



# Evidence of secondary sulfate production in the mineral soil of a temperate forested catchment in southern Québec, Canada

Charles Marty<sup>a,\*</sup>, Daniel Houle<sup>b</sup>, Louis Duchesne<sup>b</sup>, Christian Gagnon<sup>a</sup>

<sup>a</sup> Science and Technology Branch, Environment and Climate Change Canada, 105 McGill St., Montréal, QC H2Y 2E7, Canada

<sup>b</sup> Direction de la Recherche Forestière, Ministère des Forêts, de la Faune et des Parcs du Québec, 2700 Einstein, Québec, QC G1P 3W8, Canada

## ARTICLE INFO

Editorial handling by Prof. M. Kersten

### Keywords:

S deposition

Sulfate

Temperate forests

Stable isotopes

$\delta^{18}\text{O}\text{-SO}_4$

$\delta^{34}\text{S}\text{-SO}_4$

## ABSTRACT

The analysis of sulfate stable isotope ratios ( $\delta^{18}\text{O}\text{-SO}_4$  and  $\delta^{34}\text{S}\text{-SO}_4$ ) in different hydrological compartments of forested catchments has revealed the major role of the humus layer in recycling atmospherically derived sulfur (S). The contribution of the mineral soil to S recycling is still uncertain and may vary among forest types. Here, seasonal variations in  $\text{SO}_4$  concentration,  $\delta^{18}\text{O}\text{-SO}_4$  and  $\delta^{34}\text{S}\text{-SO}_4$  were investigated over a period of two and a half years in precipitation and at various depths in the soil solutions in a temperate forest catchment dominated by sugar maple in southern Québec, Canada.  $\delta^{18}\text{O}\text{-SO}_4$  declined from precipitation (11.8‰) to the humus solution (4.8‰) and to the soil solution beneath the upper B-horizon (1.2‰). No decline was observed below the upper B-horizon. This decline from precipitation to the humus layer reflected a production of secondary sulfate through chemical oxidation of  $\text{SO}_2$  in the canopy, while the decline in the soil resulted from microbial mineralization of organic S. In contrast with findings at other boreal and temperate forest sites, lower  $\delta^{18}\text{O}\text{-SO}_4$  in the upper mineral soil than in the humus layer was indicative of microbial transformations of S not only in the humus layer but also deeper in the upper part of the mineral soil. Significant seasonal variations were found for  $\delta^{18}\text{O}\text{-SO}_4$  in precipitation and in soil solutions beneath the humus and the top mineral horizon, reflecting the influence of both hydrological and microbiological factors. Higher  $\delta^{18}\text{O}\text{-SO}_4$  in the soil solution in fall than in spring and summer resulted from the release of primary sulfate from snow cover in early spring, which was subsequently recycled by soil microorganisms during the growth season, resulting in a higher proportion of secondary sulfate in fall than in spring and summer.

## 1. Introduction

Human activities and natural events such as wildfires and volcanism release large amounts of sulfur (S) to the atmosphere, from which it is constantly removed by both wet and dry deposition. The main form of S in wet deposition is the sulfate ion ( $\text{SO}_4$ ), while dry S deposition is mainly composed of  $\text{SO}_2$  (Lindberg and Lovett, 1992; Marty et al., 2012; Vet et al., 2004). The fate and the effects of S deposited in forested catchments have been extensively studied over the last three decades (Alewell et al., 1999; Campbell et al., 2006; Houle et al., 2014; Houle and Carignan, 1992; Likens et al., 1996, 2002; Marty et al., 2012; Mayer et al., 1995b, 1995a; Mitchell et al., 2001; Mitchell and Likens, 2011). A relatively small fraction of atmospheric S is taken up by trees directly by the canopy in the form of  $\text{SO}_2$  or  $\text{SO}_4$  (Marty et al., 2012), while the fraction reaching the forest floor rapidly goes through immobilization-mineralization cycles by soil microorganisms. A small fraction of soil  $\text{SO}_4$  is taken up by trees through root uptake (Giesemann et al., 1995),

while the remaining fraction accumulates in the soil in the form of  $\text{SO}_4$  or organic S after microbial immobilization. In the absence of lots of mineral surfaces, S is stored in the humus layer. However, at sites with a deep mineral soil, most S is stored in mineral horizons either dissolved or adsorbed to mineral surfaces (Houle and Carignan, 1992; Marty et al., 2011). Smaller amounts of S accumulate in the comparatively thin organic soil (Houle et al., 2014; Marty et al., 2011). Although some dissolved organic S (DOS) is found in soil solutions and drainage outlets, most S outputs from catchments occur in the form of  $\text{SO}_4$  through drainage (Homann et al., 1990; Houle et al., 2014).

Studies analyzing both S and oxygen (O) isotopes in the  $\text{SO}_4$  molecule ( $\delta^{34}\text{S}\text{-SO}_4$  and  $\delta^{18}\text{O}\text{-SO}_4$ , respectively) in several hydrological components of catchments have shown that atmospherically-derived  $\text{SO}_4$  undergoes several chemical and microbiologically mediated transformations before being leached from the catchments, both in the canopy and in the upper soil horizons (Campbell et al., 2006; Houle et al., 2014; Marty et al., 2012). In the absence of bacterial

\* Corresponding author.

E-mail address: [charles.marty@gmail.com](mailto:charles.marty@gmail.com) (C. Marty).

<https://doi.org/10.1016/j.apgeochem.2018.12.006>

Received 6 August 2018; Received in revised form 3 December 2018; Accepted 4 December 2018

Available online 05 December 2018

0883-2927/ Crown Copyright © 2018 Published by Elsevier Ltd. All rights reserved.

dissimilatory sulfate reduction (BDSR), which occurs under anoxic conditions,  $\delta^{34}\text{S}$  is relatively stable during S transformations and can therefore be used to identify S sources within components of the environment (Krouse and Grinenko, 1991; Mayer et al., 2010, 1995b; 1995a; Martin Novák et al., 2001a,b; Novák et al., 2000; Nriagu et al., 1987). In contrast,  $\delta^{18}\text{O}\text{-SO}_4$  shifts during redox reactions because O atoms from surrounding  $\text{H}_2\text{O}$  and  $\text{O}_2$  are incorporated in the  $\text{SO}_4$  molecule. Newly produced  $\text{SO}_4$  from the oxidation of  $\text{SO}_2$  or organic S is called secondary sulfate and is characterized by low  $\delta^{18}\text{O}\text{-SO}_4$  values (Caron et al., 1986; Gélinau et al., 1989; Holt et al., 1982; Jamieson and Wadleigh, 1999; Novák et al., 2007).

As a consequence, temporal or seasonal changes in  $\delta^{18}\text{O}\text{-SO}_4$  and  $\delta^{34}\text{S}\text{-SO}_4$  reflect changes in the magnitude and the loci of S transformation as well as in the source of S (Houle et al., 2014; Kang et al., 2014; Marty et al., 2012; Miles et al., 2012; Zhang et al., 1998). For instance, precipitation  $\delta^{34}\text{S}\text{-SO}_4$  can vary among seasons as a result of biogenic emission of dimethyl sulphide (DMS) to the atmosphere, resulting in lower  $\delta^{34}\text{S}\text{-SO}_4$  in the growing season than in the dormant season (Nriagu et al., 1987; Zhang et al., 1998). Lower precipitation  $\delta^{18}\text{O}\text{-SO}_4$  in winter has also been reported at the Hubbard Brook Experimental Forest as a result of the effect of low temperature on precipitation  $\delta^{18}\text{O}\text{-H}_2\text{O}$  (Miles et al., 2012). Seasonal variations in  $\text{SO}_4$  isotopes have also been reported in the soil solution. At Hubbard Brook, soil solution  $\delta^{34}\text{S}\text{-SO}_4$  was significantly higher during the growth season than in the dormant season (Zhang et al., 1998). At a boreal site in Quebec, humus  $\delta^{18}\text{O}\text{-SO}_4$  was shown to be higher in spring (2.0‰) than in summer (1.1‰) and fall (0.3‰) as a result of snow melt, which released primary  $\text{SO}_4$  (with high  $\delta^{18}\text{O}\text{-SO}_4$ ) in the soil (Houle et al., 2014). In contrast, seasonal variations of both isotopes are generally low in stream or lake water due to the buffering effect of S cycling within the catchments (Miles et al., 2012). Seasonal variations in the mineral soil were also shown to be smaller than in the humus because of larger adsorbed  $\text{SO}_4$  pool, which is in dynamic equilibrium with the soluble phase through rapid adsorption/desorption reactions (Houle et al., 2014; Houle and Carignan, 1995). Low seasonal variation in  $\delta^{18}\text{O}\text{-SO}_4$  in the mineral soil solution could also result from the absence of microbial production of secondary sulfate in this horizon. Recently, a study conducted at two boreal forests of eastern Canada, showed a strong decline in  $\delta^{18}\text{O}\text{-SO}_4$  between the throughfall and the humus layer solution, but no further decrease beneath the humus layer and the mineral soil solution, suggesting that the humus horizon was the only locus of S biochemical transformations in the soil (Houle et al., 2014). However, temperate forests may differ in this respect due to, for instance, the predominance of hardwoods and higher annual soil temperatures, which might lead to higher rates of S microbial transformation deeper in the soil profile.

In the present study,  $\text{SO}_4$  concentrations and isotopic ratios ( $\delta^{34}\text{S}\text{-SO}_4$  and  $\delta^{18}\text{O}\text{-SO}_4$ ) were analyzed in precipitation and soil solutions at several depths in a temperate sugar maple forest in southern Quebec, Canada, between 2003 and 2006. Variation in bulk soil  $\delta^{34}\text{S}$  with soil depth was also measured at six soil pits dug at the proximity of soil lysimeters. The aims of this study were to 1) identify the loci of secondary sulfate production in the soil; and 2) assess seasonal variations in  $\delta^{34}\text{S}\text{-SO}_4$  and  $\delta^{18}\text{O}\text{-SO}_4$  in precipitation and soil solutions, which may reveal variations in S sources and in the intensity and location of S biogeochemical cycling. We hypothesized that in contrast with two boreal forest sites previously studied by Houle et al. (2014): 1) the organic horizon would not be the only locus of S microbial transformation in the soil; 2) seasonal variability in soil solutions' isotopic composition would decrease with soil depth as a result of decreasing microbial activity and rapid equilibration and dilution of secondary  $\text{SO}_4$  within the adsorbed  $\text{SO}_4$  pool; and 3) seasonal variation would be higher for  $\delta^{18}\text{O}\text{-SO}_4$  than for  $\delta^{34}\text{S}\text{-SO}_4$  in all solutions, especially in soil solutions, as a result of climate induced seasonal variation in microbial S recycling rate.

## 2. Material and methods

### 2.1. Studied site

The lake Clair watershed is located approximately 50 km NW of Québec City (46°57'N; 71°40'W) at an elevation ranging from 270 to 390 m a.s.l. The dominant tree species is sugar maple (*Acer saccharum*). Mean annual temperature was 3.58 °C, mean annual precipitation 1338 mm and open field bulk S deposition 6.4 S ha<sup>-1</sup> yr<sup>-1</sup> during the decade from 1999 to 2008 (Marty et al., 2011). The soil has developed from Precambrian charnockitic gneiss covered by sandy till (about 1.3 m depth and mainly composed of gneiss and granite) and are classified as Orthic Humo-Ferric Podzol in the Canadian classification system, which corresponds to a humic cryorthod in the American classification. This podzolic soil has a thin and discontinuous slightly eluviated Ae horizon about 5 cm thick. Below, the B-horizon is a sandy loam with an overall thickness of at least 50 cm (Oumet and Duchesne, 2005). The soil is well-drained and characterized by low pH (~3.1 in LFH and < 4.3 for upper B-horizons) and as well as low inorganic nitrogen concentrations in soil water (Houle and Moore, 2008; Moore and Houle, 2009).

### 2.2. Sampling and laboratory analyses

The lake Clair watershed has been the subject of a research program run by the Quebec government since 1989. Bulk precipitation was sampled weekly using three collectors (2-L plastic bottles fitted with 577 cm<sup>2</sup> plastic funnels) located in an open area at the border of the watershed. During the winter months (November to April, inclusive), bulk deposition collectors were replaced by three 708-cm<sup>2</sup> pails for snow collection. Soil sampling was conducted within a study plot (25 × 50 m) which was established in the catchment to monitor nutrient fluxes and vegetation (Fig. 1). The soil solution percolating from the forest floor was sampled with five zero-tension lysimeters located beneath the humus layer. Within the mineral soils, soil solutions were sampled using two sets of four tension lysimeters located at a depth of 25 cm (Top B) and 73 cm (Low B) beneath the humus layer. All the soil solutions were sampled weekly in the absence of snowpack and frost, i.e., approximately from the beginning of May to the end of October. Precipitation  $\text{SO}_4$  concentration values were weighted by weekly precipitation in order to yield monthly concentration for the January 2003–December 2006 period. Seasons were delimited as follows: spring (April to June), summer (July to September), Fall (October to December) and winter (January to March).

For isotopic analyses, soil water collected in the lysimeters located at the same depth (five and four lysimeters in the humus layer and in the mineral soil, respectively) were pooled. In addition, monthly samples from up to three consecutive months were pooled in order to reduce the cost of analyses and make sure that the minimum mass of S required for isotopic analyses was reached. The resulting pooled sample was given the median date and the corresponding season accordingly. For instance, when solutions collected in September, October and November were pooled, the sample was given October as a sample date and fall as a season.

### 2.3. Soil S storage

Soil samples (LF, H, Ae, Bhf1, Bhf2, Bf, BC) were collected from 20 pits randomly located within a 0.5 ha (50 m × 100 m) surface adjacent to a study plot of the same size equipped with soil lysimeters (Houle et al., 1997). Stainless steel cores were inserted laterally in the distinct horizons to obtain density measurements. The soil samples were air dried and sieved at 2 mm. The soil mass was corrected for coarse fragments contents. The total S concentration was determined with a LECO SC-132 analyzer. Total extractable  $\text{SO}_4$  was obtained by shaking soil samples (5:1 solution:soil ratio) for 1 h with  $\text{NaH}_2\text{PO}_4$  (0.016 M).

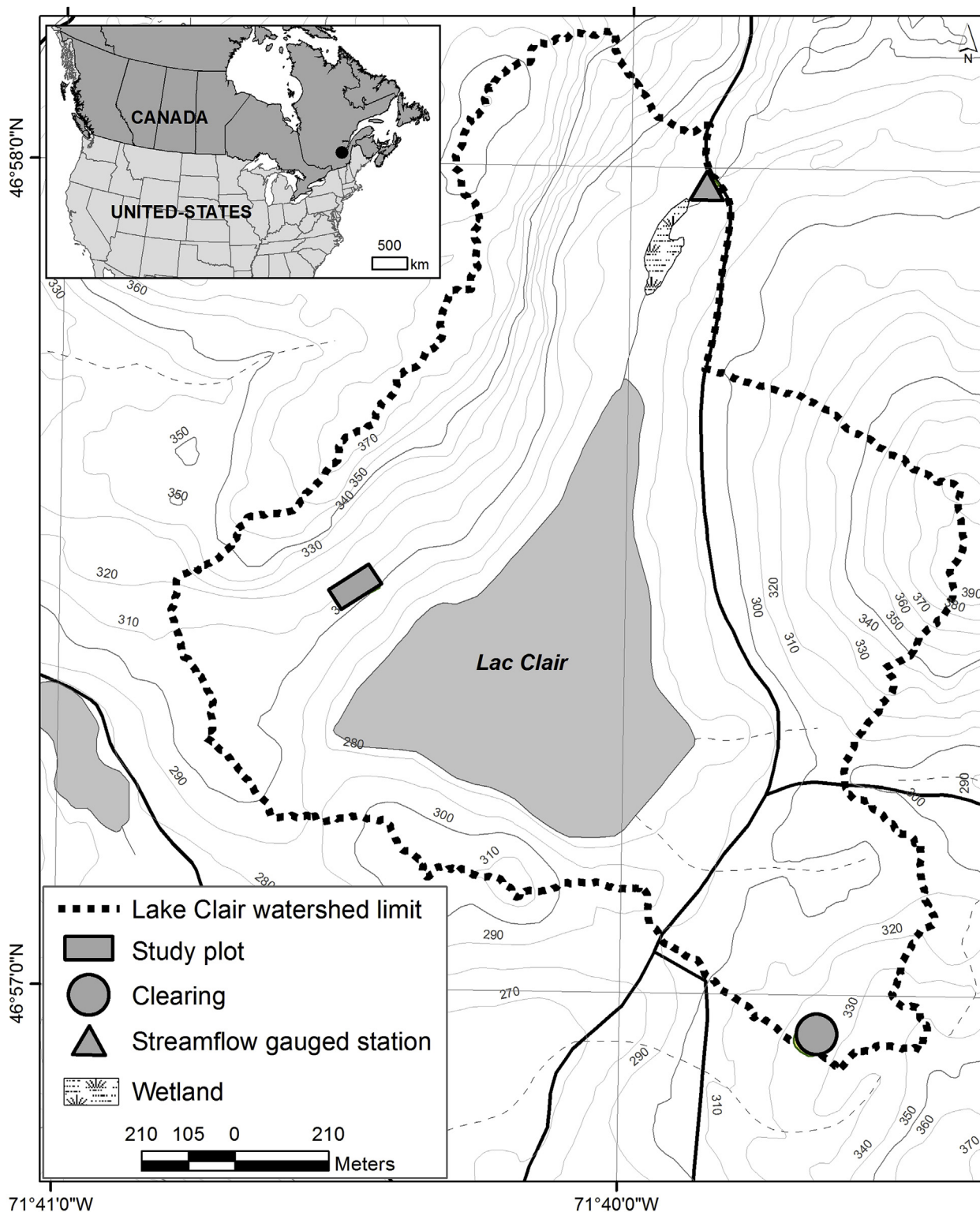


Fig. 1. Map of the Lake Clair watershed, Quebec, Canada.

The extracts were centrifuged (20 m, 21 000 g), filtered (Nuclepore membrane 0.4 mm) and analyzed for SO<sub>4</sub> by ion chromatography. Soil density was multiplied by concentrations to yield S stocks in the different compartments.

2.4. Procedures for isotopic analyses

Isotopic analyses were conducted on bulk precipitation and soil

solution samples collected from January 2003 to December 2006 for δ<sup>18</sup>O-SO<sub>4</sub> and from January 2003 to December 2005 for δ<sup>34</sup>S-SO<sub>4</sub>. After filtration through 0.45-µm membranes (Nuclepore), solutions were eluted through 9 cm high, 2 ml of prepacked analytical grade 1-X8 resin (Poly-Prep® Prefilled Chromatography Columns; Bio-Rad), using a high precision multichannel pump (IP, Ismatec, Glattbrugg, Switzerland) at a flow rate of 6–10 mL min<sup>-1</sup>. The pH of the samples was not modified and the volume of eluted solution (typically between 200 and 500 ml)

was determined according to samples'  $\text{SO}_4$  concentrations so that enough  $\text{SO}_4$  would be collected for isotopic analyses. The cartridges were then stored at 4 °C before being sent to the Isotope Science Lab at the University of Calgary, Alberta, Canada for analysis as described in Campbell et al. (2006). Sulfate ions adsorbed onto the resin were eluted with 15 mL of 3M HCl and precipitated as barium sulfate ( $\text{BaSO}_4$ ) by addition of 1 mL of 0.2 M  $\text{BaCl}_2$  solution. The precipitate was recovered by filtration through a 0.45-mm membrane filter and air-dried at room temperature ( $\sim 24$  °C).

For all soil solution samples, the dried precipitates of  $\text{BaSO}_4$ -S were converted to  $\text{SO}_2$  by high temperature reaction in an elemental analyzer and  $^{34}\text{S}/^{32}\text{S}$  ratios were determined using an isotope ratio mass spectrometer in continuous flow mode (CF-IRMS) (Mayer and Krouse, 2004). Raw  $\delta^{34}\text{S}$  values were normalized to the Vienna Canyon Diablo Troilite (VCDT) scale using as international reference materials NBS-127 (Barium Sulfate) and the following International Atomic Energy Agency materials: IAEA-S1, IAEA-S2, IAEA-S3, IAEAS-O5, IAEAS-O6. The precision of  $\delta^{34}\text{S}$  measurements was generally better than  $\pm 0.25\%$ , based on the daily reproducibility test (one standard deviation of 10 daily lab standards).

The  $^{18}\text{O}/^{16}\text{O}$  ratio of  $\text{BaSO}_4$  was determined using a high temperature reactor coupled to an isotope ratio mass spectrometer in continuous flow mode as described in Campbell et al. (2006). During this procedure,  $\text{BaSO}_4$ -O is quantitatively converted to CO at temperature between 1350 and 1500 °C in a pyrolysis reactor (HEKAtech HT oxygen analyzer) before being swept by a carrier gas into the mass spectrometer. The “raw”  $\delta^{18}\text{O}$  values are drift corrected and normalized using the USGS laboratory information management system (<http://water.usgs.gov/software/LIMS/>). Accuracy and precision of  $\delta^{18}\text{O}$ - $\text{BaSO}_4$  was generally better than  $\pm 0.3\%$  (one standard deviation based on  $n = 50$  lab standards). The resulting  $\delta^{18}\text{O}$  value is the average stable isotopic composition of the four oxygens in the  $\text{BaSO}_4$ .

Stable isotopic compositions were expressed as  $\delta$  values (‰) and calculated as follows:

$$\delta x = [(R_{\text{sample}}/R_{\text{std}}) - 1] \times 1000 \quad (1)$$

$\delta x$  is the isotope ratio in delta unit relative to a standard, and  $R_{\text{sample}}$  and  $R_{\text{std}}$  are the isotope ratios of the sample and standard, respectively. S and O international standards are VCDT and VSMOW (Standard Mean Ocean Water), respectively.

## 2.5. Statistical analyses

Mean  $\delta^{18}\text{O}$ - $\text{SO}_4$  and  $\delta^{34}\text{S}$ - $\text{SO}_4$  values in the different analyzed solutions were compared with a one-way ANOVA followed by a Tukey's HSD test of multiple comparisons of means after the normality of data (Shapiro-Wilk's test) and the homogeneity of variance (Fligner test) were confirmed. When these two conditions were not satisfied, a Kruskal-Wallis rank sum test was performed followed by a post hoc Dunn test.

## 3. Results

### 3.1. Variation in $\text{SO}_4$ concentration among analyzed solutions and seasons

Mean  $\text{SO}_4$  concentrations increased from bulk precipitation ( $1.35 \pm 0.53 \text{ mg L}^{-1}$ ) to the humus solution ( $2.85 \pm 0.32 \text{ mg L}^{-1}$ ), the upper mineral soil solution (Top B;  $3.36 \pm 0.59 \text{ mg L}^{-1}$ ) and the lower mineral soil solution (Low B;  $3.62 \pm 0.25 \text{ mg L}^{-1}$ ) (Fig. 2). Monthly variation was much higher in bulk precipitation ( $\text{CV} = 0.39$ ) than in the soil solutions ( $\text{CV}$  range from 0.07 in the low B horizon to 0.17 in the top B horizon). Concentration in precipitation was significantly lower in winter than in spring and summer ( $0.97 \text{ mg L}^{-1}$  vs.  $1.60 \text{ mg L}^{-1}$ ) (Fig. 3). There was in contrast no significant seasonal variation in the soil solutions.

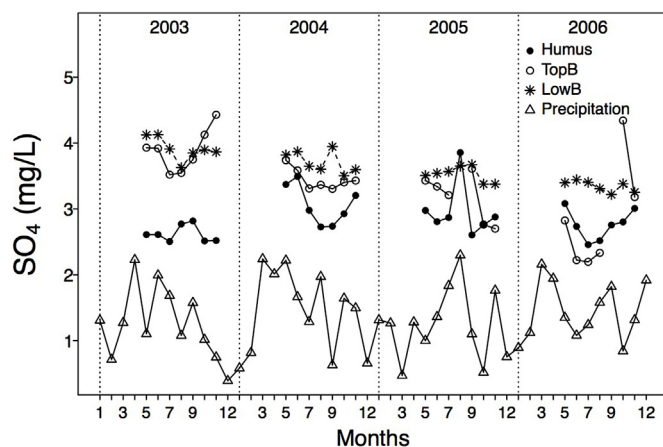


Fig. 2. Variations in  $\text{SO}_4$  concentration ( $\text{mg L}^{-1}$ ) in precipitation and soil solutions at different depths (humus, top B- and low B-horizons) sampled between 2003 and 2006 at the Lake Clair watershed, Quebec, Canada.

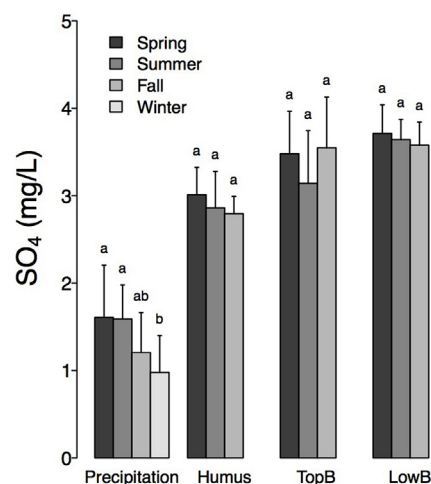


Fig. 3. Seasonal variations in  $\text{SO}_4$  concentration ( $\text{mg L}^{-1}$ ) in precipitation and soil solutions at different depths (humus, top B- and low B-horizons) sampled between 2003 and 2006 at the Lake Clair watershed, Quebec, Canada. Values (mean  $\pm$  SD) not sharing the same letters differ significantly ( $P < 0.05$ ; One-way ANOVA followed by a HSD Tukey test).

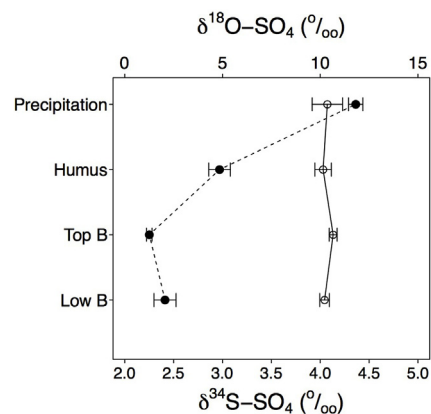


Fig. 4. Variations in  $\delta^{34}\text{S}$ - $\text{SO}_4$  (‰) (open circles) and  $\delta^{18}\text{O}$ - $\text{SO}_4$  (‰) (black circles) among the different sampled solutions (precipitation, humus, top B- and low B-horizons) at the Lake Clair watershed, Quebec, Canada. Values (mean  $\pm$  SE) not sharing the same letters differ significantly ( $P < 0.05$ ; One-way ANOVA followed by a HSD Tukey test).

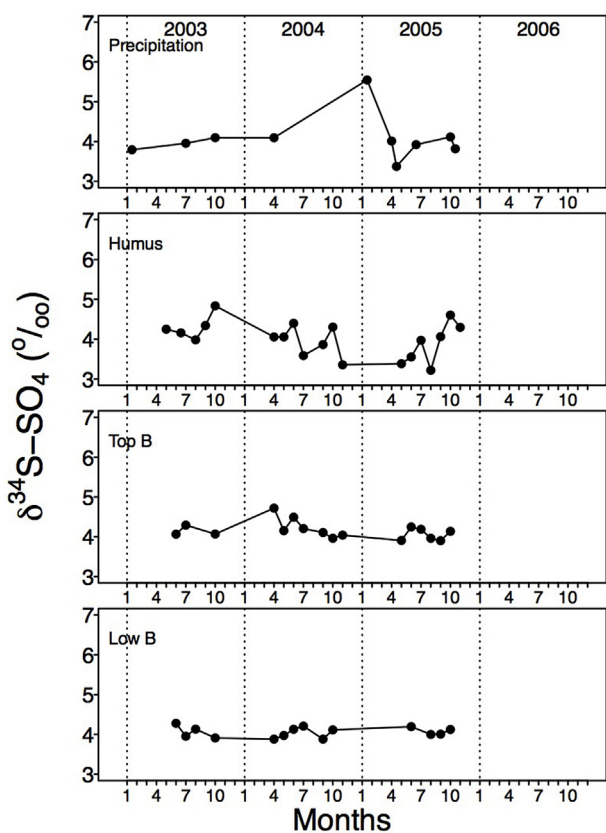


Fig. 5. Variations in  $\delta^{34}\text{S-SO}_4$  (‰) in the different sampled solutions (precipitation, humus, top B- and low B-horizons) between 2003 and 2005 at the Lake Clair watershed, Quebec, Canada.

### 3.2. Variation in $\text{SO}_4$ isotopic values among analyzed solutions

There was no difference in  $\delta^{34}\text{S-SO}_4$  values between precipitation ( $4.1 \pm 0.6\text{‰}$ ) and the soil solutions sampled beneath the humus layer and within the mineral soil (range of 4.0–4.1‰) (Fig. 4). In contrast, there was a first steep decline in  $\delta^{18}\text{O-SO}_4$  from precipitation ( $11.8 \pm 1.3\text{‰}$ ) to the humus layer solution ( $4.8 \pm 2.8\text{‰}$ ) and a second decline from the humus to beneath the upper B-horizon (Top B;  $1.2 \pm 0.7\text{‰}$ ) (Fig. 4).  $\delta^{18}\text{O-SO}_4$  values in the solution beneath the lower B-horizon (Low B;  $2.1 \pm 2.2\text{‰}$ ) were not significantly different from the value obtained beneath the upper B-horizon.

### 3.3. Seasonal variations in $\text{SO}_4$ isotopic ratios

Temporal variation in  $\delta^{34}\text{S-SO}_4$  over the studied period was smaller in the mineral soil solutions (coefficient of variation:  $\text{CV} < 5\%$ ) than in precipitation and in the humus layer ( $\text{CV} > 10\%$ ) (Fig. 5). Temporal variation in  $\delta^{18}\text{O-SO}_4$  was higher than temporal variation in  $\delta^{34}\text{S-SO}_4$  in all sampled solutions (Fig. 6). However, the variation was much lower in precipitation ( $\text{CV} < 20\%$ ) than in soil solutions ( $\text{CV} > 50\%$ ), especially in the humus and Top B horizon.

There was no seasonal variation in  $\delta^{34}\text{S-SO}_4$  for both precipitation and soil solutions (Fig. 7; top panel). In contrast, there was a significant  $\delta^{18}\text{O-SO}_4$  seasonal variation in precipitation and soil solutions beneath the humus and in the Top B horizon. In precipitation,  $\delta^{18}\text{O-SO}_4$  was significantly lower in winter ( $9.7 \pm 1.3\text{‰}$ ) than for the remainder of the year ( $> 12.0\text{‰}$ ). In the soil solutions beneath the humus and in the Top B horizon,  $\delta^{18}\text{O-SO}_4$  values were significantly lower in fall ( $3.3 \pm 2.5\text{‰}$  and  $0.7 \pm 0.6\text{‰}$ , respectively) than in spring and summer ( $> 5.7\text{‰}$  and  $> 1.6\text{‰}$ , respectively). In contrast, no seasonal variation was found for the soil solution in the low B horizon

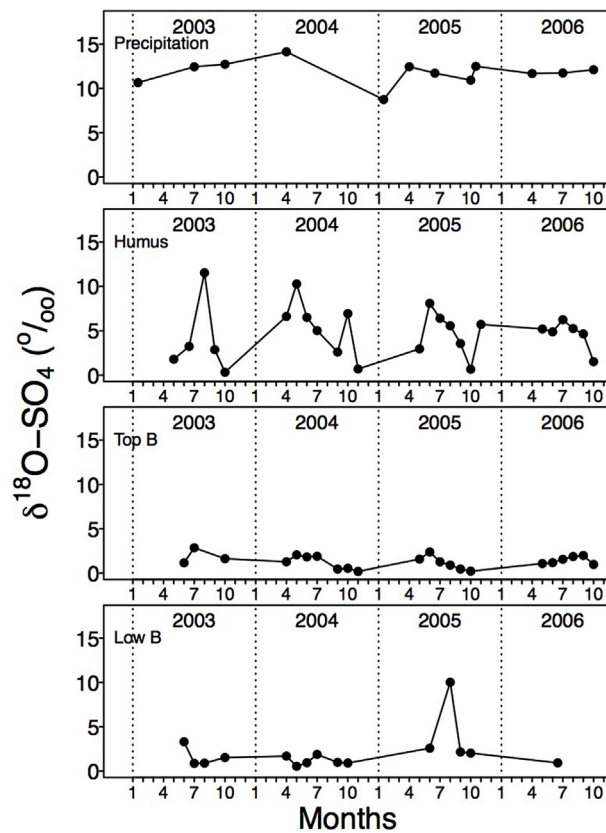


Fig. 6. Variations in  $\delta^{18}\text{O-SO}_4$  (‰) in the different sampled solutions (precipitation, humus, top B- and low B-horizons) between 2003 and 2006 at the Lake Clair watershed, Quebec, Canada.

( $P > 0.05$ ).

### 3.4. Soil S storage

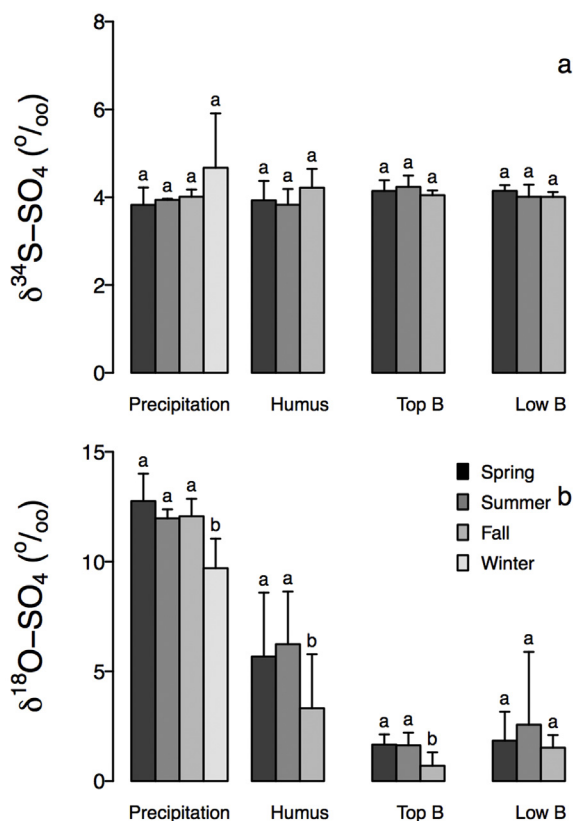
The soil in the catchment stored on average  $1455 \text{ kg S ha}^{-1}$ , mostly in the mineral horizons (Fig. 8). About 87% of the S stored in the soil was present as organic S ( $1271 \text{ kg ha}^{-1}$ ) vs. 13% ( $184 \text{ kg ha}^{-1}$ ) as sulfate. Other measurements have shown that approximately 80% of the S- $\text{SO}_4$  reservoir was adsorbed to mineral surfaces vs. 20% in the soil solution (data not shown).

## 4. Discussion

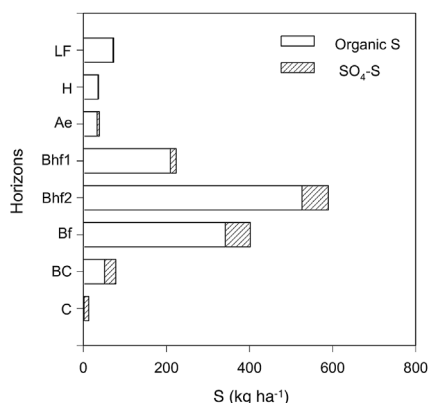
### 4.1. $\text{SO}_4$ sources in the catchment

Sulfate concentration more than doubled from precipitation ( $1.35 \pm 0.53 \text{ mg L}^{-1}$ ) to the humus layer ( $2.85 \pm 0.32 \text{ mg L}^{-1}$ ) and then slightly increased with soil depth to reach a maximum average value of  $3.62 \pm 0.25 \text{ mg L}^{-1}$  in the lower B-horizon. Precipitation  $\text{SO}_4$  concentration was in the range of those observed by Houle et al. (2014) at two boreal sites in Quebec between 1999 and 2008 ( $\sim 1 \text{ mg L}^{-1}$ ). In contrast,  $\text{SO}_4$  concentrations in soil solutions were slightly higher at the present site ( $3.4\text{--}3.7 \text{ mg L}^{-1}$  vs.  $1.0\text{--}3.1 \text{ mg L}^{-1}$ ), reflecting higher S atmospheric inputs over the last decades than at the two boreal sites (Marty et al., 2011). Nevertheless,  $\text{SO}_4$  concentrations in soil solutions were low compared to other eastern North American sites. For instance, soil solution  $\text{SO}_4$  concentrations collected in lysimeters were found to range from 75 to  $109 \mu\text{mol L}^{-1}$  (corresponding to  $7.2\text{--}10.5 \text{ mg L}^{-1}$ ) in uplands to  $24\text{--}53 \mu\text{mol L}^{-1}$  (corresponding to  $2.3\text{--}5.1 \text{ mg L}^{-1}$ ) in wetlands in the Adirondacks (Mitchell et al., 2006).

Precipitation  $\delta^{34}\text{S-SO}_4$  values ( $4.1 \pm 0.6\text{‰}$ ) were similar to those



**Fig. 7.** Seasonal variations in  $\delta^{34}\text{S-SO}_4$  (top panel) and  $\delta^{18}\text{O-SO}_4$  (bottom panel) values in precipitation and the soil solutions at different depths (humus, top B- and low B-horizons). Values (mean  $\pm$  SD) not sharing the same letters differ significantly ( $P < 0.05$ ; One-way ANOVA followed by a HSD Tukey test). See Fig. 1 for the legend.



**Fig. 8.** Organic S and SO<sub>4</sub>-S reservoirs in the different horizons of the lake Clair watershed.

reported for two previously studied boreal forests in Quebec (3.5–4.0‰; Marty et al., 2012), but slightly lower than those reported for the Hubbard Brook Experimental Forest by Zhang et al. (1998) (5.0–5.9‰) and by Campbell et al. (2006) (4.6–5.3‰). Differences among sites can result from the contribution of marine influences (Novák et al., 2001a,b), soil biogenic emissions such as dimethyl sulphide (Nriagu et al., 1987) and various local or remote pollution sources (Wadleigh and Blake, 1999). The absence of significant difference in  $\delta^{34}\text{S-SO}_4$  between precipitation and all sampled soil solutions (4.0–4.1‰) is consistent with the absence of lithogenic source of S at this site (Houle and Carignan, 1995), and confirms that precipitation is the main source of S in the catchment. This contrasts with other sites

where  $\delta^{34}\text{S-SO}_4$  was found to be lower in stream water than in precipitation due to mineral weathering of sulphide minerals such as pyrite (Campbell et al., 2006). It also confirms that the soil is well drained at the site, preventing dissimilatory SO<sub>4</sub> reduction, which causes <sup>34</sup>S enrichment in soil water SO<sub>4</sub> (Alewell and Novak, 2001; Eimers et al., 2004).

SO<sub>4</sub> represented a small fraction of total S in both organic and mineral horizons and < 15% of total S storage in the soil. In addition, most SO<sub>4</sub> was adsorbed to mineral surfaces. This adsorbed S-SO<sub>4</sub> pool was estimated at 150 kg ha<sup>-1</sup> in the catchment, which is much higher than annual S-SO<sub>4</sub> deposition, averaging ~5 kg S-SO<sub>4</sub> ha<sup>-1</sup> yr<sup>-1</sup> in the last decade (data not shown). Therefore, the adsorbed S-SO<sub>4</sub> reservoir, due to the high rate of exchange between the soluble and adsorbed compartments, has the potential to buffer S-SO<sub>4</sub> concentration variations in the soil solution and in S-SO<sub>4</sub> exports from the catchment in response to changes in atmospheric deposition S-SO<sub>4</sub> rates. This buffering power explained the low seasonal variations in SO<sub>4</sub> concentrations in the soil solution (lysimeters) in the B-horizons.

#### 4.2. SO<sub>4</sub> transformation in the catchment

There was a large decline in  $\delta^{18}\text{O-SO}_4$  between precipitation and the B-horizons ( $\Delta\delta^{18}\text{O-SO}_4 = -9.7\text{‰}$  to  $-10.6\text{‰}$ ). Declines in the same order of magnitude have been reported in North American forested catchments (Campbell et al., 2006; Caron et al., 1986; Houle et al., 2014; Shanley et al., 2005). This decline occurred in two steps: from precipitation to the humus solution ( $\Delta\delta^{18}\text{O-SO}_4 = -7\text{‰}$ ), and from the humus solution to the solution beneath the upper B-horizon ( $\Delta\delta^{18}\text{O-SO}_4 = -3.6\text{‰}$ ). Mechanisms causing this decrease in  $\delta^{18}\text{O-SO}_4$  have been well described (Caron et al., 1986; Gélinau et al., 1989; Holt et al., 1982; Jamieson and Wadleigh, 1999; Novák et al., 2007). Several studies have shown that the decrease between precipitation and the humus solution results from two processes: 1) a chemical production of secondary SO<sub>4</sub> with low  $\delta^{18}\text{O-SO}_4$  from gaseous SO<sub>2</sub> and atmospheric water within the canopy (Houle et al., 2014; Marty et al., 2012); and 2) from a biochemical production of secondary SO<sub>4</sub> resulting from the mineralization of soil organic matter by microorganisms in the humus (Campbell et al., 2006; Houle et al., 2014; Mayer et al., 1995b, 1995a), which incorporates O atoms from surrounding water with very negative  $\delta^{18}\text{O}$  ( $\delta^{18}\text{O-H}_2\text{O}$  in precipitation and soil water averaged  $-10.1\text{‰}$  at the site) and atmospheric gaseous oxygen into newly produced SO<sub>4</sub> molecules. In this study, throughfall was not collected, preventing us from estimating the relative magnitude of these two mechanisms. However, previous studies in the region have shown that the oxidation of SO<sub>2</sub> within the canopy could result in decreases in precipitation  $\delta^{18}\text{O-SO}_4$  by up to ~5‰ (Houle et al., 2014; Marty et al., 2012).

The second decrease in  $\delta^{18}\text{O-SO}_4$  (i.e., between the humus solution and the upper mineral soil solution) supports our hypothesis of secondary SO<sub>4</sub> production at the top of the mineral soil at this temperate site, which resulted from the mineralization of organic S by soil microorganisms. Similar decreases in  $\delta^{18}\text{O-SO}_4$  have been observed in the mineral soil solutions at other temperate forest sites. Mayer et al. (1995a) have for instance found decreases of 4.5–6.3‰ within the 20–30 cm uppermost of soil profiles in Germany. In contrast,  $\delta^{18}\text{O-SO}_4$  was shown to reach a minimum value in the humus layer at two boreal sites located nearby, indicating that S was not processed by soil microorganisms in the mineral soil (Houle et al., 2014). Similarly, Campbell et al. (2006) have shown that  $\delta^{18}\text{O-SO}_4$  decreased by ~12‰ from throughfall water to soil water at a depth of 10 cm and did not further decrease with soil depth in the Adirondack Mountains. Recently, the percentage of deciduous trees in forest sites was shown to be associated with a larger C isotope enrichment factor with soil depth, suggesting a positive effect of deciduous trees on the rate and the depth of SOM mineralization (Marty et al., 2015a). Therefore, deeper microbial production of SO<sub>4</sub> at the temperate site than at the boreal sites may partly result from the predominance of deciduous trees at the

temperate site. In the studied region, the percentage of deciduous trees is also associated with higher mean annual temperature (Marty et al., 2015a, 2015b), which may also have a positive impact on microbial activity deeper in the soil profile.

#### 4.3. Seasonal variations in sulfate isotopic signatures and concentrations

As hypothesized, temporal variation was much higher for  $\delta^{18}\text{O}\text{-SO}_4$  than for  $\delta^{34}\text{S}\text{-SO}_4$ , especially in the soil solutions. The lack of strong seasonal variation in precipitation  $\delta^{34}\text{S}\text{-SO}_4$  is consistent with what was found at two boreal forest sites in Québec (Marty et al., 2012) but contrasts with other studies that found lower  $\delta^{34}\text{S}\text{-SO}_4$  in spring and summer precipitation as a result of S biogenic emission of dimethyl sulphide (Nriagu et al., 1987; Zhang et al., 1998). Precipitation  $\text{SO}_4$  concentration was higher in spring and summer than in winter, suggesting that S biogenic emissions may nevertheless occur at the site.

Although there was some temporal variability in both humus solution  $\text{SO}_4$  concentration and  $\delta^{34}\text{S}\text{-SO}_4$  (CV > 10%), this variation was not associated to seasonal changes. The statistically insignificant seasonal variation in soil solution  $\delta^{34}\text{S}\text{-SO}_4$  contrasts with findings from Hubbard Brook where higher values were found during the growing season (4.0–8.9‰) than during the dormant season (3.4–5.6‰), presumably due to differences in biological activity (Zhang et al., 1998).

In contrast with  $\delta^{34}\text{S}\text{-SO}_4$ , significant seasonal variations were observed for  $\delta^{18}\text{O}\text{-SO}_4$  in precipitation on the one hand, and humus and upper mineral soil solutions on the other hand. Such seasonal variability in the soil solutions was expected, at least for the humus layer, since it reflects changes in microbial activity, which is temperature and moisture dependent. However, in contrast with our expectations, isotopic values were lower in fall than in spring and summer. This pattern may result from two mechanisms. First, higher  $\delta^{18}\text{O}\text{-SO}_4$  values in spring and summer may partly result from the influence of snow. The snow cover accumulates large amounts of atmospheric  $\text{SO}_4$  characterized by higher  $\delta^{18}\text{O}\text{-SO}_4$  (Fig. 7), which may dilute low- $\delta^{18}\text{O}$  microbially-produced secondary  $\text{SO}_4$  as snow melts in early spring. This effect may last for a few months until snow  $\text{SO}_4$  is progressively replaced by microbially produced secondary  $\text{SO}_4$  characterized by lower  $\delta^{18}\text{O}\text{-SO}_4$ . Second, vertical movements and adsorption/desorption mechanisms onto mineral surfaces might delay the capture of newly produced secondary  $\text{SO}_4$  by the lysimeters. Small temporal variations of  $\delta^{18}\text{O}\text{-SO}_4$  in the lower B-horizon soil solution is consistent with recent findings at a boreal site in Québec (Houle et al., 2014) and supports the absence of major secondary  $\text{SO}_4$  production deep in the mineral soil.

#### 5. Conclusion

Sulfate concentration more than doubled from precipitation to the low B horizon. This increase in  $\text{SO}_4$  concentration was accompanied by a decrease in  $\delta^{18}\text{O}\text{-SO}_4$ , which resulted from the chemical production of  $\text{SO}_4$  from  $\text{SO}_2$  in the canopy and from the mineralization of organic S into  $\text{SO}_4$  in the soil. In contrast with other studies, our data show evidence of microbial production of  $\text{SO}_4$  not only in the humus layer but also in the upper 25 cm of the mineral horizon. Large temporal and seasonal variations in  $\delta^{18}\text{O}\text{-SO}_4$  in these two soil solutions reflect the influence of hydrological processes as well as variation in microbial S recycling activity with soil hydro-climatic conditions. In both solution types,  $\delta^{18}\text{O}\text{-SO}_4$  was higher in spring and summer than in fall. This probably resulted from the release of primary sulfate with high  $\delta^{18}\text{O}\text{-SO}_4$  from the snow cover in early spring, which subsequently went through microbial transformations (immobilization and mineralization) during the growth season, resulting in higher proportion of secondary  $\text{SO}_4$  in fall.

#### Acknowledgment

This research was funded by the Clean Air Regulation Act (CARA),

Charles Marty wishes to acknowledge the Natural Sciences and Engineering Research Council of Canada for the award of a post-doctoral fellowship. We also thank the field technicians for sample collection and the laboratory staff of the Ministry of Forests, Wild life, and Parks.

#### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apgeochem.2018.12.006>.

#### References

- Alewel, C., Mitchell, M.J., Likens, G.E., Krouse, H.R., 1999. Sources of stream sulfate at the Hubbard Brook Experimental Forest: long-term analyses using stable isotopes. *Biogeochemistry* 44, 281–299. <https://doi.org/10.1007/BF00996994>.
- Alewel, C., Novak, M., 2001. Spotting zones of dissimilatory sulfate reduction in a forested catchment: the 34S-35S approach. *Environ. Pollut.* 112, 369–377.
- Campbell, J.L., Mitchell, M.J., Mayer, B., 2006. Isotopic assessment of NO<sub>3</sub>- and SO<sub>4</sub>-mobility during winter in two adjacent watersheds in the Adirondack Mountains, New York. *J. Geophys. Res.* 111. <https://doi.org/10.1029/2006jg000208>.
- Caron, F., Tessier, A., Kramer, J.R., Schwarcz, H.P., Rees, C.E., 1986. Sulfur and oxygen isotopes of sulfate in precipitation and lakewater, Quebec, Canada. *Appl. geochemistry* 1, 601–606.
- Eimers, M., Dillon, P., Schiff, S., 2004. A S-isotope approach to determine the relative contribution of redox processes to net SO export from upland, and wetland-dominated catchments. *Geochem. Cosmochim. Acta* 68, 3665–3674.
- Gélineau, M., Carignan, R., Tessier, A., 1989. Study of the transit of sulfate in a Canadian Shield lake watershed with stable oxygen isotope ratios. *Appl. geochemistry* 4, 195–201.
- Giesemann, A., Jäger, H.-J., Feger, K.H., 1995. Evaluation of sulphur cycling in managed forest stands by means of stable S-isotope analysis. In: Nilsson, L., Hüttel, R., Johansson, U. (Eds.), *Nutrient Uptake and Cycling in Forest Ecosystems: Proceedings of the CEC/IUFRO Symposium Nutrient Uptake and Cycling in Forest Ecosystems Halmstad, Sweden, June, 7–10, 1993*. Springer Netherlands., Dordrecht, The Netherlands, pp. 399–404.
- Holt, B.D., Kumar, R., Cunningham, P.T., 1982. Primary sulfates in atmospheric sulfates: estimation by oxygen isotope ratio measurements. *Science* 217, 51–53 (80- ).
- Homann, P.S., Mitchell, M.J., Van Miegroet, H., Cole, D.W., 1990. Organic sulfur in throughfall, stem flow, and soil solutions from temperate forests. *Can. J. For. Res.* 20, 1535–1539.
- Houle, D., Carignan, R., 1995. Role of SO<sub>4</sub> adsorption and desorption in the long-term S budget of a coniferous catchment on the Canadian Shield. *Biogeochemistry* 28, 161–182.
- Houle, D., Carignan, R., 1992. Sulfur speciation and distribution in soils and aboveground biomass of a boreal coniferous forest. *Biogeochemistry* 16, 63–82.
- Houle, D., Marty, C., Duchesne, L., Gagnon, C., 2014. Humus layer is the main locus of secondary SO<sub>4</sub> production in boreal forests. *Geochem. Cosmochim. Acta* 126, 18–29. <https://doi.org/10.1016/j.gca.2013.10.038>.
- Houle, D., Moore, J.D., 2008. Soil solution, foliar concentrations and tree growth response to 3-year of ammonium-nitrate addition in two boreal forests of Québec, Canada. *For. Ecol. Manage.* 255, 2049–2060. <https://doi.org/10.1016/j.foreco.2007.10.056>.
- Houle, D., Paquin, R., Camire, C., Ouimet, R., Duchesne, L., 1997. Response of the lake Clair watershed (Duchesnay, Quebec) to changes in precipitation chemistry (1988–1994). *Can. J. For. Res. Can. Rech. For.* 27, 1813–1821.
- Jamieson, R.E.E., Wadleigh, M.E.A., 1999. A study of oxygen isotopic composition of precipitation sulphate in eastern Newfoundland. *Water Air Soil Pollut.* 110, 405–420.
- Kang, P.G., Mitchell, M.J., Mayer, B., Campbell, J.L., 2014. Isotopic evidence for determining the sources of dissolved organic sulfur in a forested catchment. *Environ. Sci. Technol.* 48, 11259–11267. <https://doi.org/10.1021/es502563n>.
- Krouse, H.R., Grinenko, L.N., 1991. Stable Isotopes: Natural and Anthropogenic Sulphur in the Environment-SCOPE 43. John Wiley & Sons, Chichester.
- Likens, G.E., Driscoll, C.T., Buso, D.C., 1996. Long-Term effects of acid Rain: response and recovery of a forest ecosystem. *Science* 244–246 (80- ). 1940.
- Likens, G.E., Driscoll, C.T., Buso, D.C., Mitchell, M.J., Lovett, G.M., Bailey, S.W., Siccamo, T.G., Reiners, W.A., Alewell, C., 2002. The biogeochemistry of sulfur at Hubbard Brook. *Biogeochemistry* 60, 235–316.
- Lindberg, S.E., Lovett, G., 1992. Deposition and forest canopy interactions of airborne sulfur- Results from the integrated forest study. *Atmos. Environ. PART A-GENERAL Top.* 26, 1477–1492.
- Marty, C., Houle, D., Duchesne, L., Gagnon, C., 2012. Canopy interaction with precipitation and sulphur deposition in two boreal forests of Quebec, Canada. *Environ. Pollut.* 162, 354–360. <https://doi.org/10.1016/j.envpol.2011.12.007>.
- Marty, C., Houle, D., Gagnon, C., 2015a. Effect of the relative abundance of conifers vs. hardwoods on soil d13C enrichment with soil depth in eastern Canadian forests. *Ecosystems* 18, 629–642.
- Marty, C., Houle, D., Gagnon, C., 2015b. Variation in stocks and distribution of organic C in soils across 21 eastern Canadian temperate and boreal forests. *For. Ecol. Manage.* 345, 29–38. <https://doi.org/10.1016/j.foreco.2015.02.024>.
- Marty, C., Houle, D., Gagnon, C., Duchesne, L., 2011. Isotopic compositions of S, N and C in soils and vegetation of three forest types in Quebec, Canada. *Appl. Geochemistry* 26, 2181–2190.

- Mayer, B., Feger, K.H., Giesemann, A., Jäger, H.J., 1995a. Interpretation of sulfur cycling in 2 catchments in the Black-Forest (Germany) using stable sulfur and oxygen-isotope data. *Biogeochemistry* 30, 31–58.
- Mayer, B., Fritz, P., Prietzel, J., Krouse, H., 1995b. The use of stable sulfur and oxygen isotope ratios for interpreting the mobility of sulfate in aerobic forest soils. *Appl. Geochem.* 10, 161–173.
- Mayer, B., Krouse, H.R., 2004. Procedures for sulfur abundance studies. In: In: De Groot, P.A. (Ed.), *Handbook of Stable Isotope Analytical Techniques 1* Elsevier, Amsterdam.
- Mayer, B., Shanley, J.B., Bailey, S.W., Mitchell, M.J., 2010. Identifying sources of stream water sulfate after a summer drought in the Sleepers River watershed (Vermont, USA) using hydrological, chemical, and isotopic techniques. *Appl. Geochem.* 25, 747–754. <https://doi.org/10.1016/j.apgeochem.2010.02.007>.
- Miles, G.R., Mitchell, M.J., Mayer, B., Likens, G., Welker, J., 2012. Long-term analysis of Hubbard Brook stable oxygen isotope ratios of streamwater and precipitation sulfate. *Biogeochemistry* 111, 443–454. <https://doi.org/10.1007/s10533-011-9670-3>.
- Mitchell, M.J., Likens, G.E., 2011. Watershed sulfur biogeochemistry: shift from atmospheric deposition dominance to climatic regulation. *Environ. Sci. Technol.* 45, 5267–5271.
- Mitchell, M.J., Mayer, B., Bailey, S.W., Hornbeck, J.W., Alewell, C., Driscoll, C.T., Likens, G.E., 2001. Use of stable isotope ratios for evaluating sulfur sources and losses at the Hubbard Brook Experimental Forest. *Water Air Soil Pollut.* 130, 75–86.
- Mitchell, M.J., Piatek, K.B., Christopher, S., Mayer, B., Kendall, C., Mchale, P., 2006. Solute sources in stream water during consecutive fall storms in a Northern Hardwood forest watershed: a combined hydrological, chemical and isotopic approach. *Biogeochemistry* 78, 217–246.
- Moore, J.D., Houle, D., 2009. Soil solution and sugar maple response to NH<sub>4</sub>NO<sub>3</sub> additions in a base-poor northern hardwood forest of Québec, Canada. *Environ. Monit. Assess.* 155, 177–190. <https://doi.org/10.1007/s10661-008-0427-y>.
- Novák, M., Bottrell, S.H., Prechová, E., 2001a. Sulfur isotope inventories of atmospheric deposition, spruce forest floor and living Sphagnum along a NW–SE transect across Europe. *Biogeochemistry* 53, 23–50.
- Novák, M., Jacková, I., Prechová, E., 2001b. Temporal trends in the isotope signature of air-borne sulfur in Central Europe. *Environ. Sci. Technol.* 35, 255–260.
- Novák, M., Kirchner, J.W., Groscheová, H., Havel, M., Cerny, J., Krejci, R., Buzek, F., 2000. Sulfur isotope dynamics in two Central European watersheds affected by high atmospheric deposition of SO<sub>x</sub>. *Geochem. Cosmochim. Acta* 64, 367–383.
- Novák, M., Mitchell, M.J., Jacková, I., Buzek, F., Schweigstillová, J., Erbanová, L., Prikryl, R., Fottová, D., 2007. Processes affecting oxygen isotope ratios of atmospheric and ecosystem sulfate in two contrasting forest catchments in Central Europe. *Environ. Sci. Technol.* 41, 703–709.
- Nriagu, J.O., Holdway, D.A., Coker, R.D., 1987. Biogenic sulfur and the acidity of rainfall in remote areas of Canada. *Science* 237, 1189–1192.
- Quimet, R., Duchesne, L., 2005. Base cation mineral weathering and total release rates from soils in three calibrated forest watersheds on the Canadian Boreal Shield. *Can. J. Soil Sci.* 85, 245–260.
- Shanley, J.B., Mayer, B., Mitchell, M.J., Michel, R.L., Bailey, S.W., Kendall, C., 2005. Tracing sources of streamwater sulfate during snowmelt using S and O isotope ratios of sulfate and 35S activity. *Biogeochemistry* 76, 161–185.
- Vet, R., Brook, J., Ro, C., Shaw, M., Narayan, J., Zhang, L., Moran, M., Lusia, M., 2004. Atmospheric response to past emission control programs. In: *Canadian Acid Deposition Science Assessment*. Environment Canada.
- Wadleigh, M.A., Blake, D.M., 1999. Tracing sources of atmospheric sulphur using epiphytic lichens. *Environ. Pollut.* 106, 265–271.
- Zhang, Y., Mitchell, M.J., Christ, M., Likens, G.E., Krouse, H.R.O.Y., 1998. Stable sulfur isotopic biogeochemistry of the Hubbard Brook experimental forest, New Hampshire. *Biogeochemistry* 41, 259–275.