

Isotopic Evidence for Determining the Sources of Dissolved Organic Sulfur in a Forested Catchment

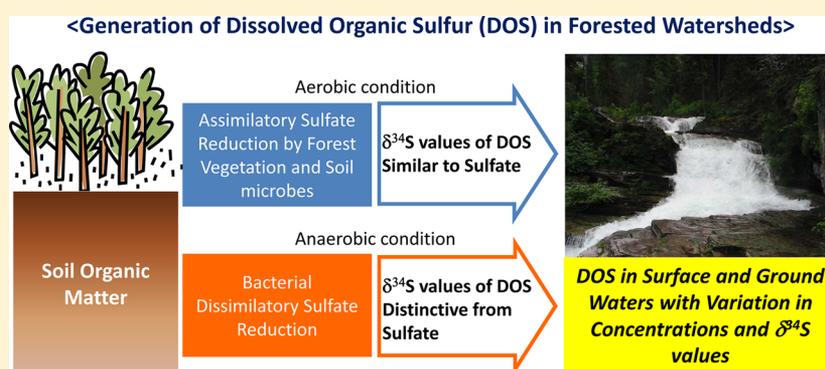
Phil-Goo Kang,^{*,†,‡} Myron J. Mitchell,[†] Bernhard Mayer,[§] and John L. Campbell^{||}

[†]Department of Environmental and Forest Biology, State University of New York-College of Environmental Science and Forestry (SUNY-ESF), Syracuse, New York 13210, United States

[‡]Research Strategy & Planning Division, National Institute of Environmental Research, 42 Hwangyong-ro, Seo-gu, Incheon 404-708, Republic of Korea

[§]Department of Geoscience, University of Calgary, Calgary, Alberta Canada T2N 1N4

^{||}U.S. Forest Service, Northern Research Station, 271 Mast Road, Durham, New Hampshire 03824, United States



ABSTRACT: Understanding sulfur (S) biogeochemistry, especially in those watersheds subject to elevated levels of atmospheric S inputs, is needed for determining the factors that contribute to acidification, nutrient losses and the mobilization of toxic solutes (e.g., monomeric aluminum and methylmercury). S is found in a variety of both organic and inorganic forms undergoing a range of biotic and abiotic transformations. In watersheds with decreasing atmospheric S inputs, internal cycling is becoming dominant in affecting whether there is net loss or retention of S. Little attention has been given to the role of dissolved organic S (DOS) in affecting S biogeochemistry. DOS originates from assimilatory and bacterial dissimilatory S reduction (BDSR), the latter of which produces ^{34}S -depleted S. Within groundwater of the Archer Creek Catchment in the Adirondack Mountains (New York) there was reoxidation of reduced S, which was an important source of SO_4^{2-} . DOS in surface waters had a higher variation of $\delta^{34}\text{S}$ -DOS values (-6.0 to $+8.4\text{‰}$) than inorganic S with $\delta^{34}\text{S}$ - SO_4^{2-} values ranging from $+1.0$ to $+5.8\text{‰}$. Inverse correlations between $\delta^{34}\text{S}$ values of SO_4^{2-} and DOS suggested that BDSR played an important role in producing DOS.

INTRODUCTION

The role of sulfur (S) biogeochemistry has received considerable attention due to its dominant role in contributing to “acid rain” from atmospheric deposition of S compounds. Sulfur inputs to watersheds have resulted in acidification of soils and surface waters, the mobilization of toxic metals (e.g., monomeric aluminum and methylmercury) and other chemical changes in these systems.¹ Recently there have been marked declines in S deposition in North America² and Europe³ and hence internal watershed processes are playing a critical role in S mass budgets including determining whether there is a net loss or retention of S. One factor linked to S concentrations and mass balances in forested watersheds is associated with changing moisture conditions that can affect a variety of biogeochemical processes including redox changes⁴ and the mobilization of S from the soil.⁵ A high loss of aqueous SO_4^{2-} from forested ecosystems in the northeastern U.S. and Europe has been observed and one of the possible reasons is the

mineralization of organic S to inorganic SO_4^{2-} .⁶ Most of the S in ecosystems including forests,⁷ wetlands,⁸ and grasslands⁹ is found in solid organic matter. However, another important form of organic S is dissolved organic S (DOS), which comprises a substantial portion of the total dissolved S in forest throughfall and soil water¹⁰ and surface waters.¹¹ There has also been increased interest in the S dynamics of watersheds because of the importance of dissimilatory SO_4^{2-} -reducing bacteria¹² and the formation of methylmercury.¹³ In forested ecosystems, there are two mechanisms for the generation of DOS: the release and hydrolysis from S-containing organic matter in plants and soils that was formed via assimilatory SO_4^{2-} -reduction and the incorporation of S produced via anaerobic

Received: May 26, 2014

Revised: September 10, 2014

Accepted: September 10, 2014

Published: September 11, 2014

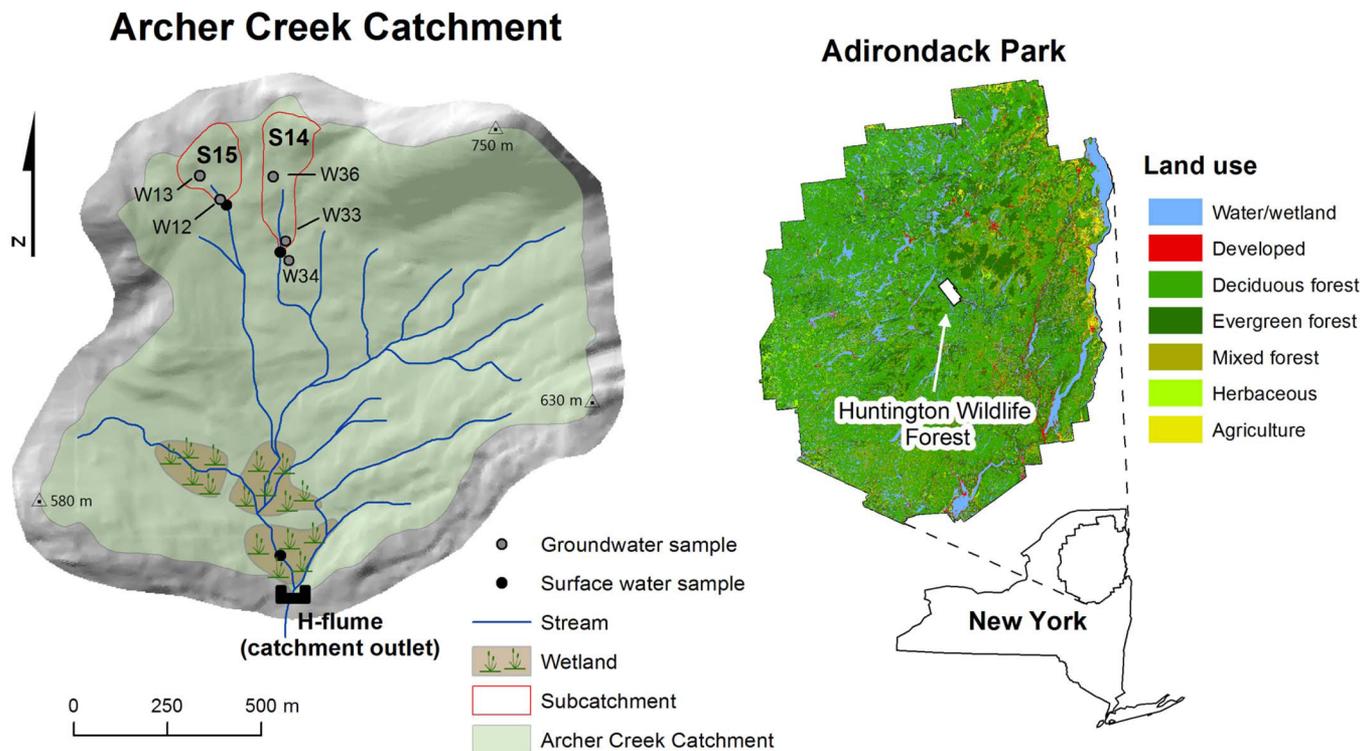


Figure 1. Site map showing the location of groundwater and surface water sampling sites in the Archer Creek Catchment, Huntington Wildlife Forest, New York State.

bacterial dissimilatory SO_4^{2-} reduction (BDSR) into dissolved organic matter (DOM). Determining stable S ($\delta^{34}\text{S}$) and oxygen ($\delta^{18}\text{O}$) isotope ratios of SO_4^{2-} and DOS provides information about the sources and fate of both inorganic and organic S in terrestrial and aquatic ecosystems.¹² Regarding the production from plant and soil organic S (SOS), most processes in the aerobic S cycle linked to assimilatory production by plants and microbes occur with small (<2‰) S isotope fractionation.¹⁴ A major source of organic S formed by assimilatory processes is plant litter, which is converted into soil organic matter that often shows progressively increasing $\delta^{34}\text{S}$ values with soil depth due to the preferential release of slightly ^{32}S -enriched SO_4^{2-} during mineralization.¹⁵ Since hydrolysis of solid organic S to DOS proceeds with little S isotope fractionation, the resultant $\delta^{34}\text{S}$ –DOS values are similar to those of the solid organic matter.¹⁶

BDSR, a bacterial process reducing dissolved SO_4^{2-} (an electron acceptor) to reduced inorganic S under anaerobic conditions to obtain energy, causes substantial isotopic fractionation. This process preferentially metabolizes ^{32}S and ^{16}O resulting in the formation of ^{34}S -depleted reaction products including H_2S and HS^- due to the strong kinetic isotope effects associated with BDSR.¹⁷ Consequently, the remaining reactant SO_4^{2-} becomes enriched in ^{34}S and ^{18}O resulting in elevated $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ values.¹⁸ Reoxidation of the ^{32}S -enriched reduced S reaction products to SO_4^{2-} has been shown to occur with little S isotope fractionation,¹⁹ thereby generating SO_4^{2-} with often distinctly negative $\delta^{34}\text{S}$ values. In marine environments with high SO_4^{2-} concentrations, ^{34}S -depleted HS^- produced by BDSR is incorporated into DOM during the early diagenesis of organic matter in anaerobic marine sediments.²⁰ This newly generated DOS has markedly lower $\delta^{34}\text{S}$ values than the initial SO_4^{2-} prior to BDSR because of the large S isotope fractionation effect associated with BDSR.²¹ Canfield et al.²¹

studied the dynamics of organic S diagenesis in sediments in Mangrove Lake, Bermuda, and found that sulfide generated from BDSR was rapidly incorporated into organic matter constituting 40–70% of the organic S.

In forested catchments including wetlands, the isotopic changes due to internal processes such as BDSR and peat maturation were initially studied using total S in soil with depth.¹⁵ Through isotope analyses of different S compounds, Mandernack et al.²² showed that SO_4^{2-} is produced by the reoxidation of reduced S compounds derived from BDSR in a peat wetland. Recently, Giesler et al.¹⁶ analyzed $\delta^{34}\text{S}$ values of DOS samples in surface waters in Sweden, and suggested the potential importance of S isotopic variations of DOS as a tracer of DOM. They developed a conceptual model of S biogeochemistry based on the isotopic S changes, and showed that $\delta^{34}\text{S}$ –DOS values of around -4‰ in mire-dominated stream were due to BDSR. For the Archer Creek Catchment in the Adirondack Mountains (U.S.), it has been shown that the reoxidation of HS^- produced from BDSR or a mineral weathering source of S may be responsible for decreasing $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ values associated with increasing SO_4^{2-} concentrations.²³ These results suggest that BDSR may potentially also affect the DOS in this catchment. Further support of the potential importance of BDSR and DOS is the observation of high concentrations of DOS compared to DON in the catchment.²⁴

Therefore, the major goal of our study was to evaluate the sources of DOS in the Archer Creek Catchment using a combination of chemical and isotopic measurements on groundwater and surface water samples, including upland and wetland streams. We demonstrate how BDSR-derived S compounds contribute to the formation of DOS providing further understanding of DOM dynamics in forested catchments. We also develop a conceptual model of the S cycle in

forest ecosystems that shows the assimilatory and dissimilatory production of DOS and associated S isotope fractionation effects.

MATERIALS AND METHODS

Site Description. The Archer Creek Catchment (135 ha) is located in the Huntington Wildlife Forest in the Adirondack Mountains, NY, USA and is the major tributary to Arbutus Lake (43°58'48"N, 74°13'48"W) (Figure 1). The elevation ranges from 513 to 748 m.²⁵ The average annual air temperature is 4.8 °C and total annual precipitation is 1,080 mm.²⁵ The overstory vegetation on the upper slopes consists of mixed northern hardwoods including American beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*), red maple (*Acer rubrum*), yellow birch (*Betula alleghaniensis*), and white pine (*Pinus strobus*). Lower slopes close to the lake are often dominated by conifer stands including eastern hemlock (*Tsuga canadensis*), red spruce (*Picea rubens*), and balsam fir (*Abies balsamea*).²⁵ The bedrock is largely composed of Precambrian rock consisting primarily of granitic gneiss with some gabbro-amphibolite.²⁶ Archer Creek is located within the Anorthosite Massif, a large intrusive igneous rock complex characterized by 90% calcium-rich feldspar.²⁷ The surficial geology consists of thin to medium thick deposits (<3 m deep) of glacial til with high sand (75%) and low clay (10%) contents with an abundance of cobbles and boulders.²⁷ Upland catchment soil depth varies, but typically is less than 1 m and includes Becket-Mundall series sandy loams (course-loamy, mixed, frigid, typic Haplorthods).²⁶

The two upland subcatchments (S14 and S15) in the Archer Creek Catchment are nearly adjacent and have similar physiographical features, but differ in soil chemistry and forest vegetation.²⁶ The S14 subcatchment (3.5 ha) has higher concentrations of elemental Ca²⁺ and extractable NO₃⁻ in soil, and a higher soil pH than the S15 subcatchment (2.5 ha). The S14 subcatchment also contains a higher percentage of base-rich indicator tree species of the total basal area (species area per unit catchment area) including sugar maple, American basswood (*Tilia americana*), eastern hophornbeam (*Ostrya virginiana*), and smaller percentages of tree species indicative of low base availability, such as American beech.²⁶ Stream water chemistry in S14 and S15 reflects these differences in soil and vegetation attributes in pH values (7.3 and 6.7, respectively), concentrations of Ca²⁺ (851 and 73 μmol L⁻¹, respectively) and concentrations of NO₃⁻ (427 and 26 μmol L⁻¹, respectively).²⁶ Wetlands compose 4% of the area of the Archer Creek Catchment including a palustrine wetland (Greenwood Mucky peats) that is located in the valley bottom (Figure 1).²⁸ These wetlands play an important role in controlling dissolved organic carbon (DOC) concentrations in surface waters at various locations within this catchment.²⁹

For groundwater sampling, wells constructed of 5.08 cm-diameter PVC pipes were installed in subcatchments S14 and S15 in 2000 (Figure 1).³⁰ In S14, wells 33 and 34 are located near the outlet and W36 is located in a hillslope hollow approximately 150 m upstream from the outlet. Wells 33, 34, and 36 were installed to a depth of 212, 91, and 232 cm with 34, 33, and 38 cm of screening, respectively. In S15, wells 12 (located near the outlet) and 13 (located in a hillslope hollow) were installed to depths of 173 and 90 cm with 59 and 46 cm of screening, respectively.³⁰ Using piezometer nests installed near wells, Christopher³⁰ determined that wells 12 and 36 were classified as deep hillslope groundwater wells and W34 was

characterized as a shallow discharging groundwater well. Wells 13 and 36 were the same groundwater sampling locations used by Campbell et al.³¹ where concentrations and stable S and O isotope ratios of SO₄²⁻ were measured during winter and spring snowmelt from 2002 to 2003.

For stream surface water samples, S14 and S15 samples were collected at the weirs preinstalled at the outlets of the S14 and S15 subcatchments,³¹ and the wetland sample was collected by grab samples (Figure 1). Stream discharge was measured at an H-flume at the outlet of the Archer Creek Catchment (Figure 1).³⁰

Sampling and Chemical Analysis. Water samples were collected 12 times over a 14-month period (May, 2009 to June, 2010) from the five groundwater wells (wells 12 and 13 in the S15 subcatchment and wells 33, 34, and 36 in the S14 subcatchment) and three surface water locations (S14, S15 and wetland) as shown in Figure 1. Two liters of groundwater were hand pumped into polyethylene bottles that had been acid-washed and rinsed with ultraclean deionized water. Surface water samples were collected at the weir and placed into 20 L flexible plastic prewashed bottles and sealed. All sample bottles were sealed tightly in order to minimize any oxidation of reduced S compounds. The water samples were kept on ice and transported to the Biogeochemistry Laboratory at SUNY-ESF in Syracuse, NY, and then refrigerated at 4 °C. Immediately before analyses, samples were filtered with a GF/F filter (Whatman). Sampling collection dates and the daily discharge at the Archer Creek Catchment outlet are shown in Figure 2.

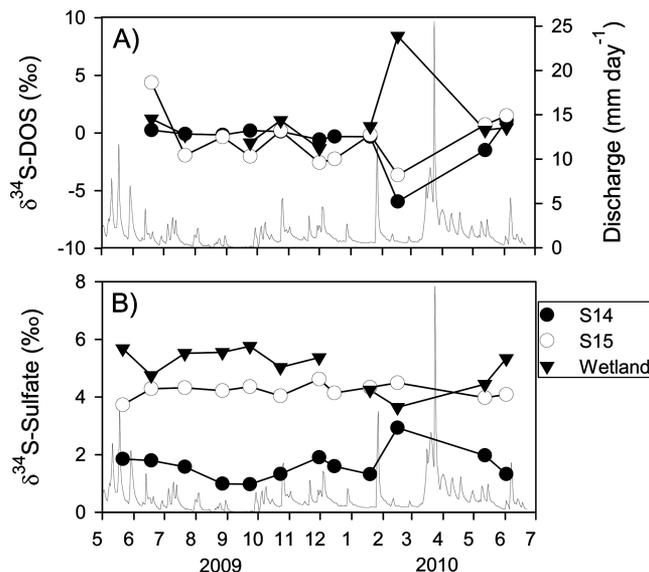


Figure 2. Daily discharge (solid line) at the H-flume in the Archer Creek Catchment and the variation of $\delta^{34}\text{S-SO}_4^{2-}$ and $\delta^{34}\text{S-DOS}$ in surface waters (S14, S15, and Wetland).

Concentrations of NO₃⁻ and SO₄²⁻ were determined using a Dionex ion chromatograph with analytical precisions (and detection limits) of $\pm 3.9\%$ (1.1 μmol L⁻¹) and $\pm 2.0\%$ (0.25 μmol L⁻¹), respectively. NH₄⁺ concentration was measured with an autoanalyzer using the Berthelot Reaction followed by colorimetric analysis with analytical precisions (and detection limits) of $\pm 3.2\%$ (1.4 μmol L⁻¹). Total dissolved S (TDS) was determined using inductively coupled plasma spectrometry (model: PerkinElmer Optima 3300DV), with an analytical precision of $\pm 3.9\%$. DOS was calculated as TDS minus SO₄²⁻.

S. The S portion of DOS and SO_4^{2-} to the TDS averaged 21% and 79%, respectively. The calculated error for the DOS concentration was $\pm 4.4\%$ (square root of the sum of the squared analytical precision of TDS and SO_4^{2-}).²⁴

Isotopic Analysis of SO_4^{2-} . The isotopic composition of SO_4^{2-} was analyzed in duplicate using 1 L of sample for each determination. After filtration, 1 mL of 3 molar HCl was added to the filtrate to remove HCO_3^- . The filtrates were passed through anion exchange resin columns (Bio-Rad Polyprep, AG 1X-8, Bio-Rad Laboratories, Hercules, CA) to retain SO_4^{2-} .³² Ion chromatographic analysis of SO_4^{2-} in the effluent confirmed that all the SO_4^{2-} had been retained on the resins. The resin columns that contained adsorbed SO_4^{2-} were shipped to the Isotope Science Laboratory (ISL) at the University of Calgary (Alberta, Canada) for isotopic analyses. At ISL, SO_4^{2-} was eluted from each column three times using 5 mL (in total 15 mL) of 3 molar HCl. Approximately 5 mL of a 0.5 molar BaCl_2 solution was added to the eluent to quantitatively precipitate BaSO_4 . The BaSO_4 precipitate was subsequently recovered by filtration using Millipore HAWP01300 or HTBP01300 (nominal pore size: 0.45 μm), and the filters were rinsed with deionized water, air-dried, weighed, and stored. Further details on the procedure are provided in Kang et al.³²

Sulfur dioxide for mass spectrometric analyses was generated by thermal decomposition of BaSO_4 in an elemental analyzer. Sulfur isotope ratios were determined by continuous flow isotope ratio mass spectrometry (CF-IRMS).³³ For O isotope analyses of SO_4^{2-} , BaSO_4 -oxygen was converted to CO at 1,450 °C in a pyrolysis reactor (Finnigan TC/EA, Thermo Electron Bremen, Germany). The resultant gas was subsequently swept with a He stream into a mass spectrometer (Finnigan MAT delta plus XL) for isotope ratio determinations in continuous-flow mode (CF-IRMS).³³ All isotope ratios are expressed in the conventional 'delta (δ) notation' in per mil (‰):

$$\delta(\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3$$

where R_{sample} and R_{standard} are the $^{34}\text{S}/^{32}\text{S}$ or $^{18}\text{O}/^{16}\text{O}$ ratios of the sample and the standard, respectively. The internationally accepted standards are Vienna-Canyon Diablo Troilite (V-CDT) for S isotope ratios and Vienna Standard Mean Ocean Water (V-SMOW) for O isotope ratios. The $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ values were calibrated with the international reference materials NBS127 (+21.1‰ and +8.6‰), IAEA S05 (+0.5‰ and +12.0‰) and IAEA S06 (−34.1‰ and −11.3‰, respectively). Analytical precision (i.e., sample pretreatment, gas preparation, and mass spectrometric analyses) determined by long-term monitoring of international and laboratory-internal standards and reference materials was $\pm 0.5\text{‰}$ for both $\delta^{34}\text{S}$ and for $\delta^{18}\text{O}$.³²

Isotopic Analysis of Dissolved Organic Sulfur. $\delta^{34}\text{S}$ values of DOS were determined for all surface water samples, but not for groundwater samples due to insufficient amounts of DOS in the groundwater samples used to isolate DOS using the tangential flow ultrafiltration method. For isolating DOS in surface water, 10 to 14 L of sample water was filtered through a precombusted (550 °C, 2 h) GF/F filter (Whatman). The filtrate was then passed through a tangential flow ultrafiltration system with a 1k Da cutoff (Millipore Pellicon 2 Cassettes) to separate the retentate (>1k Da) and the permeate (<1k Da). The average volume of retentate was 400 mL (120 to 800 mL range). For each sample, 10 mL of retentate was diluted 10-fold

with deionized water, and analyzed for TDS and SO_4^{2-} concentrations used to calculate DOS concentrations. Twenty to 40 mL of retentate was subsampled in an attempt to measure $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ values of SO_4^{2-} , but the amount of SO_4^{2-} in these subsamples was insufficient to provide accurate isotopic values. The remaining retentate was frozen, freeze-dried and then shipped to the ISL for $\delta^{34}\text{S}$ analysis. The permeate was analyzed by the same procedure as the retentate.

To maintain the capability of the ultrafiltration system and to eliminate carryover effects from previous samples, the cassette membrane was cleaned between samples as follows: (1) washing with 7 L of NaOH solution (pH 11); (2) washing with 10 L of deionized water; (3) washing with 7 L of HCl solution (pH 2); and (4) washing with 10 L of deionized water.¹⁶ Water volumes of filtrate, retentate, and permeate were measured to determine water recovery rates (calculated as (retentate + permeate)/filtrate) of the ultrafiltration system, which averaged 99% recovery. The ultrafiltration procedure yielded sufficient amounts of S (0.1–1.4 mg) to analyze the S isotope ratio of TDS in the retentate.

For the calculation of $\delta^{34}\text{S}$ in DOS, the following mass balance equations (eq 1 to 3) were used:

$$\text{TDS} = \text{DOS} + \text{SO}_4^{2-} = \text{TDS}_{\text{ret}} + \text{TDS}_{\text{per}} \quad (1)$$

$$\text{TDS}_{\text{ret}} = \text{DOS}_{\text{ret}} + \text{SO}_4^{2-}_{\text{ret}} \quad (2)$$

$$\text{TDS}_{\text{per}} = \text{DOS}_{\text{per}} + \text{SO}_4^{2-}_{\text{per}} \quad (3)$$

where "ret" and "per" indicated retentate and permeate, respectively.

We assumed that "DOS_{per}" in eq 3 was negligible, because the contribution of the DOS_{per} to the TDS was small (average 13%), and hence, the determination of $\delta^{34}\text{S}$ in the DOS_{per} was not possible due to the technical limitations for concentrating DOS_{per} samples for isotopic analysis. Therefore, assuming "DOS_{per}" is negligible, it follows from eqs 1 and 3 that

$$\text{DOS} = \text{TDS}_{\text{ret}} + \text{SO}_4^{2-}_{\text{per}} - \text{SO}_4^{2-}_{\text{ret}} \quad (4)$$

Equation 4 was then used to develop chemical and isotopic mass balances with measured or calculated $\delta^{34}\text{S}$ values and the mass of S in TDS, DOS, or SO_4^{2-} as follows:

$$\begin{aligned} M_{\text{DOS}} \times \delta^{34}\text{S}_{\text{DOS}} &= M_{\text{TDS}_{\text{ret}}} \times \delta^{34}\text{S}_{\text{TDS}_{\text{ret}}} \\ &+ M_{\text{sulfate}_{\text{per}}} \times \delta^{34}\text{S}_{\text{sulfate}_{\text{per}}} \\ &- M_{\text{sulfate}_{\text{ret}}} \times \delta^{34}\text{S}_{\text{sulfate}_{\text{ret}}} \end{aligned} \quad (5)$$

where M is a mass calculated as the product of the concentration and volume for each term.

Thus, the final equation for determining the $\delta^{34}\text{S}$ value of DOS is

$$\begin{aligned} \delta^{34}\text{S}_{\text{DOS}} &= (M_{\text{TDS}_{\text{ret}}} \times \delta^{34}\text{S}_{\text{TDS}_{\text{ret}}} + M_{\text{sulfate}_{\text{per}}} \\ &\times \delta^{34}\text{S}_{\text{sulfate}_{\text{per}}} - M_{\text{sulfate}_{\text{ret}}} \times \delta^{34}\text{S}_{\text{sulfate}_{\text{ret}}}) / M_{\text{DOS}} \end{aligned} \quad (6)$$

The total S in the sample material was oxidized to SO_4^{2-} by Parr bomb oxidation for subsequent determination of $\delta^{34}\text{S}_{\text{TDS}_{\text{ret}}}$.³³ This technique converts total S to SO_4^{2-} , which is subsequently precipitated as BaSO_4 by adding 0.25 molar BaCl_2 solution. The precipitate was filtered, weighed, and converted to SO_2 in an elemental analyzer followed by CF-

Table 1. Average (Standard Deviation) of Isotope Ratios of $\delta^{34}\text{S}-\text{SO}_4^{2-}$, $\delta^{18}\text{O}-\text{SO}_4^{2-}$, and $\delta^{34}\text{S}-\text{DOS}$ and Concentrations of SO_4^{2-} , DOS , NO_3^- , and NH_4^+ in Groundwater and Surface Waters^a

	S15 subcatchment			S14 subcatchment				wetland (11)
	W12 (11)	W13 (11)	S15 (12)	W33 (11)	W34 (10)	W36 (10)	S14 (12)	
$\delta^{34}\text{S}-\text{SO}_4^{2-}$ (‰)	+4.1 (0.5) ^B	+4.0 (0.3) ^B	+4.3 (0.3) ^B	+4.0 (0.6) ^B	^I +18.6 (13.0) ^A	+2.1 (0.3) ^B	+1.7 (0.6) ^B	+5.1 (0.7) ^B
$\delta^{18}\text{O}-\text{SO}_4^{2-}$ (‰)	+2.6 (0.5) ^B	+2.3 (0.5) ^B	+2.8 (0.6) ^B	+3.2 (1.0) ^B	^{II} +11.6 (3.6) ^A	+0.8 (0.5) ^C	+0.8 (0.8) ^C	+2.4 (0.8) ^B
$\delta^{34}\text{S}-\text{DOS}$ (‰)	n/a	n/a	-0.6 (2.3)	n/a	n/a	n/a	-0.7 (1.9)	+0.9 (2.8)
SO_4^{2-} ($\mu\text{mol L}^{-1}$)	56.8 (3.9) ^{B,C}	56.8 (1.8) ^{B,C}	90.8 (14.2) ^A	45.6 (9.9) ^C	8.2 (7.0) ^D	86.7 (8.3) ^A	85.4 (9.9) ^A	60.5 (16.8) ^B
DOS ($\mu\text{mol L}^{-1}$)	5.2 (2.4) ^E	5.4 (2.5) ^E	^{III} 12.6 (2.8) ^{C,D}	14.4 (10.3) ^C	10.1 (1.5) ^{C,D,E}	29.8 (2.9) ^A	^{III} 21.8 (2.5) ^B	^{III} 8.3 (1.2) ^{D,E}
NO_3^- ($\mu\text{mol L}^{-1}$)	0.2 (0.4) ^F	7.3 (1.8) ^{C,D,E}	10.3 (2.4) ^{C,D}	12.5 (7.4) ^C	2.0 (0.8) ^{E,F}	40.4 (6.2) ^A	30 (3.2) ^B	6.6 (5.2) ^{D,E}
NH_4^+ ($\mu\text{mol L}^{-1}$)	1.5 (1.3) ^B	1.2 (0.7) ^B	1.2 (1.2) ^B	2.3 (2.7) ^B	25.6 (3.9) ^A	1.3 (1.3) ^B	1 (0.6) ^B	2.5 (3.7)

^aSample size is shown in parentheses with a site name unless noted by ^I(sample size=6) or ^{II}(sample size=3). ^{III}Data from Kang and Mitchell³³.
^cA,B,C,D,E Grouping information based on one-way ANOVA using Tukey's pairwise comparisons across sites of each concentration or isotopic item.

IRMS. The calculated error for $\delta^{34}\text{S}_{\text{DOS}}$ values was $\pm 0.9\%$ (square root of the sum of the squared analytical precision of $\delta^{34}\text{S}_{\text{TDS}_{\text{rev}}}$, $\delta^{34}\text{S}_{\text{sulfate}_{\text{per}}}$ and $\delta^{34}\text{S}_{\text{sulfate}_{\text{fate}}}$ which is $\pm 0.5\%$ each).²³

Statistical Analyses. We used a one-way analysis of variance (ANOVA) in conjunction with Tukey's pairwise difference test to elucidate average concentration and statistical differences in stable isotopic values across sites at $\alpha = 0.05$ level using Minitab (Version 16, Minitab, Inc.). Linear regression models were used at $\alpha = 0.05$ level using SigmaPlot (Version 11.0, Systat Software, Inc.).

RESULTS AND DISCUSSION

Isotopic Evidence of SO_4^{2-} in Groundwater Derived from Microbial Processes. We observed lower $\delta^{18}\text{O}-\text{SO}_4^{2-}$ values of groundwater from wells 12, 13, and 33 (+2.6, +2.3, and +3.2‰, respectively. Table 1) compared to values in bulk precipitation from a previous study.³¹ This suggested that SO_4^{2-} in groundwater from wells 12, 13, and 33 was derived primarily from oxidation of carbon-bonded S, since oxygen from soil water with negative $\delta^{18}\text{O}$ values is predominantly incorporated during the formation of the resultant mineralized SO_4^{2-} .^{31,34} A comparison at the Hubbard Brook Experimental Forest in New Hampshire of precipitation and streamwater $\delta^{18}\text{O}-\text{SO}_4^{2-}$ values also found that the soil water values were lower than those of precipitation.³⁵ Moreover, the study of Campbell et al.³¹ found a decrease in the $\delta^{34}\text{S}-\text{SO}_4^{2-}$ values from precipitation and streamwater, respectively,³⁶ suggesting the production of some SO_4^{2-} with $\delta^{18}\text{O}$ values of -10 to +4‰ due to the oxidation of sulfide minerals.³⁷ Other soil processes, such as hydrolysis of ester SO_4^{2-} and adsorption/desorption of SO_4^{2-} in soil, have been shown to cause minor or negligible isotopic fractionation resulting in little to no change in the $\delta^{34}\text{S}$ and $\delta^{18}\text{O}$ values of SO_4^{2-} .¹²

Groundwater from W36 showed significantly lower ($F_{7,75} = 60.91$, $p < 0.001$) $\delta^{18}\text{O}-\text{SO}_4^{2-}$ values ($\sim +0.8\%$) than groundwater $\delta^{18}\text{O}-\text{SO}_4^{2-}$ values from wells 12, 13, and 33, and higher SO_4^{2-} concentrations ($F_{7,83} = 78.39$, $p < 0.001$) and DOS concentrations ($F_{7,83} = 44.25$, $p < 0.001$) (Table 1). Our results for W36 are also consistent with the study of Campbell et al.,³¹ and suggest the weathering of S-bearing minerals is responsible for the observed $\delta^{34}\text{S}-\text{SO}_4^{2-}$ and $\delta^{18}\text{O}-\text{SO}_4^{2-}$ values (+0.8 and +2.1‰, respectively) and high SO_4^{2-} concentrations ($87 \mu\text{mol L}^{-1}$).³⁷ Mineral weathering processes might have influenced groundwater from all wells, but our results suggest that water in W36 was most influenced by S mineral weathering processes.^{31,37} The significantly high concentration of NO_3^- in W36 might be the result of the

mineralization of organic nitrogen followed by nitrification that may have occurred concomitantly with the mineralization of organic S, and therefore the mineralization of DOS or SOS might have also contributed to the lower $\delta^{34}\text{S}-\text{SO}_4^{2-}$ value in W36 (Table 1). Groundwater from W34 had significantly higher $\delta^{34}\text{S}-\text{SO}_4^{2-}$ (range: +14.3 to +16.5‰) and $\delta^{18}\text{O}-\text{SO}_4^{2-}$ (range: +8.4 to +10.8‰) values than groundwater from all other sampling sites ($\delta^{34}\text{S}-\text{SO}_4^{2-}$: $F_{7,76} = 17.17$, $p < 0.001$; $\delta^{18}\text{O}-\text{SO}_4^{2-}$: $F_{7,75} = 60.91$, $p < 0.001$) (Table 1). This result strongly suggests the occurrence of BDSR in W34, since SO_4^{2-} -reducing bacteria preferentially metabolize the lighter isotopes ³²S and ¹⁶O resulting in increasing $\delta^{34}\text{S}-\text{SO}_4^{2-}$ and $\delta^{18}\text{O}-\text{SO}_4^{2-}$ values in the remaining SO_4^{2-} .¹⁷ In addition, most W34 groundwater samples had a strong "rotten egg odor" (e.g., characteristic of H_2S), indicating the occurrence of anaerobic conditions required for BDSR. Compared to other wells, groundwater from W34 had lower SO_4^{2-} concentrations (mean $8.2 \mu\text{mol L}^{-1}$; Table 1) but similar DOS concentrations (mean $10.1 \mu\text{mol L}^{-1}$; Table 1) resulting in very high (>1) molar $[\text{DOS}]/[\text{SO}_4^{2-}]$ ratios, suggesting that anaerobic conditions likely contributed to the conversion of SO_4^{2-} to sulfide. The latter would be retained within the aquifer and soil system unless converted to the more mobile DOS . It is likely that any S gaseous losses would be very small compared to solute fluxes.³⁸

Other factors also need to be considered in the interpretation of our results. Despite the high (average molar ratio = 1.2) $\text{DOS}:\text{SO}_4^{2-}$ ratios in groundwater from W34 compared to ratios in the other wells (range: 0.1 to 0.3), DOS concentrations in waters from W34 ($10.1 \mu\text{mol L}^{-1}$) were lower than concentrations in wells 33 and 36 (14.4 and $29.8 \mu\text{mol L}^{-1}$, respectively; Table 1). Additionally in W34, NO_3^- concentrations were detectable ($>0.7 \mu\text{mol L}^{-1}$, Table 1), suggesting that the well water was not completely anoxic; under anoxic conditions NO_3^- would have been denitrified before BDSR proceeds.⁴⁹ Groundwater from W34 also had significantly higher NH_4^+ concentrations ($F_{7,83} = 153.70$, $p < 0.001$; Table 1) than the other wells, so the solutes in groundwater from W34 were likely from various sources. For example, some of the area contributing to the well water chemistry could be highly anaerobic, resulting in bacterial SO_4^{2-} reduction and affecting DOS production, and also be a net source of NH_4^+ that would accumulate under anaerobic conditions. The significantly higher NH_4^+ concentrations in waters from W34 compared to wells 33 and 36 might also have been due to reduction of NO_3^- to NH_4^+ .³⁹ Some BDSR bacteria such as *Desulfovibrio desulfuricans* and *Desulfovibrio vulgaris* are known to reduce NO_3^- to NH_4^+ .⁴⁰

Change of Concentrations and $\delta^{34}\text{S}$ Values of SO_4^{2-} in Surface Waters. In the wetland and S14 streamwater, $\delta^{34}\text{S}-\text{SO}_4^{2-}$ values ranged from +3.6 to +5.8‰ and from +1.0 to +2.9‰, respectively, and SO_4^{2-} concentrations ranged from 50.3 to 66.3 $\mu\text{mol L}^{-1}$ and from 68.3 to 104.7 $\mu\text{mol L}^{-1}$, respectively. We observed that $\delta^{34}\text{S}-\text{SO}_4^{2-}$ values in both stream sites varied within a range of approximately +2‰ and generally increased with decreasing concentration, which is consistent with BDSR selectively metabolizing ^{32}S resulting in an increase in $\delta^{34}\text{S}-\text{SO}_4^{2-}$ values for the remaining SO_4^{2-} (Figure 3A).¹⁷ Although these regression relationships were not

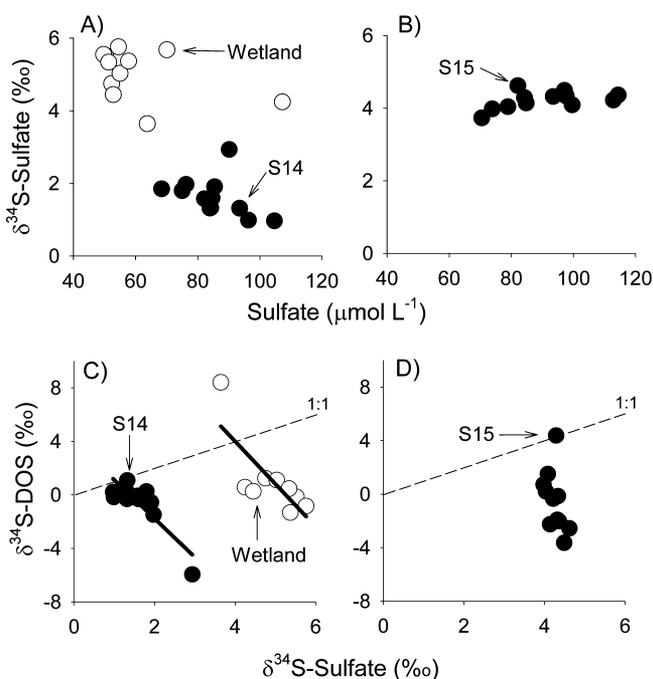


Figure 3. Relationship between the SO_4^{2-} concentrations and $\delta^{34}\text{S}-\text{SO}_4^{2-}$ in surface waters at (A) wetland and S14 (slope = -0.02 , $r^2 = 0.17$, $n = 12$, $p = 0.18$) and (B) S15, and the relationship between stable S isotope ratios of SO_4^{2-} and DOS in (C) S14 and the wetland and (D) S15. Linear regression lines are given for S14 (slope = -2.9 , $r^2 = 0.73$, $n = 11$, $p < 0.001$) and the Wetland (slope = -3.2 , $r^2 = 0.62$, $n = 10$, $p < 0.01$). Broken lines indicate a 1:1 relationship between $\delta^{34}\text{S}$ values.

statistically significant, previous studies at the Archer Creek Catchment have shown similar significant negative relationships between $\delta^{34}\text{S}-\text{SO}_4^{2-}$ values and SO_4^{2-} concentrations.¹⁹ These results along with the measurements of other solutes suggested that previously reduced S that was formed under wet conditions was later reoxidized with decreasing moisture levels and then mobilized to streamwater during storm events.¹⁹ In addition, the observation of high $\delta^{34}\text{S}-\text{SO}_4^{2-}$ values in surface water samples in the wetland compared to those from S14 (Table 1; Figure 3) was likely caused by BDSR and its remaining SO_4^{2-} with progressively enriched in ^{34}S .

In S15 streamwater, $\delta^{34}\text{S}-\text{SO}_4^{2-}$ values remained relatively constant over a range of SO_4^{2-} concentrations (Table 1; Figure 3B) and the variation in $\delta^{34}\text{S}-\text{SO}_4^{2-}$ values in S15 was relatively small (range: +3.7 to +4.6‰; Table 1) compared to S14 and the wetland. These results suggest that the streamwater SO_4^{2-} in S15 was less affected by BDSR and/or differences in S sources. The concentrations and $\delta^{34}\text{S}-\text{SO}_4^{2-}$ and $\delta^{18}\text{O}-\text{SO}_4^{2-}$ values in S15 were similar to those of groundwater from wells

12 and 13 (Table 1). Christopher³⁰ indicated that W12 yielded deep groundwater based on analyses of a range of solutes. Additionally, Campbell et al.³¹ suggested the importance of the oxidation of carbon-bonded S as a dominant sulfate source in groundwater at W13 based on $\delta^{18}\text{O}-\text{SO}_4^{2-}$ values ($\sim +2.2$ ‰).

The importance of chemical weathering of S-containing minerals affecting SO_4^{2-} and $\delta^{34}\text{S}$ values has been demonstrated by Bailey et al.⁴¹ for a range of forested catchments in the northeastern U.S. In our study, water samples from S14 and W36 had significantly lower $\delta^{34}\text{S}-\text{SO}_4^{2-}$ ($F_{6,71} = 87.95$, $p < 0.001$) and $\delta^{18}\text{O}-\text{SO}_4^{2-}$ values (Table 1) compared to S15 and wells 12, 13, and 33, likely partially caused by oxidation of sulfide minerals with lower $\delta^{34}\text{S}$ values. Since sulfides subject to oxidative weathering processes often have low $\delta^{34}\text{S}$ values, the produced SO_4^{2-} will usually retain the lower $\delta^{34}\text{S}-\text{SO}_4^{2-}$ value. Hence, some of the low $\delta^{34}\text{S}-\text{SO}_4^{2-}$ values may be partly affected by the oxidation of pyrite and pyrrhotite.³¹

Sulfur Isotope Variations of DOS in Surface Water Due to BDSR. The lowest $\delta^{34}\text{S}-\text{DOS}$ values in S14 and the wetland were -6.0 and -1.3 ‰, respectively, and associated with the highest $\delta^{34}\text{S}-\text{SO}_4^{2-}$ values (Figure 2 and 3). The lowest $\delta^{34}\text{S}-\text{DOS}$ values in S14 and S15 were observed following winter discharge in January to February, and may be due to the mobilization of DOS that was formed earlier under reduced oxygen availability. The overall range of $\delta^{34}\text{S}-\text{DOS}$ values in our study was -6.0 to $+8.4$ ‰ (Figure 3), which is comparable to results from the study of Giesler et al.¹⁶ that found $\delta^{34}\text{S}-\text{DOS}$ values of -5.2 to $+9.6$ ‰. Elevated $\delta^{34}\text{S}-\text{SO}_4^{2-}$ values would be a direct function of the SO_4^{2-} remaining from BDSR becoming enriched in ^{34}S .²⁰ In contrast, the incorporation of ^{34}S -depleted H_2S and HS^- into DOS results in low $\delta^{34}\text{S}-\text{DOS}$ values. Hence, the low $\delta^{34}\text{S}-\text{DOS}$ values observed in our study indicate the incorporation of ^{34}S -depleted H_2S or HS^- originating from BDSR.²¹ A marked increase of $\delta^{34}\text{S}-\text{DOS}$ values ($\sim +8.4$ ‰) in the wetland in February (Figure 2) was observed. The increase in $\delta^{34}\text{S}-\text{DOS}$ values might have resulted from either the hydrolysis of soil organic matter with elevated $\delta^{34}\text{S}$ values. The relative contributions of DOS sources with different $\delta^{34}\text{S}$ values would likely vary as a function of watershed connectivity.⁴² Research by Kang and Mitchell²⁴ in the Archer Creek Catchment at the same time as the current research found a similar increase of $\delta^{13}\text{C}-\text{DOC}$ values from -28.0 ‰ (January) to -27.0 ‰ (February). The lowest $\delta^{34}\text{S}-\text{SO}_4^{2-}$ value observed in February in the wetland might be due to the reoxidation of previously reduced S that was subsequently oxidized and mobilized during this period.

It is likely that BDSR varies spatially in the catchment, but in those areas where there is strong evidence of BDSR based on isotopic and concentration analyses of SO_4^{2-} , our results suggest that BDSR produces considerable amounts of sulfide that may serve as a precursor in the dissimilatory production of DOS. Some DOS may also be derived from assimilatory sulfate reduction that forms S-containing organic matter, including the amino acids, cysteine and methionine.⁴³ The formation of DOS from this assimilatory pathway would result in a small change (< 2 ‰) in the isotopic composition of the organic S.^{14,44,45} Unlike the results from S15, S14 and the wetland sites showed a significant relationship between SO_4^{2-} concentrations and $\delta^{34}\text{S}-\text{DOS}$ values indicating the importance of BDSR at these latter two locations (Figure 3C). In S15, the relatively constant $\delta^{34}\text{S}-\text{SO}_4^{2-}$ values ($\sim +4.5$ ‰) suggest that S sources have similar $\delta^{34}\text{S}$ values and that BDSR might not play a major

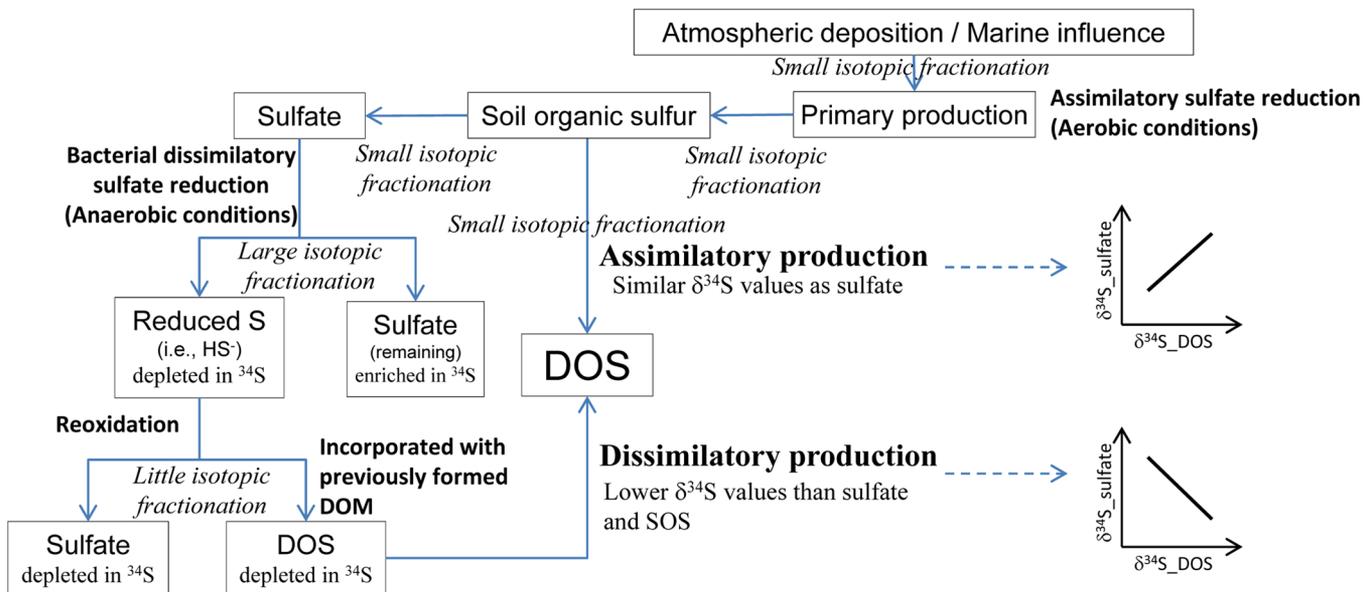


Figure 4. A conceptual model of the production of DOS and SO_4^{2-} showing isotopic changes based on assimilatory and dissimilatory production from forest soils and groundwater under aerobic and anaerobic conditions. We assume that the dissimilatory production of DOS is associated with the utilization of products (H_2S and HS^-) derived from bacterial dissimilatory SO_4^{2-} reduction (BDSR). Modified from Giesler et al.,²⁴ Mandernack et al.³⁰ and Werne et al.²¹

role. The SO_4^{2-} sources in the stream were sufficiently large to mask any isotopic differences caused by S transformations in the surface waters and associated sediments. In brief, other studies previously have shown that $\delta^{34}\text{S}$ values of SO_4^{2-} vary in surface waters. This variation can be attributed to S mineral sources,⁴¹ differences in the $\delta^{34}\text{S}$ values of atmospheric deposition and soil organic matter coupled with isotopic changes due to biological cycling of S.^{6,46} However, $\delta^{34}\text{S}$ –DOS values below 0‰ in our study suggest that formation of DOS was influenced by H_2S and HS^- from BDSR in portions of the watershed where redox conditions were suitable for this process to occur.

Importance of BDSR in the Forested Ecosystem: A Conceptual Model. Kang and Mitchell²⁴ showed that the spatial and temporal patterns of DOS within the Archer Creek Catchment vary greatly. We are only beginning to understand the mechanisms controlling DOS dynamics in watersheds. Whereas many previous studies in forested watersheds focused on understanding dynamics of SO_4^{2-} derived from assimilatory processes and relevant in-soil processes such as mineralization,⁶ our study demonstrates the importance of dissimilatory processes such as BDSR in affecting S biogeochemistry. BDSR clearly plays an important role in changing the concentrations and isotopic composition of SO_4^{2-} in streams within the catchment.²³

We have developed a conceptual model of S dynamics at the Archer Creek Catchment (Figure 4). The S isotope composition of DOS is affected by two-different pathways, an “assimilatory production” and a “dissimilatory production” pathway (Figure 4). In an assimilatory production-dominated system, the $\delta^{34}\text{S}$ –DOS value is similar to that of SO_4^{2-} that is taken up and converted to organic S compounds, because small (<2‰) S isotope fractionation occurs during the conversion of SO_4^{2-} to organic S and subsequently DOS.^{14,47} Therefore, a positive relationship between $\delta^{34}\text{S}$ of SO_4^{2-} and DOS is expected if assimilation is the dominant pathway for the incorporation of S into DOS. In contrast, if DOS formation is

dominated by dissimilatory processes (i.e., BDSR), the resultant DOS will have substantially lower $\delta^{34}\text{S}$ values (Figure 3C). Where dissimilatory processes dominate, the $\delta^{34}\text{S}$ values may be influenced by a broad range of factors including hydrological conditions, but generally a negative correlation is expected between $\delta^{34}\text{S}$ values of SO_4^{2-} and DOS (Figure 4), with DOS having the lower $\delta^{34}\text{S}$ values. Results from S15 displaying large variations in $\delta^{34}\text{S}$ –DOS versus $\delta^{34}\text{S}$ – SO_4^{2-} values indicate that both processes may occur simultaneously in watersheds on different spatial or temporal scales.

Future research using isotopic approaches should include more detailed analyses of the role of BDSR in affecting DOS formation in forested catchments including how the formation of DOS varies both within and among catchments. In addition, weathering reactions from various geological sources containing sulfide minerals such as pyrite (FeS_2)³⁰ should be further investigated to determine their contributions to S inputs to soil and seepage water SO_4^{2-} and its isotopic composition. The influence of hydrological controls in affecting groundwater and surface water S sources needs further understanding including the effects of changing redox conditions on the net retention or loss of SO_4^{2-} and DOS.^{5,48} Future studies should also make concomitant use of other stable isotopic signatures of DOM including $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ that may help provide a more comprehensive understanding of DOM dynamics.^{16,49} As S inputs from atmospheric deposition are decreasing and climatic factors are becoming more important in controlling S budgets of watersheds, a better understanding of the internal S transformations and cycling is needed. Such understanding is important for evaluating the recovery of watersheds from acidification and providing information on which factors are most important in controlling S budgets of watersheds.^{5,48} This information can be employed in policy decisions such as those that effect the emissions of S to the atmosphere.

AUTHOR INFORMATION

Corresponding Author

*Phone: +82 (0)32 560 7083; fax: +82 (0)32 568 2033; e-mail: philgkang@korea.kr, philgkang@gmail.com.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Stephen Taylor (University of Calgary) for analytical assistance, Patrick McHale and David Lyon (SUNY-ESF) for sampling and analytical assistance as well as Dr. Pamela Edwards (USDA Forest Service) for her review. We appreciate the helpful comments of Professor Reiner Giesler and two anonymous reviewers. This work was supported by U.S. National Science Foundation (Ecosystem Studies, LTER, Hubbard Brook), the Natural Sciences and Engineering Research Council of Canada (NSERC), and the New York State Energy Research and Development Authority (NYSER-DA).

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