-300 equipped with an AS14 column using electrical suppression, Dionex Corp. Sunnyvale, CA).

Statistics

For statistical comparisons between catchment and periods, two-way analysis of variance (ANOVA) was used with catchment and period as fixed factors.

Table 2. Resin bags with amounts of $SO^{2-}{}_4$ extracted larger than 50 mmol m^{-2}.

Period	Site					
	G1 Roof	*n*	F1 Control	*n*	G2 Nitrex	*n*
1	14 > 15 > 20 > 13	20	2 > 1 > 3 > 4	16	1	12
2	13 > 18[a]	16	2 > 1 > 3 > 4 > 8 > 7 > 6 > 5	16	8 > 1 > 10 > 7	7
3	20 > 14 > 15	20	1 > 2	15	12 > 1 > 5	10
4	20 > 19 > 15 > 14 > 18 > 13	20	3 > 2 > 1 > 7 > 8 > 6[b]	15	4 > 1	6

The number denotes the position of the resin bag within the catchment in decreasing order of extracted SO_4^{2-}. A resin bag from one position with a high amount of exchangeable SO_4 tend to have high amounts at more than one occasion, *n* denotes total number of resin bags recovered.

[a] (20,19,14,15) missing.

[b] 4 missing.

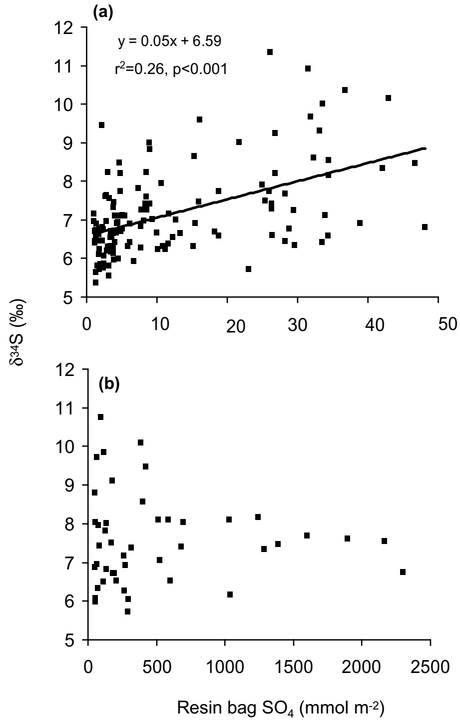

Figure 2. The relationship between $\delta\ ^{34}S$ and the amount of exchangeable SO_4 eluted from the resin bags after a period in the field, showing (a) amounts below 50 mmol m^{-2} and (b) values above 50 mmol m^{-2}. There appears to be a positive relationship between $\delta\ ^{34}S$ and the amount of SO_4 in the former, but no correlation in the latter.

Multiple comparisons in variance analyses were performed with Tukey's test. Probability values obtained using Pearson correlations were Bonferroni-adjusted. Statistical analyses was performed using SYSTAT statistical software (SYSTAT Inc., Evanstone, IL). Differences were regarded as significant for probability levels <0.05 unless otherwise stated.

Results

Relationships between amounts of SO_4^{2-} in the resin bags and $\delta\ ^{34}S$

The amount of SO_4^{2-} extracted from the resin bags showed large variations (Figure 2). Amounts of more than 500 mmol m^{-2} had an average $\delta\ ^{34}S_{SO_4}$ value of +7.5‰, ranging between +6.2‰ and +8.2‰. Amounts less that 500 mmol m^{-2} showed larger variations, with values ranging between +5.4‰ and +11.3‰. Excluding values above 50 mmol m^{-2} gave a weak positive

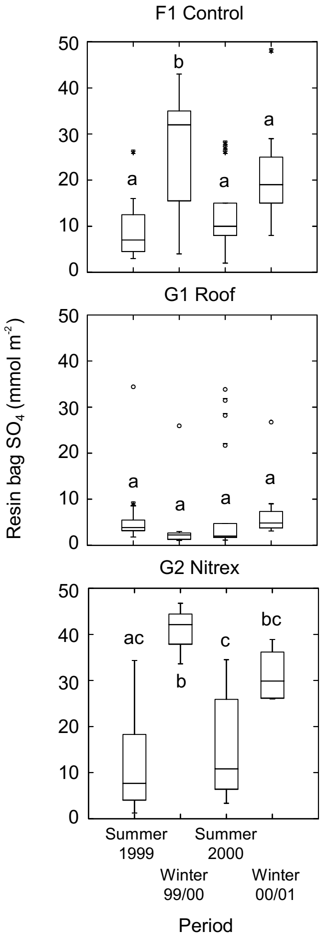

Figure 3. Box plot showing the exchangeable amount of SO_4 in the resin bags for four periods in the field. Periods 2 and 4 are "winter" periods whereas 1 and 3 are "summer" periods. The box encloses 50% of the data and the horizontal bar marks the median. Whiskers extend to the outermost data-points or 1.5 times the inter-quartile distance when outlying vales are present. Outlying values are defined as values greater than 1.5 times (*) or three times (○) the inter-quartile distance.

linear relationship between the $\delta\,^{34}S_{SO_4}$ value and the amount of SO_4^{2-} extracted ($r^2 = 0.26, p < 0.001$; Figure 2), whereas no relationship was observed for values greater than 50 mmol m^{-2}. The average amount extracted was equivalent to 4% of the total exchange capacity of the resin bags (median 0.4%), however, in some extreme cases the amount corresponded to as much as 56% of the total exchange capacity. Samples with large amounts of extractable SO_4^{2-} were generally found in resin bags placed close to the same position

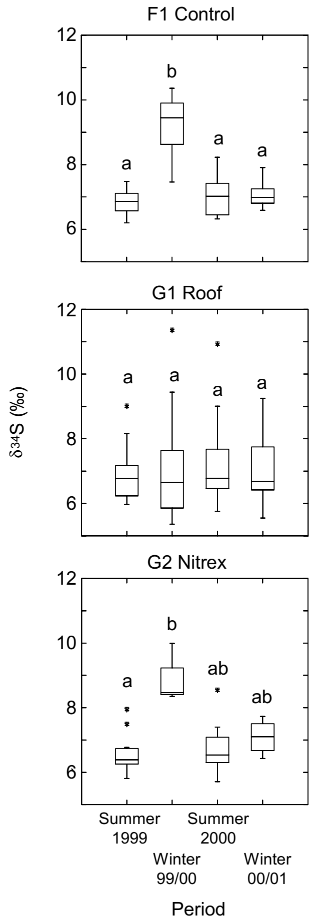

Figure 4. Box plot showing the $\delta\ ^{34}S$ values of resin bag SO_4 for four periods in the field. Periods 2 and 4 are "winter" periods whereas 1 and 3 are "summer" periods. The box encloses 50% of the data and the horizontal bar marks the median. Whiskers extend to the outermost data-points or 1.5 times the inter-quartile distance when outlying values are present. Outlying values are defined as values greater than 1.5 times (*) or three times (○) the inter-quartile distance.

Table 3. Correlation coefficients between $\delta\ ^{34}S$ values from different sampling periods.

Comparison between periods	G1 Roof	F1 Control	G2 Nitrex
1 vs. 2	0.52^{ns}	-0.22^{ns}	0.58^{ns}
2 vs. 3	0.80^{**}	-0.35^{ns}	0.45^{ns}
2 vs. 3	0.67^{*}	0.58^{ns}	-0.01^{ns}

* and ** denotes significant difference at 0.05, 0.01 and the 0.001 level.
ns denotes not significant.

within the catchment, regardless of the sampling period (Table 2). However, on some occasions (mostly in the winter periods) some resin bags gave high amounts that were not correlated with high previous or past determinations. The following results applies only to values below 50 mmol m^{-2} ($n = 128$), excluding 45 observations since we suspect that higher extractable amounts most likely are related to inputs that are not restricted solely to soil solution leachate from the humus layer but also reflect inputs from other sources.

The amounts of SO_4^{2-} extracted from the resin bags were larger in the first winter period compared to the previous summer period in the F1 Control and G2 Nitrex catchments (Figure 3). The same trend was observed in the second year. In the G1 roof catchment no such trend was found. The amounts extracted from the F1 Control and G2 Nitrex catchments were larger than those from the G1 Roof (Figure 3).

$\delta\,^{34}S_{SO_4}$ in resin bags

The most striking result is that the mean $\delta\,^{34}S_{SO_4}$ value in the resin bags was more than 12‰ lower compared to the sprinkler water input in the G1 roof catchment (Figure 4) even after almost 10 years of excluding anthropogenic SO_4^{2-}. No trend was detected between the sampling periods in the G1 Roof catchment, and the average $\delta\,^{34}S_{SO_4}$ value was +7.1‰. The average $\delta\,^{34}S_{SO_4}$ values in the other two catchments were similar: +7.6‰ and +7.4‰ respectively, for the F1 Control and G2 Nitrex catchments. The slightly higher average values in these catchments were due to the higher $\delta\,^{34}S_{SO_4}$ values found in the first "winter"-period (Figure 4). The average values during this period

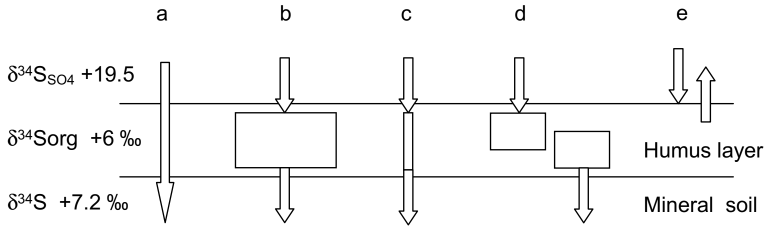

Figure 5. The figure illustrates different pathways for SO_4^{2-} and the effect on $\delta\,^{34}S_{SO_4}$ isotope values in the G1 Roof catchment. Arrows illustrates fluxes and boxes the organic S pool in the humus layer. (a) No gross immobilization or mineralization would rapidly change the $\delta\,^{34}S_{SO_4}$ value in the upper mineral soil. (b) A rapid incorporation of a thoughfall SO_4^{2-} in a large organic S pool (i.e. organic S pool ≫ throughfall input) will dilute the isotope value. (c) The dilution effect in a small "reactive" organic S pool is small (i.e. organic S pool ≍ throughfall input) and should increase the $\delta\,^{34}S_{SO_4}$ value in the upper mineral soil. (d) A rapid incorporation in an organic S pool decoupled from an organic S pool that contributes to the release of SO_4^{2-} will result in no change in $\delta\,^{34}S_{SO_4}$ values in the upper mineral soil. (e) Uptake of SO_4^{2-} and redistribution of S in litter may also be decoupled in time and delay a change in $\delta\,^{34}S_{SO_4}$ isotope values.

were +9.2‰ and +8.9‰, respectively, for the F1 Control and G2 Nitrex catchments. No increase in $\delta\ ^{34}S_{SO_4}$ was observed in the second winter period.

The $\delta\ ^{34}S_{SO_4}$ values ranged from +5.6‰ to +11.4‰ in the G1 roof catchment, the highest value being found in the second period. In the F1 Control and G2 Nitrex catchments the $\delta\ ^{34}S_{SO_4}$ values ranged from +5.6‰ to +10.8‰, the highest values again being found in the second sampling period. Linear correlations in $\delta\ ^{34}S_{SO_4}$ values were found in some cases between different sampling periods (Table 3) in the G1 Roof catchment. In these cases values obtained for successive sampling periods were correlated.

Discussion

Our results clearly demonstrate that the transfer of SO_4^{2-} through the humus layer has a large impact on the $\delta\ ^{34}S_{SO_4}$ value. In Figure 5 we illustrate how different pathways may affect the isotope signature of SO_4^{2-} during the transition through the humus layer. In the following text we discuss how shifts in $\delta\ ^{34}S_{SO_4}$ values may relate to seasonal changes in SO_4^{2-} deposition and immobilization/mineralization and the implications of the results for the interpretation of $\delta\ ^{34}S_{SO_4}$ in field studies.

Sulfate immobilization and mineralization

Rapid recycling of SO_4^{2-} through immobilization and mineralization reactions can change the isotopic value by fractionation or dilution. However, for dilution to be a significant factor, there are three requirements. The organic S pool participating in the immobilization/mineralization processes must be large compared to the amounts of incoming SO_4^{2-}. Immobilization rates must be high, and there must be either a distinct difference between the two end-members, i.e. the organic S pool and the incoming SO_4^{2-}, or the immobilization and mineralization must be decoupled.

The organic S pool in the humus layer of the G1 Roof catchment was about +6‰ in 1991 at the start of the roof treatment compared to +19.5‰ for the sprinkler water input (Torssander and Mörth 1998) so there was a distinct difference between the two end-members. We also assume that the gross immobilization rate is high in the humus layer. This hypothesis is supported by the results of previous studies in which $^{35}SO_4$ was used to determine S immobilization rates in the humus layer (Strickland and Fitzgerald 1984; Fuller et al. 1986; Houle et al. 2001). Yearly sprinkling water inputs in the G1 Roof catchment amounted to about 12 mmol SO_4^{2-} m^{-2} $year^{-1}$ (Hultberg and Skeffington 1998), whereas the size of the humus layer organic S pool was about 1100 mmol m^{-2} (C.M. Mörth et al. submitted) in 1990. The total input of sprinkler water $SO_4^{2-}S$ applied during 8 years of treatment between 1991 and 1999. when the resin bags were installed, was approximately 96 mmol

SO_4^{2-} m^{-2} $year^{-1}$, equivalent to 9% of the total S pool in the humus layer (C.M. Mörth et al. submitted). The 0.04 M $NaHCO_3$-extractable SO_4^{2-} pool in the humus layer is about 55 mmol m^{-2} (C.M. Mörth et al. submitted) and thus represents a small fraction of the total humus layer S pool. If there had been no immobilization or mineralization of SO_4^{2-}, there would probably have been a complete exchange of the 0.04 M $NaHCO_3$-extractable SO_4^{2-} pool, and the $\delta\ ^{34}S_{SO_4}$ value of the $SO^{2-}{}_4$ passing through the humus layer is likely to have increased. In contrast, incorporation of all the sprinkler water SO_4^{2-} into the soil organic S pool would have given a $\delta\ ^{34}S$ value of $+7.2‰$ in the humus layer, assuming that no change in pool size occurred and that all the SO_4^{2-} that was leached from the humus layer originated from the pre-roof organic S pool.

Immobilization due to plant uptake may result in a decoupling in time between the immobilization and the mineralization processes, since S incorporated in plant tissues will not be released into the soil until a later stage. The biomass pool is also large and likely to dilute the isotope value. However, the yearly gross tree uptake of S is estimated to be about 2 mmol S $year^{-1}$, which is insufficient to account for the observed change in $\delta\ ^{34}S_{SO_4}$ between the sprinkler water and resin bags. Microbial immobilization is thus likely to play an important role.

Important issues to address are whether there is a "reactive" organic S pool, and whether we can deduce anything about its size (if present). A small "reactive" organic S pool participating in the turnover of S would inevitably increase the $\delta\ ^{34}S_{SO_4}$ value in the ieachate from the humus layer since the input of marine SO_4 would increase the $\delta\ ^{34}S_{SO_4}$ value of this pool more rapidly than if the total organic S pool participates in the turnover of S. Using a simple mixing model ($[A \times \delta\ ^{34}S_A + B \times \delta\ ^{34}S_B]/A + B$), the capital letters denoting pool size or flux and the suffix denoting the isotope value of the pools/fluxes, we have calculated the isotope value in the organic S pool and the soil water passing the humus layer after 8 years in the G1 Roof catchment. We assume no net mineralization and pool change and the calculation is done for each consecutive year since the isotope value will increase gradually. We used a organic S pool size of 100 mmol S m^{-2} (approximately 10% of the total organic S pool in the humus layer) with a $\delta\ ^{34}S_{SO_4}$ value of $+6‰$ and a yearly sprinkling water input of 11.5 mmol S m^{-2} with a $\delta\ ^{34}S_{SO_4}$ value of $+19.5‰$. This gives a $\delta\ ^{34}S_{SO_4}$ isotope value of $+13.8$ for the soil water passing the humus layer after 8 years of the roof treatment. A rapid turnover from this "reactive" pool is thus likely to give a higher $\delta\ ^{34}S_{SO_4}$ value than those measured in the ion exchange resin. A similar calculation shows that at least 70% of the organic S pool has to be involved in the turnover to achieve the average measured isotope values in the resin bags. Houle et al. (2001) suggested that the "active" organic S reservoir in a whole soil profile they investigated was equivalent to less than 1% of the organic S of the total organic S pool. However, this estimate does not seem to be valid for our humus soil since our data indicate that a larger part of the organic S pool must be involved.

Incorporation of SO_4^{2-}S into either plant or microbial biomass involves a transformation of SO_4^{2-} to carbon-bound S and ester-bound SO_4^{2-}. In humus soil the reduced carbon-bound forms of S seems to dominate (accounting for 50–78% of the total) as demonstrated by Skyllberg et al. (in press) using S K-edge X-ray absorption near-edge structure (XANES) spectroscopy. Differences in the amount incorporated and the residence times between different forms of organic S could theoretically result in a lag phase between changes in the $\delta\ ^{34}S_{SO_4}$ values in the SO_4^{2-} immobilized and the SO_4^{2-} released during mineralization and may contribute to the phenomena we observed. This remains, however, to be tested.

It has been suggested that fractionation during mineralization of organic S is likely to cause slight reductions in $\delta\ ^{34}S_{SO_4}$ values in the soil solution with increasing soil depth (Novak et al. 1996; Alewell and Gehre 1999; Novak et al. 2000; Norman et al. 2002). Novak et al. 2000 found significant difference in $\delta\ ^{34}S_{SO_4}$ between deeper soil solutions and the incoming throughfall but not between soil water sampled from directly beneath the humus layer and throughfall. Similarly, differences between soil solution samples measured at 90–100 cm depth and throughfall were generally less than 1‰ in upland sites studied by Alewell and Gehre (1999). Furthermore, in laboratory incubations reported by Norman et al. (2002), changes in extracted $\delta\ ^{34}S_{SO_4}$ were <1.5‰ during a 3-week incubation. Our results do not exclude the possibility of fractionation due to mineralization, but the above results suggest that it is of minor importance, at most, and cannot explain the large differences seen in $\delta\ ^{34}S_{SO_4}$ between the sprinkler water and the resin-bag $\delta\ ^{34}S_{SO_4}$ values.

Seasonal differences and spatial variability in δ ³⁴S isotope values

The relatively high resin bag $\delta\ ^{34}S_{SO_4}$ values derived for the two reference sites, F1 Control and G2 Nitrex, in the first winter period are most likely related to the events causing a high seasalt input and reductions in immobilization rates during winter time. The $\delta\ ^{34}S_{SO_4}$ values of the deposition in the Gårdsjön area can be explained by a mixing model, with marine SO_4^{2-} as one end-member (+21.5‰ and anthropogenic SO_4^{2-} (+4.5‰ as the other end-member (Torssander and Mörth, 1998). Higher throughfall $\delta\ ^{34}S_{SO_4}$ values are observed in the winter in this area (Mörth et al. 1999) and an increase in the marine SO_4^{2-} contribution will increase the $\delta\ ^{34}S_{SO_4}$ value in the deposition. The increased $\delta\ ^{34}S_{SO_4}$ values in the resin bags found during the first winter period are thus most likely due to a combination of the high marine SO_4^{2-} inputs and decreased immobilization rates associated with lower soil temperatures (Houle et al. 2001). This hypothesis is supported by the positive relationship between the amount of SO_4^{2-} extracted in the resin bags and the $\delta\ ^{34}S_{SO_4}$ values although the degree of explanation was low (26%) and other factors most be more important. The lack of change in G1 Roof probably reflects the absence of sea salt events within the roofed catchment.

The general absence of a correlation in $\delta\ ^{34}S_{SO_4}$ values between the different sampling occasions suggests that the spatial heterogeneity within the locations where the resin bags were installed is greater than trends due to variables such as precipitation events within the locations. The positive correlation found in the G1 Roof catchment (Table 3) was due to one spot giving high $\delta\ ^{34}S_{SO_4}$ values on three sampling occasions. No significant correlation remained if these values were excluded.

The largest amounts of SO_4^{2-} extracted, especially at some positions, may reflect either a larger preferential flow through the humus layer or an inflow of groundwater in these positions. For instance, 500 mmol m^{-2} extracted from a resin bag corresponds to approximately 50% of the total S pool in the humus layer or a flux of about 160 kg SO_4-S ha^{-1}. The most extreme values were over 2000 mmol m^{-2} which corresponds to a flux of 640 kg SO_4-S ha^{-1}. Runoff from the G1 Roof and Fl Control catchments were estimated to approximately 135 mmol SO_4 m^{-2} $year^{-1}$ between 1991 and 1995 (Moldan 1999) and the resin bag amounts in the above cases are thus much higher. The average $\delta\ ^{34}S_{SO_4}$ value of 7.5 for samples above 500 mmol m^{-2} were similar to those reported for two groundwater wells from F1 by Mörth et al. (1999), 8.0 and 6.9 respectively. Results from the G1 Roof catchment shows that a high runoff also corresponds to a high groundwater table (Nyberg 1995) and it is thus likely that positions with shallower soils may have a large groundwater impact. We tried to avoid positions were we suspected that a groundwater impact might occur but we cannot exclude that local variations in for instance bedrock topography and mineral soil thickness has affected the impact of groundwater flux in some of the positions. Higher $\delta\ ^{34}S_{SO_4}$ values in resin bags with amounts ranging from about 50 to 500 mmol m^{-2} (Figure 2) does, however, indicate that groundwater inputs alone cannot explain the high amounts in the resin bags. Small depressions in micro-topography in combination with high vertical water-flow rates may contribute to both high amounts of leachate and higher $\delta\ ^{34}S_{SO_4}$ values reflecting marine $\delta\ ^{34}S_{SO_4}$, which were found mainly in the first winter period.

Implications for interpretations of $\delta\ ^{34}S_{SO_4}$ values

Changes in $\delta\ ^{34}S_{SO_4}$ values in the runoff in the Gårdsjön G1 Roof catchment between 1991 and 2001 has been minor (< 2‰; C.M. Mörth et al. submitted) despite dramatic reductions in inputs and significant decreases in both soil solution and runoff SO_4^{2-} concentrations (Giesler et al. 1996; Torssander and Mörth 1998). Our study suggests that the small change that has occurred in the isotope value in runoff is related to a rapid immobilization of sprinkler water SO_4^{2-} in the humus layer rather than to fractionation processes in the humus layer or mineral soil. This is also in agreement with resent findings by Novak et al. (2003) suggesting that soil-derived discharge must come from organic S in the upper soil horizon. A resent tracer study by Mayer et al. (2001) did also show that 50% of the added SO_4^{2-} was retained as organic S in the topsoil in a

20-month long tracer experiment adding 35 kg SO_4^{2-} ha^{-1}. The isotope values in the mineral soil and runoff can be assumed to converge with the isotopic value of the organic S pool in the humus layer when the latter approaches the $\delta\ ^{34}S_{SO_4}$ values of the throughfall. In support of this assumption are the findings of Novak et al. (2001), which show that throughfall $\delta\ ^{34}S_{SO_4}$ values are reflected in the humus layer and mineral soil. Adsorption/desorption processes in the B-horizon are likely to create a lag phase between changes in $\delta\ ^{34}S_{SO_4}$ values in the humus layer and the runoff, but this is probably minor compared to the time required to change the $\delta\ ^{34}S$ values of the larger organic S pool in the humus layer.

Conclusions

We demonstrate in this study that the $\delta\ ^{34}S$ isotope value of the humus layer has a major impact on the $\delta\ ^{34}S_{SO_4}$ value of the soil solution leachate that enters the mineral soil and clearly exclude alternative (a) in Figure 5. The shift in isotope value between the artificial "throughfall", i.e. sprinkler water in the G1 Roof catchment, and the leachate collected in the resin bags cannot be explained by fractionation, and we suggest that it must be related to a rapid immobilization of soil solution SO_4^{2-}. The SO_4^{2-} release due to mineralization is either isotopically diluted by a much larger organic S pool (alternative (b), Figure 5) or that the immobilization and mineralization processes are decoupled in time (alternative (d), Figure 5). Uptake of SO_4^{2-} by the vegetation may also give a similar effect as alternative (d) (alternative (e), Figure 5) since the input due to litter most likely is delayed in time. Uptake estimates of SO_4^{2-} does, however, indicate that this is of minor importance. We also exclude alternative (c) (Figure 5) since the isotope value in a small "reactive" organic S pool would have been changed after 10 years of treatment and this should have been reflected in the resin bags. Future studies can hopefully give us a better understanding of what processes that may be involved in the dynamics of S and especially processes in the humus layer.

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